NUTRIENT VALUES OF DIFFERENTLY PROCESSED *SENNA OBTUSIFOLIA* (LINN) SEEDS AND GROWTH PERFORMANCE OF *CLARIAS GARIEPINUS* (TEUGELS, 1982) FINGERLINGS FED THE FERMENTED MEAL

BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A DOCTOR OF SCIENCE IN FISHERIES

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FACULTY OF SCIENCE,
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DECLARATION

I hereby declare that this work, NUTRIENT VALUES OF DIFFERENTLY PROCESSED *SENNA OBTUSIFOLIA* (LINN) SEEDS AND GROWTH PERFORMANCE OF *CLARIAS GARIEPINUS* (TEUGELS, 1982) FINGERLINGS FED THE FERMENTED MEAL has been performed by me in the Department of Biological Sciences under the supervision of Prof. S.J. Oniye, Prof. J.K. Balogun and Prof. P.I. Bolorunduro. Any assistance or citation of other work has been duly acknowledged. I further declare that the results of this work have not been submitted for the award of any other degree or fellowship at any University.

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CERTIFICATION

This dissertation entitled NUTRIENT VALUES OF DIFFERENTLY PROCESSED *SENNA OBTUSIFOLIA* (LINN) SEEDS AND GROWTH PERFORMANCE OF *CLARIAS GARIEPINUS* (TEUGELS, 1982) FINGERLINGS FED THE FERMENTED MEAL by Onimisi Hassan UMARU meets the regulation governing the award of degree of Doctor of Philosophy of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To my beloved wife Mrs Onimisi Regina and Children Itopa, Adeiza, Sam, Oiza and Ohunene

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ABSTRACT

This study investigated the effects of three different processing methods (fermentation, boiling and toasting) on *Senna obtusifolia* seeds and replacement of soybean meal with fermented *Senna obtusifolia* seed meal on the growth of *Clarias gariepinus* fingerlings. Five isonitrogenous (42% crude protein) and isocaloric (25.7% NFE) diets were formulated in which fermented *Senna obtusifolia* seed meal (FSOSM) in graded levels replaced soybean meal (SBM) at 0% (FSOSM), 25% (FSOSM), 50% (FSOSM), 75% (FSOSM) and 100% (FSOSM). A commercial feed (CF) was used as reference diet. Three hundred and sixty (360) fingerlings of *Clarias gariepinus* with mean initial weight (2.85±0.01g) were stocked at twenty (20) fish per tank in three replicates per treatment and fed the experimental diet at 3% body weight twice a day for 180 days. Feeding quantity was adjusted after every weighing fortnightly. Water pH and temperature was taken daily (8.00-9.00h) using combined pH and thermometer model pH-009(111), while the Dissolved oxygen (DO) was determined using the DO meter model DO-510. Digestibility of diets was determined using the indirect method which relies on use of an inert marker (chromic oxide). At the end of the 180 days of feeding, three fish from each treatment were randomly selected for haematological analysis. Haematological parameters such as PCV, RBC and WBC were analyzed. Fermentation for 96hrs reduced the anti-nutrient compounds by 91%, 93%, 96% and 97% for phytates, tannins, cyanide and saponins, respectively, which were higher than the values obtained for boiling and toasting. Fermentation of *S. obtusifolia* seeds also had highest amount of amino acid content. The water quality measured (temperature, dissolved oxygen and pH) were within range recommended for *Clarias gariepinus*. Nutrient utilization was not affected ($p \geq 0.05$) in the control and fish fed the 25% inclusion of FSOSM. The commercial, 0% and 25% FSOSM groups had the highest mean weight gain (279 and 280 g) significantly ($P<0.05$) higher than the diets that received 50% and 75% FSOSM, while the diets that

received 100% FSOSM had the poorest mean weight gain (132.72g). Feed conversion ratios (FCR) ranged from (1.79 to 2.18), these values increased as the inclusion of FSOSM increased from 50 to 100%. The best FCR (1.79± 0.02) was recorded for fish fed 25% fermented. Protein intake among the fish fed 0 and 25% FSOSM was not affected ($p \geq 0.05$), fish fed 50, 75 and 100% FSOSM however, had dissimilar ($p < 0.05$) protein intake levels. Protein efficiency ratio (PER) exhibited significant differences ($P < 0.05$) in all treatments except in the control and fish fed the 25% SOSM. Apparent net protein utilization (ANPU) among treatments CF, 0% and 25% was not affected ($p \geq 0.05$). The ANPU was highest in fish fed 0% fermented SOSM (91.6±0.04) while fish fed 75 and 100% FSOSM had the least (71.4±0.02 and 73.4±0.03) respectively. Fermented SOSM affected ($p < 0.05$) final body carcass composition among treatments. The apparent digestibility coefficient of protein, lipid, dry matter and carbohydrates of the control and fish fed 25% FSOSM was not affected ($p \geq 0.05$). However, significant variation ($p < 0.05$) was obtained in the apparent digestibility coefficient of protein, lipid, dry matter and carbohydrates as the inclusion levels of FSOSM in the diets increased from 50 to100%. The range of PCV, RBC and Hb were 30.05 to 39.08% , 1.85 x $10^6 mm^{-3}$ to 3.10 x $10^6 mm^{-3}$ and 7.81 to 10.69g/100ml respectively, these values decreased as the level of FSOSM increased in the diet significantly ($P < 0.05$). White blood cells and lymphocyte count increased as the level of FSOSM increased in the diet and are significantly different ($P < 0.05$). The MCHC, MCH and MCV in this study recorded their highest values in fishes fed with 100% inclusion of FSOSM. The highest benefit cost ratio and net profit (1.65 and 77.01) was recorded in fish fed 25% FSOSM. Soybean meal can be substituted with FSOSM up to 25% in the diets of *Clarias gariepinus* without any negative effects on the growth and feed utilization. It was recommended that *S. obtusifolia* seed meal be fermented, rather than heat treated in

compounding feed for *C. gariepinus.* The inclusion of FSOSM in the diet of *C. gariepinus* should not exceed 25% level of substitution with soya bean.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Throughout the centuries, fish has been an important component of the population's diet in many parts of the world. Fish catches increased rapidly over the past hundred years due to improved technology, which provided more powerful engines and sonar equipment. This led to over fishing and caused a worldwide decrease in wild stocks. As a result, the growth in fish catches has reduced greatly over the years. The need to increase fish production by farming or aquaculture became therefore an urgent matter (Hassan, 2001).

Aquaculture remains one of the fastest-growing food-producing sectors in the world and is set to play a key role in meeting the rising demand for fishery products (FAO, 2014). The reported global production of food fish from aquaculture, including fin fishes, crustaceans, molluscs and other aquatic animals for human consumption, reached 66.6 million tonnes in 2012 with estimates for 2013 of 70.5 million tonnes (FAO, 2014).

Aquaculture is the fastest growing arm of the Nigerian agricultural industry **(**Ekunwe and Emokaro, 2009) and more people are now interested in fish farming as a source of livelihood or as an investment option (FAO, 2004). Profitable and sustainable fish farming all over the world is a product of scientific or technological know-how, and aquaculture has progressed beyond the primitive culture into a highly sophisticated business.

The trend in fish culture is towards intensification of culture systems whereby provision of feeds, becomes a necessity and success depends significantly on the availability of well balanced, nutritionally complete and cost effective compounded feeds (Chuang *et al*.,1986). Formulated fish feeds are available as locally produced or imported, but the cost of these feeds is relatively high. The major ingredients such as fish meal, and other additives to fish feed are generally imported and the prices are rising annually, hence to sustain the industry and make fish available to all, there is the need to seek locally available alternatives with the aim of reducing the cost of importation and production which is usually associated with the feed.

Fishmeal is a limited feed resource and serious concern exists on the future availability of this feedstuff for incorporation in fish diets (Hardy, 1996; Sargent and Tacon, 1999). Fishmeal is one of the main protein sources used in omnivorous fish diets, particularly in catfishes, because it has the most adequate amino acid profile, very good source of essential fatty acids and minerals and is highly palatable (Alceste *et al*.,2000; Oliva-Teles and Goncalves, 2001).

Plant feedstuffs, such as leucaena leafmeal, cranberry bean, and soybean (Amisah *et al*., 2009; Aremu *et al*., 2010; Fapohunda, 2012) have received most attention in recent years in African catfish feeds, however, due to amino acid imbalances, presence of anti-nutritional compounds and low palatability, a high level of replacement of fish meal with plant feedstuffs is generally not well accepted (Belgin *et al*., 2009). Profitable fish culture requires unfailing supply of formulated fish feed of low cost using the cheapest sources of proteins. To maintain the profitability of raising *Clarias gariepinus*, cutting production

costs is necessary, mainly through feeding, which has been acknowledged generally as the major cost incurred during the production cycle (Eyo, 2003; Jamu and Ayinla, 2003; Akinrotimi *et al*., 2007). Gabriel *et al*. (2007) reported that feed alone in fish farming accounted for between 60-70% of the running expense involved in the operation of fish farm as an enterprise. Reductions in feeding costs can be obtained by optimizing feeding strategies, nutrient levels in diets, and by using vegetable sources as substitutes for fish oil and fish meal (Martinez-Llorens *et al.,* 2009).

Some plant ingredients (soya seed meal, extruded peas and jack seed meal) have been studied in *Clarias gariepinus*, but jack bean and soybean meal in particular, are widely used in fish nutrition because of their high protein (40-60%) content, low cost and relative availability. Soybean meal, being the most nutritive of plant protein sources, has been used as the major protein source in many fish diets as partial or total replacement of dietary fish meal. Soybean meal has been used in the diets of Tilapia and catfish species (Soltan *et al.,* 2001; Fagbenro and Davies, 2002; Eyo, 2003; Abdelhamid *et al*., 2006; Magdy, 2006).

Senna obtusifolia also known as Tafasa in hausa is an annual plant considered a serious weed in many places and a competitive shrub that grow vigorously across the tropics (Ingweye *et al*., 2010) The plant grows to a height of about 1.5-2.5m tall and 1m wide. The pod is 10-15cm long, 3-5mm wide, slender and sickle shaped. The plant prefers well drained fertile soil. The leaves, seeds and roots are used for folk medicine especially in Asia. The roasted seeds are used for tea, coffee or food additives, it is also used as a thickener and for commercial cassia gum (Queensland Government, 2006).The proximate

composition of the seeds shows a high dry matter (95.50%), crude protein (29.54%) and crude fibre (10.18%), while the leaves contains dry matter (92.40%), crude protein (27.4%) and crude fibre (16.8%), ether extract (3.8%) and nitrogen free extract (36.8%) and high concentrations of anti-nutrients which can be detoxified through processing (Ingweye *et al*.,2010; Ayssiwede, *et al*., 2011). The dried and fresh leaves of *Senna obtusifolia* are employed in the treatment of ulcers, ring worm and other parasitic diseases in Northern parts of Nigeria (Dharmendra, *et al*., 2010). The domestication of this legume as food for the Sahelian people, gave promising seed yield of 1ton/ha (Pasternak *et al.*, 2007; Paster *et al*., 2007).

1.2 Research Problem

Fish meal (FM) is considered to be the most desirable animal protein ingredient in aquaculture diets due to its high protein content, balanced amino acid profiles, high digestibility and palatability, and an excellent source of essential polyunsaturated fatty acids (Alceste *et al*., 2000; Oliva-Teles and Goncalves, 2001; Muzinic *et al.*, 2006). But, fish meal is one of the most expensive macro-ingredients (used in high percentages) in diet of farmed fish. The high cost of fish meal and its doubtful future availability in high quantity have made it imperative for the aquaculture industry to reduce or eliminate fish meal from fish and crustacean diets (Muzinic *et al.*, 2006) and or look for alternative that will satisfy the needs of the present and future.

Soybean meal (SBM) is a widely available protein source that has relatively abundant digestible protein, high energy contents and good amino acid profile (Hertrampf and Piedad-Pascual, 2000). Soybean meal is used as a cost-effective feed ingredient and

comprises of about 50% of the diet of freshwater fish species (Yue and Zhou, 2008). The competition between human and livestock for the consumption of soybean and the increasing role of soybean in the world for biodiesel and feed stock have increased its cost and heightened the competition between human and animals for soybean (Cotula *et al*., 2008; Spore, 2008).

The general shortage of fish meal, groundnut cake and soybeans necessitate the crave for an alternative ingredient for use in aquaculture feeds, of interest is the need to focus on plant produce that are not in serious demand by human for consumption, in order to develop a cost effective feed for the production of aquaculture organisms (Olude *et al.*, 2008; Ingweye *et al*., 2010). Though the presence of anti-nutritional factors in plant based proteins especially in legumes has affected their use in fish feeds, it can however, be solved by various processing methods to deactivate the inhibitors and reduce it to a tolerable level by the fish or removed completely (Tacon and Jackson, 1985; Eyo, 2001; Glencross *et al*., 2007)

1.3 Justification of the Study

Profitable fish culture requires unfailing supply of formulated fish feed and farm inputs, but the cost of feed ingredients particularly the protein input is increasing while market cost and value of major cultivated fish species remain static. Soybean which is the main plant protein used in formulating livestock and fish feed in Nigeria, also serve as human food and it is required by various industries as raw material. These competition and needs for soybean has soared its demand and cost. Consequently, the search for a novel high quality, cheap and readily available source of plant protein to replace soybean is now a

major concern of livestock and fish nutritionists (Adeniji, *et al*., 2005; Obun and Ogunwale, 2006).

Already, domestication of *S. Obtusifolia* gave a promising seed yield of 1ton/ha, unlike soybean, its use for human consumption, animal feed formulation or as protein source in fish feed is equally unknown. Thus, formulation of low cost feeds using cheap and readily available sources of protein is essential to hasten fish production and to reduce feed cost.

1.4 Aims and Objectives

The aim of this study is to evaluate the effect of partial and complete replacement of soybean meal with varying levels of fermented *Senna obtusifolia* seed meal in the diet of *Clarias gariepinus*

1.5 Specific Objectives

The specific objectives are: To

a. Determine the proximate composition, amino acid profile, the anti-nutritive compounds and mineral contents of the raw, toasted, fermented and boiled *Senna obtusifolia* seeds

b. Evaluate the effect of replacing soybean meal with varying levels of fermented *S. obtusifolia* seed meal on growth performance and nutrient utilization of *C. gariepinus*

c. Investigate the effect of replacing soybean meal with varying levels of fermented *S. obtusifolia* seed meal on the haematological profile of *C. gariepinus*

d. Evaluate the effect of replacing soybean meal with varying levels of fermented *S. obtusifolia* seed meal on the carcass composition of *C. gariepinus*

e. Determine the economic viability of replacing soybean meal with fermented *S. obtusifolia* seed meal in the diets of *C. gariepinus*.

1.6 Hypotheses

The null hypotheses of the study are that:

1. There is no significant difference in the proximate composition, amino acid profile, minerals and the anti-nutritive compounds of the raw and fermented seeds of *Senna obtusifolia*

2. There is no significant difference in the growth performance and nutrients utilization of the *C. gariepinus* fed soybean meal and varying levels of the fermented *S. obtusifolia* seed meal

3. There is no significant difference on the haematological profile o*f C. gariepinus* fed soybean meal and varying levels of the fermented *S. obtusifolia* seed meal.

4. There is no significant difference in the carcass composition of the *C. gariepinus* fed soybean meal and varying levels of the fermented *S. obtusifolia* seed meal.

5. There is no significant difference in the economic viability of replacing soybean meal with fermented *S. obtusifolia* seed meal in the diet of *C. gariepinus*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Classification, Distribution and Agronomic Features of *Senna obtusifolia* (Linneaus)

Senna obtusifolia belongs to the *Fabaceae* (Leguminoseae) or bean (pea) family, and sub-family *Ceasalpinoideae.* It is annual weed or herb taxonomically known as *Senna obtusifolia*. Though native to India, this weed is also found in Myanmar, China, and Central America and in Nigeria. This weed is a member of the family Leguminoseae or *Fabaceae*. This is a small fetid herb growing up to an average height from 30 to 90cm (Parsons and Cuthbertson, 1992 and Holm *et al*., 1997).The stem is light green cylindrical or grooved and profusely branched. The leaves are paripinnate; leaflets are in 3- pairs and obovate-oblong. Leaves are arranged on a rachis which is grooved and up to 10 cm in length. Conical glands are found between each two lowest pairs of leaflets. The leaflets are usually in three pairs, opposite, obovate and oblong with oblique base. Flowers are found in pairs in axils of leaves. The flowers bloom in August to September. Corolla consists of five petals in yellow or pale colour. The pod is long and slender, 4 sided sharp and pointed. Each pod has 20-30 seeds that are rhombhedral in shape.

2.2 Trado-Medicinal Uses of *Senna Obtusifolia*

The plant *Senna obtusifolia* is extensively used in traditional medicine in tropical and warm sub-tropical countries. The leaves of *Senna obtusifolia*, as well as roots and seeds are used in folk medicine in Asia (Ambasta, 1986). It is believed to possess a laxative effect. Seeds of *Senna obtusifolia* are roasted and boiled in water to produce tea as folk medicine. Seeds of this plant yield a commercially valuable gum which is used as a food additive and as a thickener and "named for the Chinese Senna's former placement in the genus Cassia. Seeds of this plant have also been used as a substitute for coffee. These are also used in traditional Japanese medicine.

2.3 Proximate Composition of Raw *Senna obtusifolia*

The proximate composition of the raw seeds (Table 2.1) show a high dry matter (92.50%), crude protein (29.54%), ether extract (2.31) nitrogen free extract (NFE) (46.77) and crude fibre (10.18%) as reported by (Ingweye *et al*., 2010) while the leaves contains dry matter (92.40%), crude protein (27.4%) and crude fibre (16.8%), ether extract (3.8%) and nitrogen free extract (36.8%) as reported by (Ayssiwede, *et al*.,2011). It also contains alkaloid, saponin, tannins, oxalate and phytate contents in various concentrations. The hydrogen cyanide concentration is reported to be 6.98mg/100g. The essential amino acids (EAA) of *S obtusifolia* include phenylalanine, lysine, valine, leucine, isoleucine, threonine, methionine, cystine and tyrosine while the non-essential amino acids are aspartic acid serine, glutamic acid, proline, glycine and alanine (Table 2.2).

Table 2.1: proximate composition and antinutrients of raw *Senna obtusifolia*

Parameters	**Concentration(%DM)**
Drymatter	92.50
Crude protein	29.54
Ether extract	2.31
Crude fibre	10.18
Nitrogen free extract	46.77
Ash	3.70
Calorific value (Kcal/100g)	325.28
Phytochemicals	**Concentration (mg/100g)**
Alkaloids	360.00
Saponin	185.00
Tannin	388.50
Oxalate	83.25
Hydrogen cyanide	6.98
Phytate	240.50

Source: Ingweye *et al*. (2010).

Table 2.2: Amino acid composition of raw *Senna obtusifolia*

Amino acids	Concentration (g/100g protein)
Phenylalanine	4.50
Lysine	4.02
Valine	4.30
Leucine	7.60
Isoleucine	3.01
Threonine	2.20
Methionine	1.20
Cystine	0.92
Tyrosine	3.54
Histidine	7.11
Arginine	4.67
Aspartic acid	8.30
Serine	3.00
Glutamic acid	9.16
Proline	2.33
Glycine	4.11
Alanine	3.71

Source: Ingweye *et al*. (2010)

2.4 Nutritional Effect of Major Anti-nutrients in Plant Protein Sources

The major anti-nutrients mostly found in plant protein sources include toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins (phyto-haemagglutinins), protease inhibitors, chlorogenic acid and amylase inhibitors (Francis *et al*., 2001).

2.4.1 Tannins

Tannins, like gossypol, are a diverse group of polyphenolic compounds but in contrast to gossypol on hydrolysis, tannins yield sugar residues, phenolcarboxylic acids, and condensed tannins (Tacon, 1994). Tannins are widely distributed in nature, food crops such as sorghum contain up to 5% condensed tannins while legumes such as faba bean, lima bean, sunflower seed meal contain 1.2-2.7% chlorogenic acid (Rao and Prabhavathi, 1982; Francis *et al*., 2001). The toxic effects of chlorogenic acid can be counteracted by dietary supplementation with methyl donors such as choline and methionine, chlorogenic acid they can also be readily removed from sunflower seeds by aqueous extraction methods (Dominguez *et al*., l993). Common carp have been shown to tolerate 20 g condensed tannin powder /kg diet without any effect on feed intake and growth (Becker and Makkar, 1999). It is also speculated that the content of condensed tannins (24mg kg-1) was the cause of observed growth depression in tilapia and rohu (*Labeo rohita*) fingerlings when fed diets with 250mg/ kg or 200mg/ kg copra (Jackson *et al*., 1982; Mukhopadhyay and Ray, 1999).

2.4.2 Phytic acid

Phytic acid or the hexaphosphate of myo-inositol occurs naturally throughout the plant kingdom, and is present in considerable quantities within many of the major legumes and

oilseeds, including soybean, rapeseed and cottonseed, 62-73% and 46-73% of the total phosphorus within cereal grains and legume seeds are in the form of organically bound phytin phosphorus, respectively (Matyka *et al.*,1993). Apart from the major part of the phosphorus contained within phytic acid being largely unavailable to animals (due to the absence of the enzyme phytase within the digestive tract of monogastric animals), phytic acid acts as a strong chelator, forming protein and mineral-phytic acid complexes; the net result being reduced protein and mineral bioavailability (Erdman, 1979; Spinelli *et al*., 1983, Ketola, 1985; Davies and Gatlin, 1991; Hossain and Jauncey, 1993; NRC, 1993;). For example, phytic acid is reported to chelate metal ions such as calcium, magnesium, zinc, copper, iron, and molybdenum to form insoluble complexes that are not readily absorbed from the gastrointestinal tract (Liener, 1980, Hendricks and Bailey, 1989).

Feeding studies with diets containing high levels of purified phytic acid have been reported to have a negative effect on fish and shrimp. For instance, 0.5% phytic acid, reduced growth and feed efficiency in rainbow trout (O. *mykiss*) (Spinelli *et al*., 1983),1.*5%* phytic acid reduced zinc bioavailability in tilapia (*O. aureus*)(McClain and Gatlin, 1988) and 0.5% phytic acid reduced growth, feed efficiency, protein efficiency ratio and protein digestibility, reduced calcium and zinc bioavailability, hypertrophy and vacuolization of intestinal epithelium in common carp (*C. Carpio*) (Hossain and Jauncey, 1993) similarly, 1.5% phytic acid reduced zinc bioavailability in channel catfish (*1ctalurus punctatus*) (Gatlin and Phillips, 1989). 2.2% phytic acid reduced growth and feed efficiency, reduced zinc bioavailability (Satoh *et al*., 1989) in chinook salmon (*O. tshawytscha*), 2.58% phytic acid reduced growth, feed efficiency, protein efficiency ratio and thyroid function, increased mortality, promote cataract formation at low dietary zinc

levels, abnormal pyloric caeca structure (Richardson *et al*., 1985) in white shrimp (*Paeneus vannarnei*), 1.5% phytic acid reduced growth and feed efficiency, reduced phosphorus, calcium and zinc bioavailability, reduced muscle ash content and increased calcium ash content in tiger shrimp (*P. japonicas*) (Civera and Guillaume, 1989). Studies using oilseed meals containing phytic acid have also been reported to reduce mineral bioavailability. Gatlin and Wilson (1984) reported that the zinc requirement of channel catfish (*1. Punctatus*) fed a diet containing 50*%* soybean meal was about five times the normal requirement for growth. However, the negative effects of phytic acid on protein and mineral bioavailability can be reduced by the removal or extraction of phytic acid from the pulses or oilseed meals by the use of appropriate feed processing techniques (Han, 1988; Beleia *et al*., 1993; Prendergast *et al.,* 1994). For example, Hossain and Jauncey (1990) reported the loss of 48-72% and 51-74% of the original phytic acid content of linseed and sesame seed meal by aqueous extraction in (18hr) and autoclaving (120°C/2hr). Another and possibly more promising technique, has been the recent use of dietary phytase supplements within farm animal feeds as a means of breaking down the phytate protein/mineral complexes and thereby increasing protein and mineral bio availability for digestion (Gibson and Ullah, 1990; Naesi, 1990; Lei *et al*., 1993). Cheng and Guillaume (1984) reported a beneficial effect of purified phytic acid on growth and exoskeleton development in shrimp *P. japonicus.* Similarly, Clain and Gatlin (1988) reported reduced zinc bioavailability in tilapia *O. aureus* fed 1.5% phytate, fish growth improved compared with control fish fed no phytate. Recent studies with mammals have suggested that phytic acid may have a beneficial effect within the animal body by suppressing the incidence of colonic cancer by acting as a food antioxidant and forming

iron chelates that inhibit iron-catalyzed hydroxyl radical formation and lipid peroxidation (Empson *et al*., 1991; Graf and Eatin, 1993).

2.4.3 Saponins

Saponins are a heterogenous group of naturally occurring foam-producing triterpene or steroidal glycosides that occur in a wide range of plants, including pulses and oilseeds such as kidney bean, lentil, pea, chickpea, alfalfa, soybean, groundnut, lupin and sunflower (Liener, 1980; Price *et al*., 1987; Jenkins and Atwal, 1994). Saponins are reported to have diverse biological effects including antifungal and antiviral activity, immune stimulation, anticancer effects, antioxidant properties, inhibition of protein digestion and vitamin absorption, and glucocorticoid-like effects in fish (Francis *et al*., 2002a). Knudsen *et al*. (2006) demonstrated that soya saponins are not degraded during passage through the gastrointestinal tract in Atlantic salmon, and found a higher concentration (approximately 2.6 times) of saponins in feaces than in the diet, suggesting that bacterial degradation may be limited in fish. Francis *et al*. (2005) summarized the results of several reports of the growth promoting effects of saponins in common carp and tilapia. The authors claimed that maximum growth was obtained in carp fed diets containing 150 mg/kg saponin from *Quillaja saponaria* while maximum growth in tilapia was obtained when diets contained saponins at 300 mg/kg diet. Liener (1980) reported that saponins are readily removed from plant tissues by extraction with hot water or ethanol.

2.4.4 Alkaloids

Alkaloids represent a large and structurally diverse group of compounds present within angiosperm plants; the majority of alkaloids usually containing nitrogen in the form of a heterocyclic ring (Hendricks and Bailey, 1989). Some of the more well-known alkaloids include coniine (hemlock), nicotine (tobacco), atropine (deadly nightshade), cocaine (leaves of coca plant), quinine (cinchona bark), strychnine (seeds of *Nux vomica),* morphine (dried latex of opium poppy) and solanine (unripe potatoes and potato sprouts). Hendricks *et al*. (1981) have investigated the effect of dietary pyrrolizidine alkaloids (extracted from the tansy ragwort *Senecio jacobaea*) in rainbow trout (*O. mykiss*)*;* fish fed 100mg/kg diet of pyrrolizidine alkaloids displaying markedly reduced growth and mortality from the fourth month onwards.

Fish fed lower dietary concentrations (20mg/kg diet) displayed marked liver abnormalities, including liver megalocytosis, necrosis, fibrous tissue scarring, and veno occlusion of hepatic veins. The organoleptic properties such as bitter taste of the lupin alkaloids are believed to be responsible for decreased feed intake in fish fed diets containing high levels of alkaloids. Low-alkaloid varieties of lupins do not appear to cause palatability problems, but may reduce growth performance at higher inclusion levels (Glencross *et al*., 2004a and 2004b).

Glencross *et al*. (2006) added gramine, an indole alkaloid found in lupin to rainbow trout in diets at levels up to 10,000 mg kg-1 and reported decreased feed intake above 500 mg alkaloid kg-1 diet and above. Fish fed the highest levels consumed too little food to supply maintenance protein and energy requirements. The authors noted increasing densities of melanomacrophage centres in the head of the kidney at increasing levels of dietary gramine but attributed the differences to starvation due to a lack of evidence for a

direct toxic effect of alkaloids. No significant lesions were observed in the liver, kidney, spleen, pyloric caeca or intestine.

Chien and Chiu (2003) reported that alkaloid removal from *L. angustifolius* seed meal in diets (670 g/kg diet) for juvenile tilapia (*Oreochromis niloticus* × *O. aureus*) increased feed performance but did not affect growth. The authors offer no explanation for this effect. However, the alkaloid content of seed before (40mg/kg) and after (20mg/kg) extraction were both low. Additionally the authors noted that all of the experimental diets were deficient in methionine, lysine and threonine, but no supplementation was made. Therefore, whether the observed effects are attributable to the alkaloid content is questionable. At this time there is insufficient data to reliably predict safe levels of lupin alkaloids in diets for fish. Reduced feed intake has been observed in fish fed diets containing high levels of alkaloids due to effects on palatability. Based on the results of Glencross *et al*. (2006) the highest demonstrated level of dietary gramine that did not reduce feed intake in rainbow trout was 1000 mg/kg (reduced intake was observed at the next higher inclusion, 500 mg/kg diet). Also, it is not known whether different fish species exhibit varying sensitivities to lupin alkaloids or whether safety levels of dietary lupin of one species or cultivar may differ. At present the only practical way of reducing the toxic effect of alkaloids is either by removing them through aqueous extraction (i.e. solanine extraction from potatoes by hot water) or by the genetic selection and use of low-alkaloid varieties (Farrell, 1992).

2.4.5 Hydrogen Cyanide (HCN)

Hydrogen cyanide is toxic to plants, animals and humans and its toxicity stems from its ability to link with the cofactors iron, manganese or copper of various metalloproteins,

especially cytochrome oxidase of the mitochondrial respiratory chain (EFSA, 2007). Uptake of HCN in fish can be through ingestion, the skin or the gill respiratory epithelium, following which it rapidly distributes throughout the body through binding to metalloproteins (Beasley and Glass, 1998). The enzyme cytochrome oxidase is the main site of rapid lethal cyanide action and the brain is the major target organ of the HCN-induced cytotoxic hypoxia (Eisler, 1991). HCN forms a stable complex with, and inhibits, the enzyme cytochrome oxidase blocking the mitochondrial electron transfer chain. The result is cessation of cellular respiration causing cytotoxic hypoxia and tissue anoxia and a reactive shift from aerobic to anaerobic metabolism. Respiratory arrest due to the depression of the central nervous system by a combination of cytotoxic hypoxia and lactic acidosis is the cause of death (Eisler, 1991; Beasley and Glass, 1998).

Effects on the circulatory system are also postulated as significant contributors to fatalities, especially in severe poisoning, manifesting as reduced cardiac output and cardiac shock in extreme cases, pulmonary oedema and possibly release and detrimental activity of biogenic amines (Beasley and Glass, 1998).

The toxicity of HCN to fish is in relation to cyanide in water, mainly in eco-toxicological experiments studying contamination of natural fish habitats by cyanide in industrial effluents. These exposures result in chronic cyanide effects in the fish that affect fecundity and other aspects of fish reproduction (Eisler, 1991; EFSA, 2007). The only report on exposure of fish to cyanide through the diet is a growth study conducted with Nile tilapia fed soaked and unsoaked sun-dried cassava meal with total cyanide levels of 9.9 and 71.1 mg/kg, respectively (Ng and Wee, 1989). The cyanide did not depress growth in the fish as the two diets showed similar growth at similar levels of inclusion.

2.4.6 Fibres

Fibres, are also referred to as non-starch polysaccharides (NSP), are defined as the polysaccharides not digested by endogenous enzymes in monogastric animals. Dietary fibre is a mixture of substances derived from the structural materials of the plant cell wall and a range of non-structural polysaccharides either present naturally in foods or derived from food additives (McCleary, 2003). Effects of semi-purified and soluble NSPs, such as guar gum and alginates in fish diets, have been investigated and found to result in decreased dry matter of the faeces in both fish (Storebakken, 1985; Amirkolaie *et al*., 2005; Leenhouwers *et al*., 2006) and mammals (Zentek, 1996). Leenhouwers *et al*. (2006) found increased intestinal viscosity in African catfish, leading to decreased digestibility of main nutrients when including guar gum, at 40g/kg and 80g/kg, to the diets. Generally, no clear effect on intestinal viscosity by the presence of soluble NSPs in the diet has been observed (Amirkolaie *et al*., 2005; Leenhouwers *et al*., 2006). Leenhouwers *et al*. (2006), explained that the reduced digestibility of main nutrients was related to a reduced distribution of digestive enzymes in a viscous solution and decreased flow at the mucosal layer. Majority of NSPs in the relevant plant ingredients for use in fish feed are insoluble (Wang *et al*., 1993; Rinaldi *et al*., 2000). Extrusion of NSP-rich diets may result in increased digestibility of main nutrients; although in most cases digestibility is not altered (Thiessen *et al*., 2003; Sklan *et al*., 2004; Barrows *et al*., 2007). Dry matter in faeces and faecal output of Cu was lower for diets with the highest inclusion level of NSP, while digestibility of starch and faecal output of Mn and K were higher in the diets (Barrows *et al*., 2007). Gro-Ingunn *et al*. (2009), reported that high dietary fibres alter flow, impair interactions, affect intestinal receptors, restrict nutrient

diffusion, change microbial diversity and activities, and change absorptive surfaces in fish. Depending on the nutrient profile of the matter reaching the distal compartments, microbial species will be stimulated differently. Fiber may decrease the numbers and diversity of bacterial strains present in the intestines of fish (Tsukahara *et al*., 2002).

2.5 The Effects of Processing Methods on Some Anti-Nutritional Compounds

Anti-nutrients or anti-nutritional compounds may be defined as those substances generated in natural feedstuffs by the normal metabolism of species and by different mechanisms (for example inactivation of some nutrients, diminution of the digestive process or metabolic utilization of feed) which exerts effect contrary to optimum nutrition (Akande *et al.*, 2010).

The utilization of many plant feedstuffs is limited due to the presence of anti-nutritional factors. Among these are phytates, polyphenols, and enzyme inhibitors (Abd El-Hady and Habiba, 2003). Therefore, manipulation of processing conditions may be required to remove or reduce certain unwanted components (Alonso *et al*., 2000). Attempts to increase the utilization of plant feedstuffs have employed a wide range of processing techniques such as soaking, boiling, autoclaving, radiation, cooking, roasting, dehulling, germination, fermentation, supplementation with various chemicals and enzymes and recently extrusion cooking have been described for various plant sources (Van der Poel, 1990; Gujska and Khan, 1991; Bishnoi and Khetarpaul, 1994; Fernandez *et al*., 1997; Alonso *et al*., 1998; Alonso *et al*., 2000a and Eyo, 2003).

Cheeke and Shull (1985), reported that an anti-nutritional factor is not an intrinsic characteristic of a compound but depends upon the digestive process of the ingesting

animal and further reported that, trypsin inhibitors, which are anti-nutritional factors for monogastric animals; do not exert adverse effects in ruminants because they are degraded in the rumen. When feedstuffs are heat treated, the anti-nutritional factors present in them are either reduced or destroyed, thereby making them more digestible (Eyo, 2003).

Tacon and Jackson (1985), reported that soaking, roasting or autoclaving will reduce anti-nutritional content in Mucuna beans prior to inclusion into fish feed. The cooking (boiling) of Mango kernel has being proved to improve the feed utilization suggesting that its anti-nutritional factors were degraded (Joseph and Abolaji, 1997).

Falayi (2003), reported that the toasting of Soybeans at a controlled temperature of 100^{o}C for 45 minutes greatly reduced its toxic content. Soybeans treated by dry extrusion at temperatures ranging from 121-130^{o}C and 30 bar pressure, has been confirmed as the best for fish production by the Nigerian Institute for Fisheries Research (NIFFR), New Bussa. Agboola (2004), reported that the boiling at 100^{o}C at varying period of time and oven roasting at equivalent time periods detoxified and deactivated toxins in Castor oil seeds. Balogun *et al*. (2004), also boiled Delonix regia seeds at 100^{o}C at varying time intervals (0, 20, 40, 60, 80, minutes) to remove or to reduce toxins. (Eyo, 2003), noted that some of these anti-nutritional factors can be destroyed during processing. Anwa *et al*. (2007), subjected *Albezia lebbecks* seeds to different processing methods which include; boiling for 14, 20, 30 and 60 minutes, toasted for 1hr, 2hrs, 3hrs and roasted traditionally. The result showed that the boiling process gave the best results particularly the portion boiled for 1hr had the least concentrations of the anti-nutritional factors. However moist

heat has been reported to improve digestibility of proteins by opening up the protein structure (Abbey and Berezi, 1988).

Smith (1986), reported that proper processing of feeds require precise control of moisture content, temperature and processing time. Adequate moisture during processing facilitates destruction of the anti-nutritional factors. Both over and under toasting of feeds can result in a meal of lower nutritional quality, while under heating produces incomplete inactivation of the anti-nutritional factors and over toasting can reduce amino acid available (Smith, 1986). Therefore, digestibility is enhanced by cooking and thus the metabolizeable energy value is increased (Anwa *et al.*, 2007).

Legume extrusion cooking would allow reduction of anti-nutritional factors and therefore improve the nutritional quality at a cost lower than other heating systems (baking, autoclaving, etc.) due to a more efficient use of energy and better process control with greater production capacities (Reimerdes, 1990; Alonso *et al*., 1998; Morales and Ramos, 1998).When practically feasible, the recommended method for removal of condensed tannins is dehulling to remove the tannin rich seed coat. This also reduced the level of cellulose and lignin, which are the main constituents of seed coats (Mukhopadhyay *et al*., 2007). Tannins may also to some extent be removed by alkali treatment (Griffiths, 1991) or lactic acid fermentation (Mukhopadhyay and Ray, 1999a). Moderate heating of soybeans reduces the soy trypsin inhibitors (TI) and lectin activities to tolerable levels by fish. Thermal denaturation and inactivation of proteinase inhibitors and agglutinating lectins requires moist heat (McNaughton and Reece, 1980). Defatted soy flakes are typically toasted (steam-cooked) at 105 °C for 30 minutes to remove solvent residues

after the oil extraction procedure. This reduces the TI activity down to levels tolerable by fish. It is also paralleled by denaturation and inactivation of the lectins (Maenz *et al*., 1999).

Fermentation of raw grains yields a significant reduction in concentration of both phytic acid (Marklinder *et al*., 1996; Mukhopadhyay and Ray, 1999b; Skrede *et al*., 2002) and α- galactocide oligosaccharide (Skrede *et al*., 2001; Refstie *et al*., 2005). In processing of raw materials and of the complete fish diets may potentially alter nutritional properties of plant materials. Sandberg (1991) and Hurrell (2004) reported that fermentation can induce phytate hydrolysis via the action of microbial phytase enzymes, which hydrolyze phytate to lower inositol phosphates they further assert that such hydrolysis is important because myoinositol phosphates with, 5 phosphate groups (i.e., IP-1 to IP-4) do not have a negative effect on zinc absorption, and those with 3 phosphate groups do not inhibit non-heme iron absorption. Microbial phytases originate either from the micro-flora on the surface of cereals and legumes or from a starter culture inoculate (Sandberg, 1991).

Low-molecular-weight organic acids (e.g., citric, malic, lactic acid) are also produced during fermentation and have the potential to enhance iron and zinc absorption via the formation of soluble ligands while simultaneously generating a low pH that optimizes the activity of endogenous phytase from cereal or legume flours (Teucher *et al.*, 2004). Puztai (1991) reported that fermentation helps to reduce raffinose oligosaccharides due to the alpha-galactosidase found in the bean seeds, and also phytic acid content due to the action of phytase present in the bean seeds. Microorganisms which are responsible for the fermentation also play a role (Pusztai, 1991). Similarly in germination, most of the

changes occurring during the fermentation of foods are of catabolic nature, and they help in the hydrolysis of such components as proteins and carbohydrates. Fermentation of foods can also result in significant reduction in the quantity of certain anti-nutrients. The removal of raffinose oligosaccharides of legume during fermentation, for example, is primarily due to the alpha galactosidase activity present in legume seeds as well as in microorganisms involved in the process. Depending upon the type of legume as well as fermentation, phytic acid is also hydrolysed during fermentation to a variable degree (Deshpande, 2002). Fermentation of cereals reduces phytate content via action of phytases that catalyse conversion of phytate to inorganic orthophosphate and a series of myoinositols, lower phosphoric esters of phosphate. A 3-phytase appears to be characteristic of micro-organisms, while a 6-phatase is found in cereal grains and other plant food (Shahidi, 1997). According to Vin (2009) consumption of fermented food has been shown to improve the intestinal balance of beneficial lactic acid bacteria. Based on this and the phytase producing capability of the bacteria, the consumption of fermented foods may be another effective way to reduce the potential of dietary phytic acid impairing mineral absorption (Vin, 2009).

Gharaghani *et al*. (2008), reported the effect of gamma irradiation on anti-nutritional factors and nutritional value of canola meal for broiler chickens. Glucosinolate content was reduced to 40, 70 and 89% at irradiation dose levels of 10, 20 and 30 (Kilo gray) kGy, respectively. Their observation indicated that the irradiation treatment has a significant effect on the glucosinolate content of canola meal. Gamma irradiation treatment with its radiolytic effects can destroy glucosinolate molecules. Other

investigators reported that anti nutritional factors, such as protease inhibitors (Farag, 1989; El-Morsi et al., 1992; Farag, 1998), a-amylaseinhibitors (Al-Kahtani,1995;Abu-Tarboush,1998), phytohamagglutinins (Farag, 1989; and Farag, 1998), oligosaccharids (Rao and Vakil, 1983; Ghazy, 1990) and tannin (Abu-Tarboush, 1998) were significantly inactivated by gamma irradiation.

2.6 Biological Description of *Clarias gariepinus*

The family Clariidae is the second most important group of farmed fish in the world (Fapohunda, 2012). They are characterized by their ability to grow on a wide range of artificial and natural food, attainment of a large size in a short time, high yield potential, hardiness and tolerance to low dissolved oxygen and other aquatic conditions (Oresegun *et al.,* 2007). In Africa, Clariid catfishes are of great importance as esteemed food fish. Hence, emphasis has been on the increase on how to improve productivity of the catfish. Catfish account for over 50% of the total animal protein consumed in developing countries and less than 50% in developed countries (Omitoyin, 2007).

Feeds and feeding habits of the larvae, fry and fingerlings of the catfish have been studied and shown to influence the growth and survival of the fish (Egborge and Chigbu, 1988; Ovie, 1996; Osuji *et al*., 2003; Olaleye, 2005; Adewumi and Olaleye, 2011). Of the cannibalistic nature of *C. gariepinus,* multiple sorting is essential. As the fish grow, big ones of the same size-group are removed to another tank for rearing.

African catfish exhibits considerable growth variation both under aquaculture and in the wild (Van der Waal, 1998). The causes of such variation are still not clear, although it has

been suggested that inherent differences in feeding behaviour may contribute to this variation (Valente *et al*., 2001; Sundström *et al*., 2003; Martins *et al*., 2005 Aderolu *et al*., 2011).

The African catfish is especially known to exhibit sophisticated forms of social feeding behaviour, where the dominant fish are observed to exclude the subordinates from getting a relatively greater proportion of the food (Hecht and Uys, 1997). This phenomenon is thought to be a major cause for the feeding growth variations among individuals (Rad *et al*., 2004).

Compensatory growth is of interest in aquaculture because an understanding of its dynamics may allow for the design of feeding schedules that could further improve growth rates (Hayward *et al*., 1997). Feed and feeding practices can have significant effects on catfish production cost and hence the profitability of catfish farming. Under normal conditions catfish should typically be fed daily as much feed as they will consume without adversely affecting water quality. However, depending on water temperature, other water quality parameters and the health of the fish, it may be prudent to restrict the daily feed allowance or to feed less frequently. The quantity of feed and the frequency of feeding are decisions that must be made daily by catfish producers based on the weight of fish and capacity of the fish pond. Feed and feeding of catfishes in grow out ponds are perhaps the most documented in literature (Ayinla, 1988; Adewumi, 2005; Oresegun *et al*., 2007; Alegbeleye *et al*., 2008; Olukunle, 2009).

2.6.1 Nutrient requirement of *Clarias gariepinus*

2.6.1.1 Energy requirements

Energy is one of the most important nutrients of the diet and feeding standards for many animals are based on energy needs. According to Jantrarotai *et al*. (1994) if the dietary energy content is too high, catfish may not eat as much as expected, resulting in too low intake of essential nutrients. The absolute energy requirements for catfish are unknown; the estimates that are available have been made by measuring weight gain or protein gain of catfish fed diets with known content of energy (Henken *et al*., 1985; Hossain *et al*., 1998). Energy requirements for catfish, which have generally been expressed as a ratio of digestible energy (DE) to crude protein (DE/P), range from 31.0 to 50.2 KJ/g. Based on current knowledge, a DE/P ratio from 35.6 to 39.8 kJ g is adequate for use in catfish feeds, increasing the DE/P ratios of catfish diets above this range will increase fat deposition and reduce processed yield, and in contrast if the energy value is too low the fish will grow slowly (Nematipour *et al*., 1992a).

2.6.1.2 Protein and amino acid requirements

Protein comprises about 65-70 % of the dry weight of fish muscle (Wilson and Halver, 1986). A continual supply of protein is needed throughout life for maintenance and growth of fish. African catfish (*Clarias gariepinus*) has a relatively high dietary protein requirement. The best growth rates and food conversions are achieved with diets containing 35-42% crude protein and a calculated digestible energy level of 12 kJ g^{-1} (ADCP, 1983; Faturoti *et al.*, 1986 and Eyo, 2003). Catfish, like other animals, need nitrogen and certain amino acids rather than protein as such.Usually, the most economical

source of these elements is a mixture of proteins in feedstuffs. Using protein for energy is expensive, so catfish feeds should be balanced to ensure adequate levels of nonspecific nitrogen, amino acids, and non-protein energy are supplied in the right amounts (Phonekhampheng, 2008).

In order to obtain the best profit, the optimum dietary protein level should be changed as fish and feed prices change (Robinson, 1989). However, in practice, most catfish producers feed a diet with the same amount of protein throughout the growing period. Commercial catfish feeds used for growing food fish typically contain 28 to 32 % protein (Beem *et al.*, 1988). Diets containing lower levels of protein are adequate for maximum growth but may increase body fat. Catfish fry and fingerlings require diets with more protein. Fry diets used in the hatchery should contain 45 to 50 % protein, and fingerlings (less than 40 kg /1,000) could be fed a 35 % protein diet (Reis *et al*., 1989, Eyo and Olatunde, 2001; Eyo 2003).

Amino acids are substances derived from products of protein digestion. They are classified into two groups, the dispensable and indispensable amino acids; these are in accordance with the ability of the fish to synthesize any of the amino acid (NRC, 1993). The indispensable amino acids are those that cannot be manufactured by the fish and therefore must be supplied in the diet, also called the Essential Amino acids (EAA). These EAA include lysine, leucine, arginine, methionine, valine, histidine, isoleucine, phenylalanine, tryptophan and threonine (Nose, 1974). The dispensable amino acids can be manufactured and synthesized by fish and are usually called the non-essential amino

acids. They include alanine, aspartic acid, cystine, glutamic acid, glutamine, glycine, proline, serine and tyrosine (Hardy, 1990).

Lysine is the most important amino acid for the growth of fish and can be obtained from legumes, meat, fish, meat and bone meal. Its requirement in the diet of fish is about 7.3-8.6% (Fagbenro *et al*., 1999).Tryptophan is very important for the growth of all cells and normal development. It is used for cell proliferation. It is needed in about 4.5-5.8% in the diet of catfish (NRC, 1993). Arginine and histidine play an important role in maintaining a normal and healthy bloodstream and has other complex functions. The amount of arginine needed by fish is 1.2% and histidine 0.42%. NRC (1993), gave the following amount of amino acid requirement for catfish. Lysine 1.43% leucine 0.98%, arginine 1.2% methionine 0.64% valine 0.84%, histidine 0.42% tryptophan 0.14%, isoleucine 0.72% phenylalanine 1.40% and threonine 0.65%.

Table 2.3: Recommended dietary nutrient levels in dry matter for (*Clarias gariepinus*)

Component	Fry and Fingerlings	Growers	Broodstock
Digestible protein,%	35-40	30-35	35-40
Digestible energy(kJ/g)	12.5-16.7	10.5-14.6	12.5-16.7
Ca (min-max),%	0.8-1.5	0.5-1.8	0.8-1.5
P (min-max),%	0.6-1.0	0.5-1.0	0.6-1.0
Methionine + Cystine (min),%	1.2	0.9	1.0
Lysine (min),%	2.0	1.6	1.8

Source: ADCP, 1983.

Table 2.4: Dietary protein and energy requirements for *Clarias gariepinus*

Protein (%)	Energy (kJ/g)[a]	P/E (mg/kJ)[b]	References
>40	13 ME	31	Machiels and Henken(1985)
40-42	14-16 DE	26-29	Uys (1989)
40	11-13 GE	31-36	Degani *et al*. (1989)

[a]GE = gross energy; ME= metabolizable energy; DE= digestible energy [b] P/E= protein-energy ratio. Source: ADCP, 1983.

2.6.1.3 Carbohydrate requirements

Carbohydrates are the main form of energy stored in seeds, roots and tubers. Carbohydrates have several functions in animals. Although catfish do not have a specific requirement for carbohydrates in their diet (Raj *et al.*, 2008). Catfish feeds contain considerable amounts of carbohydrates that are supplied from grains or grain by-products that are rich in starch. Catfish feed contains 25 % or more soluble carbohydrates plus 3 to 6 % less soluble carbohydrates that are in general present as crude fiber (Hogendoorn *et al.*, 1983). African catfish cannot digest crude fiber well, so it should be kept at as low a level as possible. Commercial catfish feeds typically contain less than 5 % crude fiber.

2.6.1.4 Lipid requirements

Lipids play several important roles in an animal's metabolism, including supplying essential fatty acids (EFA), and helping to absorb fat-soluble vitamins. Inclusion of lipids in the diet may increase food intake (Murai *et al.*, 1985). Catfish apparently require 0.5 to 0.75 % omega-3 fatty acids in the diet (Robinson and Li, 1996). Lipid levels in commercial feeds for table-sized catfish rarely exceed 5 to 6 %. About 3 to 4 % of the lipids are naturally found in the feed ingredients, with the remaining 1 to 2 % being sprayed onto the finished pellets (Lovell, 1991). Spraying feed pellets with lipid increases dietary energy and helps reduce feed dust. Essential fatty acids can be supplied by marine fish oil such as menhaden oil.

Ng *et al*. (2003) examined the replacement of fish oil with palm and sunflower oil and found that complete replacement of fish oil actually improved the growth of the African catfish (*Clarius gariepinus* Burchell, 1822). This observation provides support for a very

low Essential Fatty Acid requirement, if any at all. In addition, the fact that no differences were observed between the palm oil and the sunflower oil suggests that it has a limited effect as an EFA in this species. A second study by Olurin *et al*. (2004) examined the gradual replacement of fish oil with palm oil and accordingly diluted out much of the EFA content of the diet so that a response to any EFA deficiency might be apparent. However, after a 49-day growth trial no effects on growth were observed among any of the treatments. Based on re-examination of the data approximately 40% of the dietary lipid should have come from the added oil sources, and approximately 40% from the fish meal content of the diet with other ingredients contributing the remaining 20%. It is estimated from this that 100% replacement of fish oil by palm oil would have had approximately 10 g/ kg of the diet as n-3 PUFA.

2.6.1.5 Vitamin requirements

Vitamins vary greatly in structure and function. They are generally defined as organic compounds that animals require in small amounts in their diets for normal growth, health, and reproduction (Robinson *et al*., 2003). Some vitamins may be made in the body and thus are not required in the diet. The amounts and quality of vitamins that catfish need have been fairly well determined in laboratory and pond studies (NRC, 1993). The following amounts of vitamin requirement for catfish have been reported by the NRC (1993); Vitamin A (1,000-2,000IU/kg), Vitamin D (500IU/kg), Vitamin B_6 (3mg/kg), Riboflavin (9mg/kg), Pantothenic acid (10-15mg/kg), Niacin (14mg/kg), Choline (400mg/kg), Falacin (1mg/kg) Thiamin (1mg/kg) and Vitamin C (25-50mg/kg). These vitamins are sufficiently found in fish feed ingredients and losses during storage are not a major factor if the feed is stored for a short time (Robinson, 1989).

2.6.1.6 Mineral requirements

Although mineral studies with fish are scarce, mineral needs of catfish have been suggested, and the signs of mineral deficiency have been described (Robinson and Li, 1996). Phosphorus is particularly important in fish feeds because fish require a large amount of it. Feedstuffs, especially those from plants, are poor sources of phosphorus, and fish do not get enough phosphorus from pond water, as a result, African catfish feeds are usually supplemented with phosphorus (Yan *et al*., 2007). Dicalcium and defluorinated phosphates are commonly used as phosphorus supplements in catfish feeds their feeds are typically supplemented with a trace mineral premix with enough of all essential trace minerals to meet or exceed dietary requirements however; trace mineral premix may not be needed in catfish feeds that contain 4 % or more animal protein (Robinson, 1989, Yan *et al*., 2007).

2.6.2 Food and feeding habit of *Clarias gariepinus*

Clarias gariepinus are generally classified as omnivore (Reed *et al*., 1976; Teugels, 1982). The fish was reported to feed mainly on detritus, planktons, aquatic insects, small fish, terrestrial insects, mollusks and crustaceans (Bakare, 1968 and Idodo-Umeh, 2003). In order to feed on this wide variety of food organisms in different situations *C gariepinus* is equipped with a wide array of anatomical adaptation for feeding under low visibility. These adaptation include: a wide mouth capable of considerable vertical displacement for engulfing large prey or large volumes of water during filter feeding, a broad band of curved teeth on the jaws and pharyngeal teeth preventing prey from escaping; an abundant network of sensory organs on the body, head, lips and circumoral barbels, which are extensively used for prey detection and fixation; a wide, rounded,

caudal fin, typical of fish which ambush their prey and a short and dilatable oesophagus which opens into a distinct muscular stomach (for mechanical digestion) and a simple thin walled intestine (Gertan de graaf, 1994).Olatunde (2005) reported that food selection of the fish, changes with size and age of the fish thus, *C gariepinus* is said to be a slow-moving omnivorous predatory fish which feeds on a variety of food items from microscopic zooplankton to fish half its length or 10% of its own body weight (Gertan de graaf, 1994).

2.6.3 Digestibility in *Clarias gariepinus*

Digestion is the process by which food in the digestive tract is split into simpler compounds capable of passing through the intestinal wall to be absorbed into the blood stream, this is performed primarily by the digestive enzymes which are secreted into the lumen of the alimentary canal, the digestive enzymes originate from the oesophageal, gastric, pyloric caecal and intestinal mucosa and from the pancreas (De Silva and Anderson, 1995). According to Glencross *et al.* (2007) diet design, feeding strategy, faecal collection method and method of calculation all have important implications for determination of the digestible value of nutrients from any ingredient.

2.6.3.1 Digestive capacity of Clarias gariepinus

Digestibility of the feed is a function of feed composition and is directly related to the digestive capacity (Jantrarotai *et al*., 1994). A digestibility value is a measure of the extent to which ingested food and its nutrient components have been digested and metabolized by the fish. The ability of fish and vertebrates to use nutrients depends on many factors, such as the synthesis of appropriate enzymes, production of digestive

enzymes in suitable amounts and their distribution along the gut lumen (Kuz'mina, 1990). In carnivorous fish or those with a meat-orientated omnivorous diet there is normally a definite stomach (foregut) whilst herbivorous or plant-orientated omnivores normally have no stomach but rely on a much extended mid gut area for the digestion of the food (Chou *et al*., 2001).

The African catfish (*Clarias gariepinus*), are classified as omnivores, and can be expected to utilize dietary carbohydrates more efficiently than other fish (Chow and Halver, 1980; Nematipour *et al*., 1992a, Jantrarotai *et al*., 1994). Previous studies on omnivorous fish such as common carp, tilapia and African catfish, have shown that they are able to digest carbohydrates efficiently. Studies on (*Clarias gariepinus*) have shown that they can perform equally well on isonitrogenous and isocaloric diets (40% protein, 20 kJ GE g^{-1}) with a dietary carbohydrate content ranging from 27 to 38% and with a carbohydrate to lipid ratio of 1.7 to 3.4 (Ali and Jauncey, 2004).

The total tract apparent digestibility values for protein in protein-rich feedstuffs in channel catfish are usually in the range of 75-95 % (NRC, 1993). Protein digestibility tends to be depressed as the concentration of dietary carbohydrates increases (NRC, 1993). Increased amounts of dietary lipids have been reported to produce increased protein digestibility (Takeuchi *et al.*, 1978). According to Fagbenro (1996), the apparent digestibility of protein in feedstuffs of animal origin or of plant origin in catfish (*Clarias isheriensis)* is similar. It has been reported that the protein digestibility of cottonseed meal in catfish is about 84%, but the lysine availability is only about 66% (Fagbenro *et*

al., 1998). A similar situation may exist for other feedstuffs. Therefore, if feeds are formulated on a plant protein basis, a lysine deficiency may result (Fagbenro *et al*.,1998).

The total tract apparent digestibility values for lipids in channel catfish are high and vary between feedstuffs (NRC, 1993). The lipid digestibility coefficients of carp range between 53 and 90% (Kirchgessner *et al.*, 1986), and between 64 and 94 % for channel catfish (Andrews *et al.*, 1978). Polyunsaturated fatty acids such as 20:5 or 22:6 acids are up to 100 % digested by rainbow trout (Austreng *et al.*, 1980). Lipids are a highly digestible source of concentrated energy for catfish feed and contain twice as much energy as does an equivalent amount of carbohydrates (Robinson *et al*., 2001).

Starch is an important dietary non-protein energy source for catfish, and this has to be included at suitable levels to maximize the use of dietary protein for growth (Ali and Jauncey, 2004). The ability to digest starch depends on the secretion of α-amylase into the gastrointestinal tract. It is believed that all species of fish have the ability to secrete α-amylase (Chow and Halver, 1980).Warm-water fish are able to utilize much higher levels of dietary carbohydrates than cold water or marine fish. This difference may be related to the relative amylase activity present in the digestive system of the various fish. Source, dietary level, and heat treatment affect the digestibility of carbohydrates in fish (Wilson, 1994). Channel catfish were found to digest over 70 % of the energy of raw corn starch. Cooked starch was 12.1 % more digestible than raw starch when fed at 30 % dietary level to channel catfish (Wilson, 1994).

Fibre has no functional value in fish feeds except possibly to control movement of ingested feeds through the digestive tract (Krogdhal *et al*., 2007). Lovell and Leary (1975), reported that fibre above 2.8% in catfish diet will lead to increase in feacal waste and biological oxygen demand i.e. increasing crude fibre level to 14 % will lead to decrease in growth. Hence in practical diets crude fibre should not exceed 3-6% meal (Wilson and Poe, 1985).

2.6.3.2 Factors influencing digestibility in fish

Digestion of food in fish depends on three main factors, the ingested food and the extent to which it is susceptible to the effects of the digestive enzymes the activity of the digestive enzymes and the length of time the food is exposed to the action of the digestive enzymes (Hepher, 1988). Each of these main factors is affected by a multitude of secondary factors, some of which are associated with the fish itself, such as fish species, age, size and physiological condition; some are associated with the environmental conditions, such as water temperature, and some are related to the composition of food, such as particle size and amount eaten (Hepher, 1988).

Nutrient digestibility may vary among fish species, due to differences in the digestive system and enzymes and foods consumed. Despite these differences and the lack of pepsin in fish without a stomach, variations in the digestibility of proteins and lipids among species are small. Much more pronounced are variations in digestibility of carbohydrates, especially starch. Carnivorous fish digest starch to a much lesser degree than omnivorous and herbivorous fish (Hepher, 1988). Differences in digestibility of carbohydrates can also occur among different families of carnivorous fish within the

same species (Refstie and Austreng, 1981). With respect to amylase activity, the omnivorous species show higher activity than the carnivorous species. The ratio of total amylase: Total proteolytic activities were reported to be higher in omnivorous fish species (Hidalgo *et al.*, 1999). A comparative study on digestive capabilities of the three fish species, carp, tilapia and African catfish, by Degani and Revach (1991) shows that, protein from poultry sources was digested better by tilapia than carp or catfish, while carp showed the best ability to digest fats, followed by catfish, and with tilapia having the least ability. However, tilapia digested carbohydrate better than the other two species and there was no difference in digestibility of energy among the three fish species.

Digestibility can increase with size in omnivorous and herbivorous fish due to a relative increase in intestinal length, thereby prolonging digestion and assimilation time (Ferraris *et al.*, 1986). Enzymatic activity may vary with fish age and size, and proteolytic and amylolytic activities are usually lower during the first development stage of fish than in the later stages and this may affect the digestibility of nutrients (Hepher, 1988; Kolkovski, 2001).

Stressed fish due to either excessive handling or disease, may have a disturbed digestibility. A long period of starvation may also affect enzyme secretion and digestibility. Parallel to the seasonal variations in digestive enzyme activities, seasonal variations in digestibility may also occur (Ferraris *et al.*, 1986).

Fish metabolic activities, including the activity of digestive enzymes, are reduced at low temperature and this may reduce the rate of nutrient utilization. Hence, increasing water

temperature may increase both enzyme secretions and enzyme activity (Nordlie, 1966 and Smith, 1967). Temperature may also affect the rate of absorption of digested nutrients through the intestinal wall (Smith, 1970). Generally, the higher the temperature, the more rapid is the transport of food and the shorter its exposure time to the digestive enzymes (Elliott, 1975). In trout and carp, starch and crude protein digestibility decreased with a decrease of water temperature (Yamamoto *et al.*, 2007).

The proportion of nutrients in the diet may affect the digestibility. Increased amounts of dietary lipids are known to support increased protein, carbohydrate, lipid and energy digestibility (Takeuchi *et al.*, 1978). High carbohydrate content in the diet has been reported to reduce protein digestibility (Kaushik *et al.*, 1989). The explanation for this is that the undigested portion of the carbohydrates passes more rapidly through the alimentary canal, carrying with it some of the proteins (Hepher, 1988). Digestibility of protein was found to be negatively correlated with fibre content (Ferraris *et al.*, 1986). Some feeds may contain digestive enzyme inhibitors which reduce digestibility (NRC, 1993).

The quantity and quality of feed consumed have a pronounced effect on growth rate, efficiency of feed utilization and body chemical composition (Juell and Lekang, 2001; and Bureau *et al.*, 2006). In addition, feeding levels have been reported to influence body composition and morphometry of rainbow trout, and fish on a lower ration level contained less fat and were smaller in size (Kiessling *et al.*, 1991). Lei (2006), found that digestibility of dry matter and protein was significantly affected by feeding level but not feeding frequency when the feeding level was similar. Similar results were obtained by

Yuan *et al.* (2010), who showed that apparent digestibility coefficients of dry matter, protein and energy were significantly affected by feeding level in juvenile Chinese sucker, *Myxocyprinus asiaticus*. It has been reported that the gastric evacuation rate increases with increased feeding rate (Pandian and Vivekanandan, 1985). This may result in increased rate of passage of dietary material through the digestive tract, resulting in less material digested, and lower apparent protein digestibility values. This has been noted in *Clarias batrachus* and *Clarias gariepinus*, and it has therefore been suggested that these fish should be fed two times per day when they are past the fingerling stage, in order to utilize dietary protein more efficiently (Pandian and Vivekanandan, 1985).

2.6.4 Carcass composition of *Clarias gariepinus*

Proper nutrition is one of the essential factors that influence the ability of the fish to attain genetic potential, growth, reproduction and longevity (Lall, 1991). Knowledge of the carcass composition of the fish is a good measure for assessing the growth and quality of protein and its utilization, whether the diet promoted growth or simply accumulated only fat.

Studies have been carried out on the carcass composition of some indigenous fishes. Abdullahi *et al*. (2001) reported that *C. gariepinus* has the following nutrients, moisture 75.00%, ash 10.50%, carbohydrate 10.20%, crude lipid 27.10 and crude protein 52.20%. The amino acid composition (g/100g) was also reported as lysine 10.13, histidine 2.84, arginine 4.40, aspartic acid 8.30, threonine 2.89, serine 2.07, glutamic acid 14.85, proline 2.54, glycine 8.86, alanine 4.90, cystine 2.62, valine 3.01, methionine 1.97, isoleucine 5.16, leucine 5.64, and trypsine 1.10.

2.7. Haematological Effect of Using Plant Nutrients for Catfish

Fish cultures are especially at risk to the adverse effects of stress. Blood chemistry and hematological measurements can provide valuable physiological indices that may offer critical feedback on different stressors (Binukumari *et al*., 2011). Haematological characteristics of most fish have been studied with the aim of establishing normal value range and any deviation from it may indicate a disturbance in the physiological process (Rainzapaiva *et al*., 2000). Haematological studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. The red blood cells count, haematocrit and haemoglobin concentration vary with diet and strain as well as temperature, season of the year and nutritional status of the fish (Wagner *et al.,* 1997). Haematological studies have been widely used as means of assessing the state of health of fishes and the establishment of the haemotological characteristics of fishes generally serves as a standard for physiology, pathological or toxicological studies (Wedemeyer *et al*., 1977).

Assessment of nutrient utilization and biological values of feeds and feedstuffs may seem inconclusive without adequate consideration of their implications on the physiological and health status of the animal (Anyanwu *et al*., 2011). Haematological parameters of different species of fish and screening test provide a bank of useful information from which valuable and informative conclusions could be drawn (Nlewadim and Alum, 1999).

Anyanwu *et al*. (2011) reported a range of 5.48-6.25g/100ml, 2.35-3.0x106/mm3, 2.33-24.75% and 4.48-5.03X104/mm3 for the heamoglobin, red blood cells, packed cell

volume and white blood cells values for *Clarias gariepinus* respectively, fed varied dietary inclusion levels of *Carica papaya* leaf meal.

Bhasker and Rao (1990) reported Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) values of 132.8-308.4fl, 20.90- 47.20pg and 10.90–38.10% respectively in their studies in relation to stocking and feeding conditions. Ochang *et al.* (2007) observed similar range values, although with higher MCHC values. The haematological and biochemical indices of farm fish as with other farm animals namely - haemoglobin, red blood cells, white blood cells, packed cell volume, plasma protein, blood glucose, specific gravity of blood plasma and whole blood, coagulation time etc. have been analysed and variously reported as useful tools in assessing the performance, viability and health status of fish and animals (Bhaskar and Rao, 1990; Harikrishnan *et al.*, 2003; Anyanwu *et al.*, 2003; Hemre *et al.*, 2007).

Osuigwe *et al*. (2007) reported that the Pack Cell Volume (PCV), Red Blood Cell (RBC) count, White Blood Cell (WBC) count and Haemoglobin (Hb) concentration decreased significantly with increasing dietary Jack Bean Seed Meal (JBSM) such that fish fed the control diet had the highest values that were significantly different from the values obtained from *Clarias gariepinus* fed other diets, and that Jackbean seed meal has been shown to contain anti-nutritional factors known to cause some negative effects on some haematological parameters, and concluded that the increasing presence of anti-nutritional factors in increasing dietary JBSM caused the inferior haematological parameters observed in *C. gariepinus* fed such diets. It is of importance to note that irrespective of

the reduction in the levels of haematological values observed in *C. gariepinus*, they were still within the normal ranges reported for the fish species (Erondu *et al*., 1993; Musa and Omoregie, 1999).

Osuigwe *et al*. (2007) also reported that the erythrocytes count range of 2.17-2.6, 106/mm^3 in *Clarias gariepinus* were within the range described by Gabriel *et al.* (2004) (2.3-2.9 x106 /mm^3) and Adeyemo *et al*. (2003) (1.5x106 /mm^3) and a similar observation was noted with another species such as tilapia as reported by Terry *et al*. (2000), Nilza *et al.* (2003), Adam (2004). They also reported that the mean values of packed cell volume (PCV) in *Clarias garipeinus* obtained from their study were within the range of the corresponding values described by Munkittrik and Leatherland (1983); Gabriel *et al.* (2001); Gabriel *et al.* (2004). Several of these studies attempted to determine whether significant variations from normal values of these parameters do exist and could be attributable to some internal or external factors.

A range of mean corpuscular volume (MCV,73.1-108.7ft) and mean corpuscular haemoglobin (MCH, 24.4-36.2 pg) obtained by Osuigwe *et al*. (2007) were similar to findings of Terry *et al.* (2000), Nilza *et al.* (2003) and Gabriel *et al.* (2004). Also, higher levels of MCV (145.8 ft) than those findings were obtained by Adeyemo *et al.* (2003). The haemoglobin concentration (6.29 g/dl) in studied fish was similar to those reported by Adeyemo *et al.* (2003) and Gabriel *et al*. (2004), but was lower than those of Red Tilapia (7.3 g/dl), Nile Tilapia (10.4 g/dl), Curimbata (9.7 g/dl) and hybrid Tilapia (12.7 g/dl) as reported by Terry *et al*. (2000), Adam (2004). Teixeira (2000), stated that the reference values determined for haematological elements and biochemical tests may not

represent precisely those of a certain population or animal species and should, therefore, be carefully interpreted once there was a wide range of physiological variations. According to these authors, these variations might be influenced by environmental conditions, gender, and age, origin, breeding system and feeding.

Adedeji *et al.* (2011) in a study of comparative haematological parameters of the bagrid catfish (*Chrysichthys nigrodigitatus)* and the African catfish (*Clarias gariepinus*) from Asejire dam in South-western Nigeria reported that, there was no significant difference ($p>0.05$) between the Packed Cell Volume of the two species of fish. The mean haemoglobin concentration of *Clarias gariepinus* was 9.28±1.88g/dl while C*hrysichthys nigrodigitatus* has 8.325±2.07g/dl. They assert that *Clarias gariepinus* is more active than C*hrysichthys nigrodigitatus* and have higher value of heamoglobin concentration. Eisler (1965), suggested that there was a correlation between heamoglobin concentration and activity of fish. The more active fishes tend to have higher heamoglobin values than the sedentary ones. Consequently, *Pleuronectes annectens* being a relatively quiet and sedentary species has a slightly lower heamoglobin concentration than more active as *Clarias buthupogon* whose haemoglobin is as high as 9.88g/dl Kori-Siakpero (1988). Adedeji *et al.* (2000), reported that *Clarias gariepinus* has higher number of circulating erythrocyte (RBC) than *Chrysichthys nigrodigitatus* therefore, transportation of oxygen and carbondioxide to the lung tissue will be more effective in *Clarias gariepinus* than *Chrysichthys nigrodigitatus*. According to Douglas and Jane (2010), white blood cell (WBC) and lymphocytes are the defensive cells of the body; their amount has implication in immune responses and the ability of the animal to fight infection. The species with

higher value of WBC and lymphocytes will be able to fight infection more than the other species with lesser value of leucocyte. Adedeji *et al.* (2000) while working on different fresh water species found out that there was correlation between the MCV and number of circulating RBC (The higher the number the lower the size and *vice versa*).

According to Bhaskar and Rao (1990), plasma constituents (particularly the sodium and potassium ion concentrations) rather than erythrocyte and leucocyte parameters are better indicators of the condition of fish in aquaculture. Fagbenro *et al*. (2013) reported that changes in haematological parameters due to unfavourable exogenous factors like adverse water quality, overstocking, starvation, etc., are indices of the health of cultivated fish.

Studies on the haematology of European catfish (*Silurus glains*) under natural condition recorded haematocrit in the range of 27.0-31.0%, haemoglobin concentration 8.22-9.38 (g/dL), RBC count (0.85-1.3 mil/mm3), WBC count 10.0-24.0 (1000/mm3), platelets in the range of 0-0.4 (1000/mm3), MCV range 231.5-289.2 (fl), MCH range 70.5-107.2 (pg), MCHC range 30.0-33.0 (g/dL), and total protein concentrations range 3.40-5.93 (g/dL) (Fikri *et al*., 1997). For catfish (*Clarias lazera*) the same authors recorded haematocrit 24.0-43.0%, haemoglobin 7.38-12.43 (g/dL), RBC count 1.03-1.61 (mil/mm3), WBC count 21.0-54.0 (1000/mm3) and platelets 0.0-5.0 (1000/mm3), and MCV, MCH, MCHC and total protein were reported as 188.3-303.7 (fl), 74.50-92.40 (pg), 28.0-37.0 (g/dL) and 3.30-3.79 (g/dL), respectively. Stoskopf (1993) reported that total protein concentration, RBC count, WBC count, haematocrit and haemoglobin were 2.2-4.5(g/dL), 1.6-2.4 (mil/mm3), 28.1×103/mm3), 22-39.3% and 4-8.4 (g/dL),

respectively for channel catfish (*Ictaluru punctatus*). Kori-Siakpere (1985) reported that haematological parameters for *Clarias isheriensis* were haemoglobin 14.56 (g/dL), haematocrit 31.62%, RBC count 1.55 (mil/mm3) and total protein 3.27 (g/dL).

Sex of the fish may also influence the blood parameters. Sneiszko (1960), showed that males consistently had higher packed cell volume values than the females and this has been proposed as means of sexing fish. Blood cell responses are important indicators of changes in the internal and/or external environment of animals (Adeyemo, 2007). A number of haematological indices such as Haematocrit (Ht) Haemoglobin (Hb), Red Blood Cells (RBCs) are used to assess the functional status of the oxygen carrying capacity of bloodstream and have been used as indicators of the presence of metallic pollutants in aquatic environment (Shah and Altindag, 2004). Red Blood Count (RBC), concentration of haemoglobin (MCH) and Haematocrit (Ht) have been reported to indicate secondary responses of an organism to irritants (Adeyemo *et al*., 2008) Decrease in erythrocytes has been reported to be the major and reliable indicators of various sources of stress in fish (Rainza-Paiva *et al*., 2000; O' Neal and Weirich, 2001). White blood cells are the main defense cells of the animals. Decrease in these cells indicates vulnerability to stress and infection (Sunomonu and Oyelola, 2008). Decrease in red blood cells implies reduction in level of oxygen that is being carried to the tissues and the level of carbon dioxide returned to the lungs will also be reduced. Reduction in mean haemoglobin concentration and mean corpuscular haemoglobin concentration indicates anaemia (Sunomonu and Oyelola, 2008). According to MyDr (2006), packed cell volume or haematocrit, Red Blood Cell (RBC) and Mean Cell Haemoglobin Concentration

(MCHC) are used for diagnosing anaemic condition while neutrophils and monocytes (differential counts) protects the body against bacterial invasion. Lymphocytes are involved in immune process, producing antibodies against foreign organism, protecting against microbes. Low RBC indicates malnutrition and platelets helps in diagnosing problems associated with bleeding or bruising.

2.8. Water Quality and Fish Production

The aquatic environment governs fish life, hence water quality should be suitable for fish culture. When environmental condition does not conform to optimal range for normal fish growth, then fish culture could be affected (Boyd, 1978).Water quality is a dynamic web of the physical, biological and chemical factors, which constitute the water environment and influences the production of fish and other aquatic organisms. There are many water quality variables in pond fish culture. All other things being equal, a pond with good water quality will produce more and healthier fish than a pond with poor water quality (Piper *et al*., 1982). Water quality parameters, which are of prime importance, are mainly temperature, turbidity, dissolved oxygen, Carbon (IV) oxide (CO_2), nitrogen, ammonia, pH, alkalinity and hardness.

The water temperature is considered to be one of the most important factors in aquatic environment because it affects all metabolic, physiological activities and life processes of different trophic levels of pond ecosystem. In addition, it also affects the speed of chemical changes in soil and water (Dhirendra, 2002). Water temperature plays an important role in influencing the periodicity, occurrence and abundance of phytoplankton as it had a direct relationship with total plankton (Tripathi and Pandey, 1990). Fishes are

cold-blooded animals and dependent upon the water temperature in which they live. Every fish species has an ideal temperature range within which it grows quickly. The optimum temperature range for 'cold water' and 'warm water' fishes are 14-18°C and 24-30°C respectively (Degani *et al*., 1989).

Ammonia is the first measurement to determine the health of biological converter (Binukumari *et al*., 2011). Fish are very sensitive to unionised ammonia and the optimum range is 0.02-0.05 mg/litre in the pond water. When ammonia accumulates to toxic levels, fish cannot extract energy from feed efficiently. If the ammonia concentration gets high enough, the fish will become lethargic and eventually fall into a coma and die. However, ammonia can have so-called "sub-lethal" effects such as reduced growth; poor feed conversion, and reduced disease resistance at concentrations that are lower than lethal concentrations. The source of ammonia in fish ponds are fish excretion and protein in feed constitute ultimate source of most ammonia in ponds where fish are fed (Boyd, 1990).

There are two main processes that result in the loss or transformation of ammonia. The most important is the uptake of ammonia by algae and other plants. Plants use the nitrogen as a nutrient for growth. The other important process of ammonia transformation in fish ponds is â 'nitrification'. Bacteria oxidize ammonia in a two-step process, first to nitrite (NO_2) and then to nitrate (NO_3). Aeration can also reduce ammonia toxicity (Cole and Boyd, 1986).

2.8.1 Effects of dissolved oxygen (DO) level on growth and food conversion ratios of fish

Successful fish production depends on good oxygen management. Oxygen is essential to the survival (respiration) of fish, to sustain healthy fish and bacteria which decompose the waste produced by the fish, and to meet the biological oxygen demand (BOD) within culture system. Dissolved oxygen levels can affect fish respiration, as well as ammonia and nitrite toxicity (Yovita, 2007). When the oxygen level is maintained near saturation or even at slightly super saturation at all times it will increase growth rates, reduce the food conversion ratio and increase overall fish production (Tom, 1998).

2.8.2 Effect of dissolved oxygen (DO) on fish growth

Dissolved oxygen is important in respiration and metabolism processes in any animal (Tom, 1998). In fish, the metabolic rate is highly affected by the concentration of oxygen in the rearing environment. As the dissolved oxygen concentration decreases, respiration and feeding activities also decrease. As a result, the growth rate is reduced and the possibility of a disease attack is increased, and fish is not able to assimilate the food consumed when DO is low (Tom, 1998). Overall health and physiological conditions are best if the dissolved oxygen is kept closer to saturation (Yovita, 2007). When the levels are low the growth of the fish can be highly affected by an increase in stress, tissue hypoxia, and a decrease in swimming activities and reduction in immunity to diseases (Wedemeyer, 1996). The oxygen level requirement depends on the species, but also on fish size and activity of the fish. According to Tom (1998), oxygen requirements per unit weight of fish significantly declines with increasing individual weight. In carp this reduction may be expressed by the following ratios: yearling = 1mg/l, two-year-old carp = 0.5–0.7mg/l, marketable carp = 0.3–0.4mg/l. Significant differences in oxygen demand

are also found for different species. Several studies have investigated the relationship between oxygen saturation and fish food intake. Randolph and Clemens (1976), found that feeding patterns of channel catfish varied with temperature and oxygen availability. When the oxygen content drops below 59% fish starts to lose its appetite. Rainbow trout (*Oncorhynchus mykiss*) reduced its appetite when oxygen saturation fell below approximately 60% (Jobling, 1995). Similar results have been obtained from European sea bass (*Dicentrarchus labrax, L*) (Thetmeyer *et al*., 1999) blue tilapia (*Oreochromis aureus*) (Papoutsoglou and Tziha 1996) channel catfish (*Ictalurus punctatus*) (Buentello *et al*., 2000), juvenile turbot (Pichavant *et al.*, 2001) and common carp (*Cyprinus carpio*), showed reduced growth when exposed to low oxygen levels.

2.8.3 Effect of dissolved oxygen (DO) on the food conversion ratio in fish

Tolerance for low oxygen may relate in part to the metabolic rate of a fish (Verheyen and Decleir, 1994). Fishes will regulate their metabolic rate over a range of dissolved oxygen concentrations; however, at some point, a further reduction in oxygen tension will produce a shift from a metabolic rate that is independent of oxygen concentration to one that is dependent on oxygen level. The point is referred to as the critical oxygen tension (Ultsch *et al*., 1978). Decreased oxygen availability is also considered a major factor in determining food intake. Low dissolved oxygen is a type of stress frequently found in fish farms characterised by high fish densities and polluted fresh or marine waters. The food conversion ratio (FCR) is the amount of fish food consumed to generate a given weight gain. It is the ratio between the weights gained in a given period to the total feed intake by the fish in the same period. It is the inverse of the feed intake. The food

conversion ratio is improved (lowered) at higher growth rates (Markore and Rorvik, 2001, Crampton *et al*., 2003, Norgarden *et al*., 2003). The average FCR from several recent studies of fish growth is 0.97. In most cases, the FCR appears to be slightly lower for tank studies (mean: 0.93) than for net-pen studies (mean: 1.02) (Neuman *et al*., 2004). An experiment done on tilapia (*Oreochromis niloticus*) showed that FCR was inversely proportional to the dissolved oxygen level (1.45 at higher dissolved oxygen level and 6.75 at lower dissolved oxygen level) (Tsadik and Kutty, 1987).

It is well known that tilapia can tolerate hypoxic and even anoxic conditions for short periods and are thus better suited than other species to hypereutectic conditions that may exist in static water aquaculture systems (Chorn *et al.,* 2006). It is also known that fish found both in the tropics and temperate waters have incipient limiting oxygen levels which may occur as in hypoxia. In reference to *Oreochromis niloticus,* studies have shown that the incipient oxygen requirements are between 1.39mg/l-2.92mg/l. Tsadik and Kutty (1987), revealed that experiments under the various oxygen regimes varied and that the fastest rate of growth was at high DO and the slowest growth in the low DO.

Table 2.5: Water quality requirement of catfish

Chemical and physical features	Desired levels
Salinity	100-8000mg/lit
Total dissolved gases	105%
Dissolved oxygen	Minimum 6mg/lit
Carbondioxide	10mg/lit
Calcium hardness	Minimum 20mg/lit as CaCO3

Ammonia	Minimum 0.05mg/lit
Iron	Max 0.5mg/lit
Hydrogen sulphide	0.0

Source: Boyd, 1990

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Processing of *Senna obtusifolia* Seeds

The seeds of *Senna obtusifolia* were handpicked at National Animal Production Research Institute (NAPRI) Shika Zaria, Kaduna State. Other feed ingredients were purchased from the market in Zaria. Three portions of *Senna obtusifolia* seeds of 100g each were taken and kept separately prior to treatments. The seeds were soaked in water in a 1:6 (seeds: water) i.e.1kg/6lit ratio for 24hrs, the drained soaked seeds were allowed to ferment naturally (Udensi *et al.,* 2006) without the addition of yeast. The fermentation periods were 48, 72 and 96 hrs. Corresponding to F_{48}, F_{72}, F_{96}, respectively. $F_{48\text{-}96}$ refers to the fermentation periods in hours.

The fermented seeds were air dried for two days, then ground into fine powder with hammer mill, packaged and labelled in a polythene bags in preparation for proximate analysis, amino acid profile, mineral elements and determination of anti-nutritive compounds. Similarly, three portions each containing 100g of the seed samples were taken for toasting as described by (Mugendi *et al*., 2010) at 100^{o}C for 5, 10 and 15, minutes corresponding to T_1, T_2, T_3 respectively. $T_{1\text{-}3}$ refers to the toasting time in minutes. The toasted seeds was grounded into fine powder, packaged and labelled in a polythene bags in preparation for proximate analysis, amino acid profile, mineral elements and determination of anti-nutritive compounds. Furthermore, three portions each of 100g of the seed samples was taken for cooking as described by (Kakati *et al*., 2010) at 100^{o}C for 30, 45, 60, minutes corresponding to $B_{1,}$ B_2, B_3 respectively. $B_{1\text{-}3}$

refers to the boiling periods of the seeds in minutes. The boiled seeds was sundried and grounded into fine powder, packaged and labelled in a polythene bags in preparation for proximate analysis, amino acid profile, mineral elements, and determination of anti-nutritive compounds.

3.2 Biochemical Analysis of Raw and Processed Seeds

The proximate composition (moisture, crude protein, crude lipid, and crude fibre, ash and nitrogen free extract) of the raw, fermented, toasted and boiled seeds, experimental diets, and experimental fish before and after the experiment were determined as described by the Association of Analytical Chemistry (A.O.A.C., 2000). All chemical analysis was carried out in triplicate.

3.2.1 Moisture content and dry matter

The fresh seeds were oven dried at 100^{o}C in a crucible dish for 24hrs to a constant weight for the calculation of percentage of dry matter (%DM) content.

Empty clean beakers, one for each sample, was dried in hot oven at 100^{o}C for 30 minutes, when cooled each beaker was then weighed (W1). Five grams of the sample was placed in the beaker and then reweighed (W2). The beaker with the sample was then dried in an oven at 100^{o}C for 24hrs, during which time a constant weight was attained and then transferred to a desiccator to cool. The beaker was quickly weighed with minimum exposure to atmosphere (W3). The loss in weight of the sample during the drying represents the moisture content, while the dry matter was the weight obtained after drying the sample. This is shown as:

% Moisture = W2-W3/W2-W1 x 100

% Dry matter = W3-W1/W2-W1 x 100

Where W1= Weight of empty crucible

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

W3 = Weight of oven dried sample

3.2.2 Crude protein determination

Protein determination was carried out in three stages, which comprised of digestion, distillation and titration.

1. Digestion: One gram of sample was weighed into a 50ml digestion flask and the kjeldahl mixture which acts as a digestion catalyst was added with 5ml concentrated tetraoxosulphate V1 acid (H_2SO_4). Some pumice stones (anti-bumping granules) were added. The flask containing the sample mixture was heated gently at an inclined angle in a kjeldahl digestion rack until frothing subsided. It was then boiled until the solution became colourless. Heating of the mixture released the nitrogen in the various samples which was then converted to ammonia with the concentrated H_2SO_4. It was later allowed to cool. The sample was transferred to a 100ml volumetric flask and diluted with distilled water to the mark. It was then thoroughly mixed. The mixture was further allowed to cool before distillation. A blank containing only H_2SO_4 acid and catalyst was also heated.
2. Distillation: A known aliquot (10ml) was transferred to the sample addition through the funnel of the distillation apparatus and then introduced to the sample chamber. About 10ml of 40% sodium hydroxide was added to the same sample through the funnel and released to the sample chamber at a slow rate. The

ammonia was entrapped in a receiving solution containing 10ml of 2% boric acid solution in which 4 drops of bromocresol green 2 drops of methyl red indicator had been added. Distillation continued until the pink colour turned greenish.

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

$$\% \text{ Nitrogen} = \frac{\text{Titre value (A-B) X Normality of acid X Vol. Of dilute } N_2 \text{ X } 100}{\text{Sample of weight (mg) X Volume of digest (aliquot)}}$$

$$= \frac{\text{Titre value X } 0.01 \text{ X } 100 \text{ X } 14.4007 \text{ x } 100}{\text{Sample weight (mg) X } 10}$$

Where A = Titre value for digestion sample

B = Titre value for the blank

$\% CP = \% N_2 \text{ X } 6.25$

N_2 = Nitrogen

3.2.3 Crude lipid

One gram of sample was weighed into an extraction thimble and covered with absorbent cotton. About 50 ml of solvent was added to a pre-weighed cup, which contained 5 glass balls. Both thimble and cup were attached to the extraction unit (Soxtec System 1043). Extraction unit temperature was fixed at 115°C for petroleum ether as a solvent while chloroform: methanol (2:1) was at 160°C. The sample was subjected to boiling in solvent for 30 minutes followed by rinsing for 1- 1.5 hours. Solvent was evaporated from the cup to the condensing column. Extracted lipid in the cup was placed in an oven at 110°C for 1 hour and after cooling the crude lipid was calculated using formula:

Crude lipid (%) = (extracted lipid/ sample weight) x100

3.2.4 Crude fibre (CF)

About 1g of defatted sample was placed in a glass crucible and attached to the extraction unit (Fibertec system 1020 hot Extractor). 150 ml of boiling 1.25% sulphuric acid solution and 10 drops of octanol (anti-foaming agent) were added. The sample was digested for 30 minutes and when the process was stopped, the acid was drained out and the sample washed with boiling distilled water. After this, 150ml of 1.25% sodium hydroxide solution and 10 drops of octanol were added. The sample was digested for 30 minutes then the process was stopped, the alkali was drained out and the sample washed with boiling distilled water. Finally the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was cooled in a desiccator and weighed (W1). The sample was then ashed at 550°C in a muffle furnace (Gallenkamp Muffle Furnace) for 2 hours, cooled in a dissector and reweighed (W2). Extracted fibre was expressed as percentage of the original undefatted sample and calculated according to the formula:

Fibre (%) = [(digested sample W1 – ashed sample W2) / sample weight] x100

3.2.5 Ash

About 1g of sample was weighed into a pre-weighed porcelain crucible and incinerated (Gallenkamp Muffle Furnace) for 24hrs at 600°C. The crucible was removed from the muffle furnace, cooled in a desiccator and weighed. Ash content was calculated according to the formula:

Ash (%) = (ash weight / sample weight) x 100

3.2.6 Nitrogen free extract (NFE)

Nitrogen free extract (carbohydrate) was calculated by subtracting the total percentages of moisture, crude protein, crude lipid, ash and crude fibre from 100%.

NFE (%) = 100 – (moisture* + protein + lipid + ash + fibre**)

* In case of dry matter basis, moisture was excluded

** Fibre is included here so that NFE represents potentially available carbohydrate

3.2.7 Amino acid profile determination

The Amino Acid profile in the known sample was determined using the method described by Benitez (1989). The known 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).

Defatting Sample:

A Known weight of the dried sample was weighed into extraction thimble and the fat was extracted with chloroform methanol (2:1 mixture) using soxhlet extraction apparatus as described by AOAC (2006) the extraction lasted for 15hrs.

Nitrogen Determination:

200mg of ground sample was weighed, wrapped in whatman filter paper (No 1) and put in the Kjeldhal digestion flask. Concentrated tetraoxosulphate (vi) acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), cooper sulphate ($CuSO_4$) and selenium oxide (ScO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added. The flask was then put in Kjeidhal digestion apparatus for 3 hours until the liquid turned light

green. The digested sample was cooled and diluted with distilled water to 1000ml in standard volumetric flask; A liquot (10ml) of the diluted solution with 10ml of 45%sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 1% boric acid containing 4 drops of bromoceresol green / methyl red indicator until about 70 ml of distillate was collected. The distillate was then liberated with standardize 0.01 N hydrochloric acid to grey coloured end point, the percentage nitrogen in the original sample was calculated using the formula:

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

Where:

a = Titre value of the digested sample

b = Titre value of blank sample

V = Volume after dilution (100ml)

W = Weight of dried sample (mg)

C = Aliquot of the sample used (10ml)

14 = Nitrogen constant in mg

Hydrolysis of the sample:

A known weight of the defatted sample was weighed into a glass ampoule. 7ml of 6m HCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and placed in an oven preset at $105^{o}C \pm 5^{o}C$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to

remove the fumes. Tryptophan is destroyed by 6M HCL during hydrolysis. The filtrate was then evaporated to dryness at 40^{o}C under vacuum in a rotary evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into TSM analyzer

The amount of the hydrolysate loaded was between 5 to 10 microlitres. 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 hydrolysate. The period of an analysis lasted for 76 minutes.

Method of Calculating Amino Acid Values from the Chromatogram Peaks

The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and width of the peak on the half height was 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:

Where S_{std} NE_{std} x Molecular Weight x μMAA_{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:

Concentration (g/100g protein) = NH x WNH/2 x S_{std} x C

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

Where: NH = Net height

W = Width at half height

nlμe = Norleucine

3.3. Determination of Anti-Nutritional Compounds

The anti-nutritional compounds such as tannins, saponins, hydrogen cyanide, phytate /phytic acids were determined as followed:

3.3.1. Tannin

Tannin was determined using the standard method described by A.O.A.C. (2000). Two grams (g) of the dried sample was boiled with 300ml of distilled water. This was diluted in a standard volumetric flask and filtered through a non-absorbent cotton wool. 25ml of the infusion was measured into a 2 liter 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. A few drops of 0.1N potassium permanganate were added. The titre value was multiplied by 0.0066235 to obtain the amount of tannin in the sample. The equation is as follows:

0.1N oxalic acid =m0.0066235g tannin

3.3.2. Saponin

A gravimetric method employing the use of a soxhlet extractor and two different organic solvents was used to determine the saponin. The first solvent, extracts lipids and interfering pigments, while the second solvent extracts the saponin properly. 5g of the dried ground sample was weighed into a thimble and transferred into the soxhlet extraction chamber fitted with a condenser and a round bottomed flask. Some quantity of acetone, enough to cause a reflux were poured into the flask. The sample was exhaustively extracted of its lipids and interfering pigments for 3 hours by heating the flask on a hot plate and the solvent distilled off. For the second extraction, a pre-weighed round bottom flask was fitted into the soxhlet apparatus which bore the thimble containing the sample. Then menthol was poured into the flask which was enough to cause reflux. The Saponin was then exhaustively extracted for 3 hours by heating the flask on a hot plate after which the solvent was distilled off. The flask was reweighed. The difference between the final and initial weights of the flask represents the weight of the saponins extracted.

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

3.3.3. Hydrogen cyanide (HCN)

To determine cynogenic glucosides in the samples, the alkaline titration method was used. 5 g of sample was placed in a 200ml conical flask and 50ml of distilled water was added. The contents of the flask was stirred and allowed to stand for 4 hours. The filtrate was collected into a beaker using a glass wool placed in a funnel and steam distilled into a 20ml of 2.5% sodium hydroxide. About 74ml of the distillate was collected. This was

titrated with 2N silver nitrate after the addition of 8ml of 6N ammonium hydroxide and 2ml of 2% potassium iodide. Permanent turbidity indicated end point (A.O.A.C., 1990).

3.3.4. Phytate/phytic acid

Phytate phosphorus was determined by the method of Wheeler and Ferrel (1971). A known weight of each ground sample was soaked in 100ml of 2% HCL for 5 hours and filtered. 25cm^3 of the filtrate was taken into a conical flask. 50cm^3 of 0.3% potassium thiocyanate solution was added. The mixture was titrated in a standard solution of $FeCl_3$ until a brownish-yellow colour persisted for 5 minutes. The concentration of the $FeCl_3$ was calculated.

Calculation: Mole ratio of Fe to phytate =1:1

Concentration of phytate phosphorus = $\frac{\text{liter value X0.064}}{1000}$ weight of sample

3.3.5 Oxalate

The oxalate content was determined using the procedures of Oke (1969). 2g of the sample powder was put into 250ml flask and mixed with 190ml distilled water and 10ml 6M hydrogen chloride acid. The mixture was boiled for one hour in water bath and later cooled and transferred into a 250ml volumetric flask, diluted and filtered. Four drops of methyl red indicator and concentrated ammonia were added until solution turns faint yellow. The mixture was heated to 100^oC and allowed to cool and filtered. The filtrate was boiled with 5% calcium chloride added and left overnight. 5ml of 25% tetraoxosulphate (vi) acid was added to dissolve precipitate solution was maintained at 80% and titrated with 0;5% potassium permanganate until pink colour persisted. The

amount of potassium permanganate used, was used to give the estimate of the unknown oxalate.

1ml potassium permanganate = 2.24mg oxalate

3.4. Mineral Analysis

Phosphorus was quantified using the method outlined by Allen (1989). Approximately 10 mg of sample was extracted overnight in 25mL of concentrated nitric acid. This was followed by digestion in concentrated nitric acid and then perchloric acid on a hot plate. Distilled water (20 mL) was added to the sample and boiled until white fumes appeared. This was followed by addition of 5mL of ammonia solution and further boiling until crystals were formed. The crystals were then dissolved using 20mL of acidified water and 80ml of distilled water followed by 20mL of a mixed reagent for colour development. The mixed reagent consisted of 250mL sulphuric acid–antimony, 250ml sodium molybdate, 500 mL distilled water and 2g/l ascorbic acid. The sample was left for 15 minutes for colour to develop. The quantity of phosphorus was then determined using a spectrophotometer (Cecil Elegant Technology, UK). Other minerals (potassium, calcium, iron, zinc and magnesium) were quantified using a Thermo Xseries 2 inductively coupled plasma mass spectrophotometer (ICP MS) (Thermo Scientific, USA). Samples weighing approximately 80mg were digested in nitric acid for 1hr in a Mars Xpress microwave (CEM Corporation, USA) and then diluted to 10ml with distilled water ready for quantification.

3.5. Experimental Design

3.5.1 Experimental system

The experiment was carried out in a semi flow through water system (Appendix 3.6). The system consisted of 18 units' cylindrical black plastic tanks of 380l capacity, 0.50m diameter and 1.00m depth. Water was supplied to each tank at a rate of 1l/min from a 1500l header tank. Water was drained from the tanks through central standpipes. The water in each experimental tank was aerated using air stones to augment the dissolved oxygen supply throughout the experimental period. The water in the experimental system was replaced twice a week with freshwater to adjust water quality and to avoid accumulation of faeces and uneaten feed or any excretory products.

3.5.2 Experimental fish

Fingerlings of *Clarias gariepinus* were obtained from Elegant Fisheries hatchery in Kaduna. A total of 360 fish with an average weight of 2.85g and age 8weeks were obtained and transported to the experimental site PZ, Zaria, in (2) 50 litres jerry cans. The feeding trial was conducted in (18) 380l black circular tanks located at the experimental site. Water was supplied from the overhead tank located at the site; each tank was filled up with water to ¾ marks. Fingerlings of *Clarias gariepinus* were stocked at average weight of 2.85g.Twenty (20) fish were randomly selected and stocked into each tank which comprised of six (6) treatments with three replicate per treatment in a complete randomized design (CRD); hence a total of 60 fingerlings were used per treatment. The total, standard lengths and weight of the fish was taken using meter board and a digital electronic scale. Prior to feeding, the fish were starved for 24 hours to empty their gut.

The fish were fed twice daily at 3% body weight (Lovell, 1989) for a period of (24 weeks) waste water was changed twice a week.

3.5.3 Feed formulation and pellet preparation.

The experimental diets contained fish meal (FM), soybean meal (SBM), yellow maize meal (YMM), groundnut cake meal (GNCM) and fermented *Senna obtusifolia* seed meal (FSOSM). All ingredients were ground into a fine powder using a hammer mill and sieved by using a 0.25mm sieve. Fish meal, SBM, GNCM and YMM were obtained from commercial suppliers. Prior to feed formulation, proximate compositions of these ingredients were determined (Table 3.1). On the basis of the nutrient composition of the ingredients (Table 3.2), Five isonitrogenous diets (42% CP) and Isocaloric (gross energy 2777 kcal kg^{-1}) was prepared where the fermented *Senna obtusifolia* seed meal (FSOSM) replaced soybean meal (SBM) at 0%, 25%, 50%, 75%, and 100%. While 0% (SBM0) and commercial pelleted feed (Coppens) served as the control diets.

All dietary ingredients were ground and mixed with vitamin–mineral premix and vegetable oil. Water was added gradually until a desirable paste-like consistency was obtained after which the mixture was pelleted through 3mm disc and sun-dried. The dry pelleted feed were then stored in airtight polythene bags, until required. Before feeding, the dried diets were broken and graded to produce suitable particle sizes. Diet samples were subjected to proximate analysis.

3.6. Sampling and Monitoring of Experimental Fish

Fish specimens from each tank were removed fortnightly, using scoop net after total draining of water for growth and survival records (mean body weight, mean standard length and mortality) and to adjust the ration so as to correspond with the new body weight of the fish in each treatment. The sampling exercise was carried out in the morning (7.00-9.00 am) to minimize stress due to heat.

3.7. Estimation of Digestibility

Digestibility of diets was determined using the indirect method which relies on use of an inert marker (Lovell, 1989). The marker concentrates in faeces relative to the digestible material. Digestibility was determined by the relative quantities of the marker in feed and faeces. In this study chromium (III) oxide (BDH 10234) was used as a marker at an inclusion level of 0.5%.

3.7.1 Faecal collection

Dissection

Ten hours after feeding, three fish were sacrificed by partial decapitation by cutting through the vertebral column. The gastrointestinal tract was divided into four parts (Uys, 1989). These were the stomach, the fore-gut, the mid-gut and the hind-gut. To distinguish between the fore-gut, mid-gut and the hind-gut when sampling for the faecal material and to keep the results constant, the total length of the gut was measured from the anus to the sphincter muscle leading into the stomach and this was divided by three. Each segment then represented the approximate appropriate section of the gastrointestinal tract.

The stomach was considered the region between the cardiac and pyloric sphincters. A longitudinal incision was made along the length of the abdomen from the anus to the base of the mouth. The gastrointestinal tract was then carefully removed.

The hind part of the gastrointestinal tract was then severed from the mid-gut. A longitudinal incision was then made in the hind-gut to evacuate its feacal content without scraping the rectal and intestinal tissues, and stored in a freezer. Before analysis, frozen faecal material was thawed overnight in a refrigerator. Thereafter, the faecal material was dried at 60^0C for 48hrs and then ground to a fine powder using mortar and pestle. Chromic oxide was quantified following the method of (Furukawa and Tsukahara, 1966). Approximately 50-100mg of sample was digested with 5ml of concentrated nitric acid followed by 3ml of perchloric acid which turned the digesta from green to yellow. This

Table 3.1: Proximate Composition of Ingredients determined in the Laboratory

Ingredients	**Moist%**	**CP%**	**EE%**	**CF%**	**ASH%**	**NFE%**	**GE(Kcal/j)**
Fish meal	9.78	72	7.92	-	11.03	4.89	2980
Soya meal	7.92	44	2.6	7.8	5.6	32	2400
Yellow corn	9.13	8.9	3.48	11.6	3.81	63.08	3447
GNC meal	9.09	43	5.2	6.0	5.8	30.91	2400
FSOSM	6.54	32.11	7.71	4.3	5.14	44.2	3226

Moist: % Moisture
CP: % Crude Protein
EE: % Ether Extract
CF: % Crude Fibre
ASH: % Ash
NFE: % Nitrogen Free Extract

FSOSM: Fermented *Senna obtusifoilia* Seed meal

Table 3.2: Gross Composition of the Experimental diets

Ingredients	**0%**	**25%**	**50%**	**75%**	**100%**
Maize	23	23	23	23	23
FM	24	24	24	24	24
GNC	15	15	15	15	15
SBM	35	26.25	17.5	8.75	-
FSOSM	-	8.75	17.5	26.25	35
Vitamins-mineral premix	0.25	0.25	0.25	0.25	0.25
Vegetable oil	2	2	2	2	2
Salt	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**
Cost ₦/kg	**360**	**210.65**	**203.36**	**195.30**	**180**

FM: Fish meal
GNC: Groundnut cake
SBM: Soybean meal
FSOSM: Fermented *Senna obtusifolia* seed meal

was then diluted to 100 ml of distilled water and absorbance read using Uvikon 860 spectrophotometer (Kontron Instruments) at 350nm. The weight and percentage of chromic oxide was calculated using the following formulae:

Weight of Cr2O3 = Absorbance – 0.0032/0.2089

Digestibility coefficients of diets were estimated to determine bioavailability of nutrients. Apparent digestibility coefficients (ADC) of the feeds were computed using the formula described by Maynard and Loosli (1969).

ADC (%) =100 × (%nutrient in faeces/%nutrient in feed) × (%marker in feed/marker in faeces)

Faecal material was placed in a sterile vial which was then placed in a drying oven at 40^0C until constant weight was achieved.

3.8. Determination of Haematogical Parameters

At the end of the week 24 of feeding, fish from each treatment were randomly selected for haematological analysis. Live fish was put on a tray. A damp cloth was used to cover the fish head. Blood was drawn from the caudal vein through the lateral line of fish into ethylenediamine tetra-acetic acid EDTA bottles by the use of needle and syringe for determination of the Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) as reported by Hrubec and Smith (2000).

3.8.1 Packed cell volume (PCV)

The capillary tubes were 3/4 filled with whole blood and one end sealed with plasticine. The tubes were centrifuged for 5 min in a microhaematocrit centrifuge at 12,000 rpm. The PCV was read by the use of haematocrit reader (Kelly, 1979)

3.8.2 Red blood cell (RBC) and white blood cell (WBC) counts

The RBC and total WBC counts were carried out by use of the Neubauer improved counting chamber as described by (Kelly, 1979). For red blood cell counts, blood was diluted 1:200 with Dacies fluid (99 ml of 3% aqueous solution of sodium citrate; and 1 ml of 40% formaldehyde) which keeps and preserves the shape of the red blood cell for estimation in the counting chamber Kelly (1979)

3.8.3 Total white blood cell counts

For white blood cell counts, the dilution was 1:20 using 2-3% aqueous solution of acetic acid to which tinge of Gentian violet was added. Thin blood smears were stained with Wright-Giemsa stain (Schalm *et al*., 1975). A total of 100 white blood cells were enumerated and differentiated.

3.8.4 Haemoglobin (Hb) estimation

The cyanomethaemoglobin method as described by (Schalm *et al*., 1975 and Kelly, 1979) was used in the determination of haemoglobin concentration. Well-mixed blood of 0.02 ml was added to 4 ml of modified Dabkin's solution (potassium ferricyanide, 200 mg; potassium cyanide, 50 mg; potassium dihydrogen phosphate 140 mg). The volume was made up to 1 L with distilled water at pH of 7.0. The mixture was allowed to stand for

3min and the Hb concentration was read photometrically by comparing with a cyanomethaemoglobin standard with a yellow-green filter at 625 nm.

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

MCH (gd/l) = (Hb in g 100 mL-1/RBC in millions cell mm-3) × 10

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

3.9. Water Quality Monitoring

Water temperature for each tank was taken daily (8.00-9.00hr) using combined pH and thermometer model pH-009(111), while the Dissolved oxygen (DO) was also determined using the DO meter model DO-510. Both the DO and pH measurements were taken every morning before feeding the fish.

3.10. Growth and Feed Utilization Parameters

The data collected was analysed for the following

1. Mean body weight gain

   (MWG) = W_2-W_1

   Where W_1= Mean initial body weight

   W_2= Mean Final body weight

2. Specific Growth Rate

(SGR) = 100(Ln W_2- Ln W_1)/ T---------- (Kaushik, 1998)

Where W_1= Mean initial body weight

W_2= Mean Final body weight

Ln= Natural log

3. Feed conversion Ratio

(FCR)= weight of feed fed/ weight gain by fish x 100---------- (Adikwu, 2003)

4. Protein Efficiency Ratio (PER) was determined according to the formula

PER = Mean weight gain/weight of protein fed

5. Apparent Net protein utilization (APNU) was determined as reported by (Jackson *et al*., 1982)

ANPU = fish protein gained (g) /protein consumed (g) X100

6. Carcass proximate composition of fish before and after the feeding trial was determined according to (AOAC, 2000).

3.11. Determination of Economic Viability of the Feeds

The economic evaluations of substituting soybean meal with fermented *Senna obtusifolia* seed meal in the culture of the catfish *Clarias gariepinus* were evaluated. The economic benefit of the various diets was determined using the following indices:

1. Incidence cost (IC) = Cost of feed/weight of fish produced
2. Profit index (PI) = weight of fish produced/cost of feed
3. Net profit (NP) = sales – expenditure
4. Benefit cost ratio (BCR) = total sales/total expenditure (Mazid *et al*., 1997)
5. Total cost (TC) = fixed cost(FC) + total variable cost(TVC)

Where FC= cost of fingerlings

TVC = Cost of producing Different diets (Sogbesan and Ugwumba, 2006)

Gross margin analysis and profitability ratio was used to examine the cost and returns of fish production in substituting soybean meal with fermented *Senna obtusifolia* seed meal. Gross margin analysis is given by equation vi:

6. GM = TR – TVC

Where GM = gross margin

TR = Total revenue

TVC = Total Variable Cost

The performance and economic worth of various diets was determined by the use of the following (Abu *et al*., 2010)

7. NR = TR- TC

Where NR = net revenue

TR = Total revenue

TC = Total cost

3.12. Statistical Analysis

The biweekly weight recorded and feed supplied were used to compute the growth, nutrient utilization and economic evaluation indices as described by New(1989), Morraise *et al*.(2001) and Abu *et al*. (2010).

Data collected were subjected to one way analysis of variance (ANOVA) using General Linear Model (GLM) procedure of SAS (SAS, 2004) followed by Duncan Multiple Range Test (DMRT) to test difference between levels of means and to separate means. Values of $p<0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate compositions of the experimental diets

The proximate compositions of the experimental diets are presented in Table 4.1. It showed five iso-nitrogenous (42% CP) and iso-caloric (gross energy 2985 Kcal/kg) in the diets.

4.2 Effects of Processing Methods on Nutrient Composition of *Senna obtusifolia*

The proximate compositions of raw, boiled, toasted and fermented *Senna obtusifolia* seeds meal (FSOSM) are presented in Table 4.2.The crude protein content of raw SOSM sample ranged between 21.12 ±0.05 g/100g to 32.11±0.06g/100g for fermented SOSM. The crude protein content for the fermented sample differed significantly ($p<0.05$) than the boiled, toasted and raw sample. Boiling significantly reduced the crude protein contents of the raw SOSM from 21.12% to 15.51% as the boiling time increases from 30 to 60min.Toasting initially reduce the crude protein to 14.06% but slightly increased as the toasting time increases ($P<0.05$).The crude protein of the fermented seed meal increased from 25.16% to 32.11% from 48hrs to 96hrs. In all the processing methods there was a significant differences ($P<0.05$) in crude protein amongst treatment as processing time progresses.

The crude lipid content of the raw *Senna obtusifolia* seed was (5.61%) but the boiled (12.57 ±0.02 g/100 g) and fermented (7.17 ±0.02 g/100 g) *Senna obtusifolia* seeds meal had higher lipid content. Boiling time increased the lipid contents from 11.14% to 12.57%. There was a decrease in the lipid content of the toasted seed which decreased significantly ($p>0.05$) as the toasting time increases from 5 to 15min, similarly crude

Table 4.1: Proximate compositions of the experimental diets

Composition	**CF**	**0%**	**25%**	**50%**	**75%**	**100%**
Crude protein	42.34[a]	42.35[a]	42.28[a]	42.23[a]	42.22[a]	42.24[a]
Moisture	7.86[a]	7.38[b]	7.49[b]	7.90[a]	7.38[a]	7.48[b]
Crude lipid	14.69[a]	11.47[c]	11.46[c]	11.69[b]	11.84[b]	11.79[b]
Crude fibre	2.8[c]	4.91[b]	4.62[b]	4.59[b]	5.32[a]	5.33[a]
Ash	6.7[d]	8.21[c]	8.30[b]	8.46[a]	8.14[c]	8.18[c]
NFE	25.70[a]	25.68[a]	25.85[a]	25.03[a]	25.10[a]	24.98[a]
Dry matter	92.23[a]	92.62[a]	92.51[a]	92.00[b]	91.62[c]	92.12[b]

Means on the same row with the same superscript are not significantly different ($p>0.05$)
CF: Commercial feed

Table 4.2**: Effects of different processing methods on the nutrients composition of *S. obtusifolia***

Treatment	Crude protein	Lipids	Ash	Moisture	Fibre	NFE
Raw	21.12±0.05^{d}	5.61±0.02^{g}	5.42±0.02^{b}	7.02±0.02^{a}	8.63±0.02^{a}	52.22±0.01^{e}
F 48	25.16±0.06^{b}	7.17±0.02^{e}	5.25±0.02^{d}	5.91±0.02^{d}	5.51±0.02^{g}	51.00±0.01^{f}
F 72	28.74±0.06^{b}	6.15±0.02^{d}	5.40±0.02^{b}	5.05±0.02^{e}	5.03±0.02^{i}	49.63±0.01^{h}
F 96	32.11±0.06^{a}	4.71±0.02^{f}	5.14±0.02^{e}	4.60±0.02^{f}	5.29±0.02^{h}	48.15±0.01^{i}
T 5	14.06±0.06^{i}	6.05±0.02^{h}	5.39±0.02^{c}	4.87±0.02^{f}	7.87±0.02^{b}	61.76±0.01^{b}
T 10	15.63±0.06^{h}	4.73±0.02^{j}	5.63±0.02^{a}	4.31±0.02^{g}	7.31±0.02^{c}	62.39±0.01^{a}
T 15	17.91±0.06^{f}	4.80±0.02^{i}	5.77±0.02^{a}	4.20±0.02^{h}	8.62±0.02^{a}	58.70±0.01^{c}
B 30	20.12±0.06^{e}	11.14±0.02^{c}	4.71±0.02^{f}	5.72±0.02^{d}	7.14±0.02^{e}	51.17±0.01^{g}
B 45	17.54±0.06^{g}	12.71±0.02^{a}	4.40±0.02^{f}	6.40±0.02^{c}	7.82±0.02^{b}	51.13±0.01^{g}
B 60	15.51±0.06^{h}	12.57±0.02^{b}	4.61±0.02^{f}	6.92±0.02^{b}	6.96±0.02^{f}	53.43±0.01^{d}

Means in the same column with different superscripts differed significantly ($p<0.05$)
F 48, 72 and 96(fermented seeds at different hours)
T5, 10 and 15 (toasted seeds at different minutes)
B 30, 45 and 60 (Boiled seeds at different minutes)

lipid decreased as fermentation time progressed from 48hrs to 96hrs. In all the processing methods there was significant differences ($P<0.05$) in crude lipids contents amongst treatment as processing time progresses.

The ash content of the raw SOSM (5.42 ±0.38 g/100 g) was slightly higher than that of the fermented (5.40 ±0.21 g/100 g) but insignificantly different ($p>0.05$) when compared among boiled and toasted samples. Boiling reduced the ash contents but not significantly different ($P>0.05$) as boiling progressed from 30 to 60min. Increasing toasting time from 10 to 15min had no significant difference ($P>0.05$) in the ash content. Fermentation also reduced ash content slightly but no significant difference ($P>0.05$) within the fermented treatment and the raw sample (Table 4.2).

The crude fiber contents of the raw sample was 8.63±0.35 g/100g and significantly different ($p<0.05$) from boiled sample (7.82%). Boiling treatment significantly ($p<0.05$) reduced fibre contents. Boiling for 30, 45 and 60 min. resulted in crude fibre contents of 7.14, 7.82 and 6.96% respectively. Increasing toasting time increased crude fiber from 7.87% to 8.62% but not significantly different ($p>0.05$) from the raw sample. The crude fibre content of the fermented sample was lower than the raw sample and significantly different ($P<0.05$) among treatments, as the fermentation time progressed the fibre contents decreased from 5.51 to 5.29% (Table 4.2).

The moisture content of the raw SOSM was 7.02%. The moisture content of the fermented sample progressively decreased as fermentation period increased from 48 to 96hrs. There is a significant difference ($P<0.05$) in the moisture contents among fermented treatment. The moisture content ranged from 5.72% in 30 min to 6.92% in 60

min boiled sample, increasing boiling time increases the moisture content and there was a significant difference ($P<0.05$) in the moisture content of the boiled samples.

The nitrogen free extract (NFE) of the raw sample was 52.22% but increased slightly after toasting. Though no significant difference ($p>0.05$) was observed among toasting time, but toasting at 5, 10 and 15 minutes resulted in 61.81, 62.42 and 58.67% of NFE respectively. Boiling did not affect NFE, boiling for 30, 45 and 60min resulted in NFE of 51.12, 51.10 and 53.34% respectively. The total NFE reduced during fermentation progressively at 48, 72 and 96hrs to 51.09, 50.60 and 47.88% respectively and there were significant difference ($p<0.05$) in NFE within the hours of fermentation (Table 4.2).

4.3. Anti-nutritional Compound of Raw and Processed *Senna obtusifolia* Seed Meal

The anti-nutritional compound of raw and differently processed *Senna obtusifolia* seeds meal (SOSM) samples is presented in Table 4.3. There were significant differences ($p<0.05$) in anti-nutritional compounds between raw and the differently processed seeds, the levels of these compounds reduced as processing time increased.

The phytate concentration in the raw seed was 4.22±0.13g/100g reduced significantly ($p<0.05$) to 0.28±0.01g/100g for the fermented seed at 96hrs represents 93.4% decrease in the phytate concentration. Similarly, the concentration of phytates reduced to 0.82±0.13g/100g for the seed toasted at 15min representing a reduction of 80.6% of phytates and also the concentration of phytates reduced to 0.96±0.13g/100g in the 60min boiled seeds representing 77.25% reduction in phytates respectively.

The tannin in the raw seed was 1.60 ±0.02g/100g which was significantly reduced ($p<0.05$) by 96.3% to 0.06±0.02g/100g in the 96hrs fermented seed. The tannin was also

Table 4.3: Effects of Different Processing Methods on the Anti-nutrient of *Senna obtusifolia* seed

Treatments	Phytates	Tannins	Cyanide	Oxalates	Saponins
Raw	4.22 ±0.13^{a}	1.60±0.02^{a}	0.47 ±0.01^{a}	2.44±0.01^{a}	2.14±0.08^{a}
F_{48}	0.66±0.01^{h}	0.16±0.02^{g}	0.12±0.01^{f}	0.05±0.01^{h}	0.14±0.08^{ef}
F_{72}	0.43±0.01^{i}	0.15±0.02^{g}	0.08±0.01^{g}	0.10±0.01^{i}	0.32±0.08^{ed}
F_{96}	0.28±0.01^{j}	0.06±0.02^{h}	0.04±0.01^{h}	0.00±0.01^{j}	0.06±0.08^{f}
% Reduction	93.40	96.30	91.50	100	97.20
T_{5}	2.45±0.01^{c}	0.66±0.02^{c}	0.28±0.01^{b}	0.98±0.01^{c}	0.75±0.08^{b}
T_{10}	1.72±0.01^{d}	0.45±0.02^{e}	0.22±0.01^{d}	0.47±0.01^{e}	0.48±0.08^{d}
T_{15}	0.82±0.01^{g}	0.28±0.02^{f}	0.18±0.01^{e}	0.20±0.01^{g}	0.48±0.08^{d}
% Reduction	80.60	82.50	61.70	91.80	77.57
B_{30}	2.64±0.01^{b}	0.82±0.02^{b}	0.23±0.01^{cd}	1.15±0.01^{b}	1.65±0.08^{b}
B_{45}	1.28±0.01^{e}	0.55±0.02^{d}	0.14±0.01^{f}	0.63±0.01^{d}	0.84±0.08^{c}
B_{60}	0.96±0.01^{f}	0.27±0.02^{f}	0.26±0.01^{bc}	0.39±0.01^{f}	0.42±0.08^{d}
% Reduction	77.25	83.10	44.68	84.02	80.37

Means on the same column with the same superscripts are not significantly different ($p>0.05$)
F 48, 72 and 96(Fermented seeds at different hours)
T5, 10 and 15 (Toasted seeds at different minutes)
B 30, 45 and 60 (Boiled seeds at different minutes)
% Reduction: Percentage reduction in Anti-nutritional compounds

reduced by 82.5% to 0.28±0.02g/100g in the 15min toasted seed, while the tannin in the seed boiled for 60min also reduced by 83.1% to 0.27±0.02g/100g.

The cyanide concentration was 0.47±0.01g/100g in the raw seed was reduced to 0.04±0.01g/100g for the fermented seed at 96hrs representing a reduction of 91.5% reduction. The concentration of cyanide was also reduced to 0.18±0.01g/100g in the toasted seed at 15min. The cyanide concentration also reduced significantly ($p<0.05$) to 0.26±0.01g/100g in the boiled seed at 60min.

The oxalate concentration was 2.44 ±0.020 g/100g in the raw seed meal, it was reduced to 0.00±0.003g/100g in the fermented seed at 96hrs, the concentration was reduced to 0.20±0.01g/100g in the toasted seed at 15min, and in the boiled seed at 60min the concentration was also reduced to 0.39±0.01g/100g.

Saponin concentration was 2.14±0.08g/100g in the raw seeds and it was significantly ($p<0.05$) reduced to 0.06±0.08g/100g in the fermented seed at 96hrs representing a reduction of 97.2% in saponin concentration, the concentration was also reduced to 1.65±0.08g/100g in the toasted seed at 15min and in the boiled seed at 60min the saponin was reduced by up to 80.37% to 0.42±0.08g/100g.

4.4. Amino Acid Composition of Processed *Senna obtusifolia* Seed

The amino acid (g/100g protein) composition of the raw and differently processed *Senna obtusifolia* is presented in Table.4.4. Leucine (6.23g/100g protein) had the highest value of essential amino acid while glutamic and aspartic acids (11.92 and 7.82g/100g protein respectively) were the most available non-essential amino acids in the raw seeds. Tryptophan concentration could not be determined because it was hydrolysed by the acid.

Table4.4: Amino Acid Composition of Differently Processed *Senna obtusifolia* Seeds

Trt	Ref. value	Raw	F_{48}	F_{72}	F_{96}	T_5	T_{10}	T_{15}	B_{30}	B_{45}	B_{60}	SEM
EAA												
Lys	5.80	3.48^{cd}	3.99^{b}	4.08^{b}	4.90^{a}	3.34^{d}	3.46^{cd}	3.48^{cd}	3.45^{cd}	3.56^{c}	3.48^{cd}	±0.10
Hist	2.50	2.25^{b}	2.70^{b}	3.47^{a}	3.85^{a}	2.16^{b}	2.26^{b}	2.20^{b}	2.20^{b}	2.26^{b}	2.24^{b}	±0.12
Arg	5.20	5.60^{b}	5.89^{ab}	6.03^{ab}	6.70^{a}	5.54^{b}	5.60^{b}	5.53^{b}	5.58^{b}	5.65^{b}	5.60^{b}	±0.94
Thr	3.40	3.33^{c}	3.86^{ab}	4.10^{ab}	4.35^{a}	3.18^{c}	3.31^{c}	4.00^{ab}	3.34^{c}	3.33^{c}	3.40^{c}	±0.08
Val.	5.00	3.88^{abc}	3.96^{ab}	4.05^{ab}	4.11^{a}	3.60^{b}	367^{cde}	3.63^{de}	3.86^{abcd}	3.82^{abcd}	3.87^{abcd}	±0.04
Met	2.50	0.64^{de}	0.71^{bc}	0.74^{b}	0.86^{a}	0.61^{de}	0.63^{de}	0.58^{e}	0.67^{cd}	0.63^{de}	0.61^{de}	±0.02
Iso.	2.80	3.11^{b}	3.05^{c}	3.16^{b}	3.96^{a}	3.07^{c}	3.12^{b}	3.13^{b}	3.07^{c}	3.11^{b}	3.10^{b}	±0.07
Leu	1.10	6.23^{bc}	6.78^{abc}	6.79^{ab}	7.14^{a}	6.07^{bc}	6.13^{bc}	5.87^{c}	6.14^{bc}	5.96^{c}	6.23^{bc}	±0.94
Phe.	6.30	3.54^{bc}	3.88^{bc}	4.11^{ab}	4.58^{a}	3.50^{bc}	3.56^{bc}	3.33^{c}	3.56^{bc}	3.56^{bc}	3.64^{bc}	±0.08
NEAA												
Glu.	14.70	11.97^{ab}	12.02^{ab}	12.11^{ab}	12.26^{a}	11.66^{ab}	11.80^{ab}	11.40^{ab}	11.20^{b}	11.60^{ab}	11.80^{ab}	±0.10
Ser.	7.00	3.09^{ab}	3.11^{ab}	3.26^{ab}	3.48a	3.04^{ab}	2.97^{b}	2.95^{b}	3.01^{ab}	2.92^{b}	2.56^{b}	±0.05
Asp	7.70	7.82^{abc}	7.96^{ab}	8.14^{ab}	8.36a	7.55^{abcd}	7.12^{cd}	6.94^{d}	7.35^{bcd}	7.12^{cd}	6.98^{d}	±0.11
Pro.	10.70	2.85^{bcd}	2.98^{abc}	3.16^{ab}	3.33^{a}	2.65^{cd}	2.61^{cd}	2.51^{d}	2.74^{cd}	2.71^{cd}	2.61^{cd}	±0.06
Gly.	2.20	4.28^{ab}	4.33^{ab}	4.46^{a}	4.88^{a}	3.01^{c}	3.70^{bc}	3.64^{bc}	3.64^{bc}	370^{bc}	3.71^{bc}	±0.11
Ala.	6.10	4.03^{b}	4.11^{b}	4.18^{b}	4.67^{a}	4.00^{b}	4.06^{b}	3.98^{b}	4.01^{b}	4.06^{b}	4.00^{b}	±0.05
Cys	3.00	0.79^{c}	0.86^{c}	0.98^{b}	1.52^{a}	0.60^{d}	0.63^{d}	0.60^{d}	0.65^{d}	0.68^{d}	0.66^{d}	±0.05
Tyr.	1.10	2.86^{b}	3.06^{ab}	3.12^{ab}	3.41^{a}	2.06^{c}	2.01^{c}	1.98^{c}	2.14^{c}	2.11^{c}	186^{c}	±0.11

Means on the same row with the same superscript are not significantly different ($p>0.05$)
F 48, 72 and 96: (Fermented seeds at different hours)
T5, 10 and 15: (Toasted seeds at different minutes)
B 30, 45 and 60: (Boiled seeds at different minutes)
EAA: Essential Amino Acids
NEAA: Non-Essential Amino Acids

Methionine (Met.) had the least value both in the raw and processed seeds. Boiling and toasting significantly ($p<0.05$) reduced the amino acid compositions, while fermentation significantly ($p<0.05$) improved the amino acids.

4.5: Concentration of Amino Acid (g/100gcp) of Raw and Processed seeds of *Senna obtusifolia*

The evaluation of the amino acids based on the classification of the differently processed *Senna obtusifolia* seed meal is presented in Table 4.5 revealed that the total amino acid (TAA),Total Essential Amino Acid (TEAA) with histidine and Total Sulpur Amino Acids (TSAA) of the raw seeds were 69.93, 32.24 and 1.42 respectively. Boiling and toasting reduced the TAA while fermentation enhanced the total TAA. Similarly, the TEAA and TSAA were reduced by boiling and toasting while fermentation progressively enhanced it from 1.57 to 2.38 in F_{48} and F_{96} respectively.

4.6. Effects of Different Processing Methods on the Mineral Contents of *S. obtusifolia* Seeds

Calcium (Ca), potassium (K), phosphorus (P), magnesium (Mg), iron (Fe), and zinc (Zn) contents of raw *S. obtusifolia* were 890, 1017, 976, 602, 215.37, and 50.49 mg/100g respectively. Potassium was the most abundant mineral element while zinc was the least in the raw seeds (Table 4.6).

The raw *S. obtusifolia* seed in this study contained 890mg/100g of calcium whereas, fermented samples 48, 72 and 96hrs had 945mg/100g, 942mg/100g, and 956mg/100g of calcium respectively. These variation in calcium contents was higher than in the raw samples but not significantly different $p<0.05$ from the toasted samples but significantly

different ($p>0.05$) from the boiled samples. The highest value of potassium (1022mg/100g) was in the fermented seed for 96hrs (Table 4.6).

Table 4.5: Concentration of Amino Acid (g/100gcp) of Raw and Differently Processed *Senna obtusifolia*

Amino acids	Raw	F_{48}	F_{72}	F_{96}	T_5	T_{10}	T_{15}	B_{30}	B_{45}	B_{60}
TAA	69.93	73.03	75.89	83.44	65.59	66.48	66.51	66.57	66.87	66.24
TEAA										
Histidine	32.24	34.60	36.48	40.53	31.02	31.58	30.50	31.83	31.97	31.76
No histidine	29.97	31.92	33.16	36.69	28.91	29.44	28.38	29.59	29.71	29.54
% TEAA										
Histidine	46.10	47.38	48.10	48.57	47.29	47.50	47.28	47.81	47.81	47.95
No histidine	42.86	43.71	43.70	44.00	44.08	44.28	44.00	44.45	44.43	44.60
TNEAA	37.69	38.43	39.41	42.91	34.57	34.90	34.01	34.74	34.90	34.48
%TNEAA	53.90	52.62	52.00	51.43	52.71	52.50	52.72	52.19	52.20	52.10
TSAA(met+cys)	1.42	1.57	1.72	2.38	1.21	1.27	1.18	1.30	1.30	1.77

TAA: Total Amino Acids
TEAA: Total Essential Amino Acids
TNEAA: Total Non-Essential Amino Acids
TSAA: Total Sulphur Amino Acids
F 48, 72 and 96: (Fermented seeds at different hours)
T 5, 10 and 15: (Toasted seeds at different minutes)
B 30, 45 and 60: (Boiled seeds at different minutes)

Table 4.6: Effects of Different Processing Methods on the Mineral Contents of *Senna obtusifolia* Seeds

Trt	Raw	F_{48}	F_{72}	F_{96}	T_5	T_{10}	T_{15}	B_{30}	B_{45}	B_{60}	SEM
Mineral											
Ca	890^{abc}	945.67^{a}	942.67^{a}	956^{a}	911.33^{ab}	902^{abc}	809.33^{d}	851.33^{bcd}	836.33^{cd}	806.33^{d}	±11.56
K	1017^{a}	1010.67^{ab}	1002^{ab}	1022.33^{a}	970.67^{ab}	981^{ab}	960^{ab}	993.88^{ab}	912.3^{b}	919.33^{ab}	±10.64
P	976^{a}	803.67^{ab}	803.33^{a}	805.67^{a}	784.61^{ab}	759^{b}	752^{b}	780.5^{ab}	780^{ab}	748.33^{b}	±4.80
Mg	602^{ab}	671.33^{ab}	614^{ba}	643^{a}	585^{bc}	595^{abc}	595.67^{abc}	584.53^{bc}	582.67^{bc}	550^{c}	±6.09
Fe	215.37^{d}	231.2^{ab}	228.8^{abc}	232.44^{a}	216.13^{cd}	216.33^{cd}	207^{d}	220.29^{abcd}	218.93^{bcd}	218.47^{bcd}	±1.74
Zn	50.49^{a}	50.43^{a}	50.30^{a}	49.97^{a}	46.37^{b}	46.35^{b}	46.09^{b}	45.56^{b}	48.27^{b}	47.90^{ab}	±0.42

Means on the same row with the same superscripts are not significantly different ($p>0.05$)

F 48, 72 and 96: (fermented seeds at different hours)
T 5, 10 and 15: (toasted seeds at different minutes)
B 30, 45 and 60: (Boiled seeds at different minutes)

4.7. Physicho-Chemical Parameters of Water of the Experimental Tanks

Water quality parameters in the experimental tanks are presented in Table 4.7. The water temperature ranged from 28.75 to 29.4^{o}C while the pH ranged from 6.99 to 7.06.The dissolved oxygen range was 5.71 to 5.90mg/l. There was no significant difference (P> 0.05) in the water quality parameters in the different treatment tank.

4.8. Growth Performance and Feed Utilisation of *Clarias gariepinus*

The growth performance and feed utilization of *Clarias gariepinus* fed fermented *Senna obtusifolia* seed meal (FSOSM) is presented in (Table 4.8). Fishes fed the commercial and control diets gained 278.02g and 277.23g respectively, while those fed 25%, 50% 75% and 100% inclusion of FSOSM gained 276.76g, 215.62g, 173.48g and 135.81g respectively. There was no statistical difference ($p<0.05$) in fishes fed commercial, control diet and 25% fermented SOSM but there were significantly different ($p<0.05$) from values obtained in fishes fed diet containing 50%, 75% and 100% inclusion of FSOSM.

The results for the specific growth rate (SGR) showed that fishes fed commercial feed had the highest value of 1.14%/day while fishes fed 100% FSOSM had 0.92%/day. The feed conversion ratio (FCR) ranged from 1.79-2.18. There was no significant difference ($p>0.05$)FCR in fishes fed the commercial, control diet, and 25% fermented SOSM but fishes fed diet containing 50%, 75% and 100% fermented SOSM were significantly different ($p<0.05$). The protein efficiency ratio (PER) of 1.38 was observed in fishes fed the commercial and control(0%) diet and the lowest value of 1.09 was in fishes fed diet containing 75% fermented SOSM.

Table 4.7**: Mean Water Quality Parameters of the Experimental Tanks**

Parameters	**CF**	**0%**	**25%**	**50%**	**75%**	**100%**
Mean pH	6.99±0.01	7.05±0.02	7.01±0.01	7.06±0.03	7.02±0.01	7.04±0.02
Mean Temp.	29.4±0.03	28.90±0.02	29.20±0.03	28.75±0.01	29.01±0.01	29.02±0.03
Mean DO	5.90±0.02	5.80±0.03	5.78±0.01	5.75±0.02	5.73±0.01	5.71±0.01

CF: Commercial feeds

Table 4.8: Growth performances and feed utilization of *Clarias gariepinus* fed graded levels of fermented (SOSM)

Indices	**CF**	**0%**	**25%**	**50%**	**75%**	**100%**	SEM
Mean initial weight(g)	2.84[a]	2.85[a]	2.86[a]	2.84[a]	2.87[a]	2.85[a]	±0.01
Mean final weight(g)	280.86[a]	280.08[a]	279.62[a]	218.47[b]	173.48[c]	135.81[d]	±13.91
Mean weight gain(g)	278.02[a]	277.23[a]	276.76[a]	215.6[b]	170.60[c]	135.81[d]	±13.91
SGR %/day	1.14[a]	1.12[a]	1.13[a]	1.06[b]	0.97[c]	0.92[d]	±0.02
FCR	1.81[a]	1.79[a]	1.81[a]	2.09[b]	2.18[b]	2.11[b]	±0.04
PER	1.38[a]	1.38[a]	1.36[a]	1.15[b]	1.09[c]	1.12[bc]	±0.03
ANPU	91.63[a]	91.83[a]	91.63[a]	76.35[b]	71.43[c]	71.70[c]	±2.29
Survival rate%	98.67[a]	97.33[a]	95.33[b]	87.33[c]	82.67[d]	80.67[d]	±1.72
Mean feed intake(g)	487.80[a]	483.58[b]	484.48[b]	446.69[c]	371.57[d]	279.64[e]	±18.62
Protein Intake(g)	204.88[a]	203.10[b]	203.48[b]	187.61[c]	156.07[c]	117.45[e]	±7.82

Means on the same row with the same superscript are not significantly different ($p>0.05$)
CF: Commercial Feeds

SGR: Specific Growth Rate

The highest mean feed intake was 487.80g, while the lowest was 279.64g. There was significant difference ($p<0.05$) in the mean feed intake in fishes fed the commercial, control diet, and 25% fermented SOSM. Significant variation ($p<0.05$) was observed in the mean feed intake in fish fed diets containing 50%, 75% and 100% fermented SOSM.

The protein intake values of 204.88, 203.10 and 203.48 were obtained in fishes fed commercial control diets, and 25% FSOSM respectively. These values were significantly different ($p<0.05$) when compared with fishes that were fed 50%, 75% and 100% FSOSM. Higher survival rates were in groups of fish fed 0, 25, 50 and commercial feed, respectively, but lower survival were in 75 and 100% fermented FSOSM (Table 4.8).

The biweekly weight increase of the experimental fish fed diets with varying inclusion levels of the fermented *Senna obtusifolia* seed meal is presented in (Fig.4.1). There was a uniform growth pattern of fish fed CF, 0% and 25% for the first three weeks while fish fed diets containing 100% FSOSM displayed the slowest growth pattern. Fish fed diet CF increased in weight above the other treatments between weeks four to the end of the feeding period. This was closely followed by fish fed diet 0% and 25% while fish fed diet 50-100% lagged behind others after the fourth week.

The results of the trend analysis of the experimental fish fed diets with varying inclusion levels of the fermented *Senna obtusifolia* seed meal is presented in (Fig.4.2). It showed that the optimum inclusion of FSOSM was at 25% with final mean weight gain of 297.62g, this was then followed by a sharp drop in growth as the inclusion of FSOSM increased from 50-100%

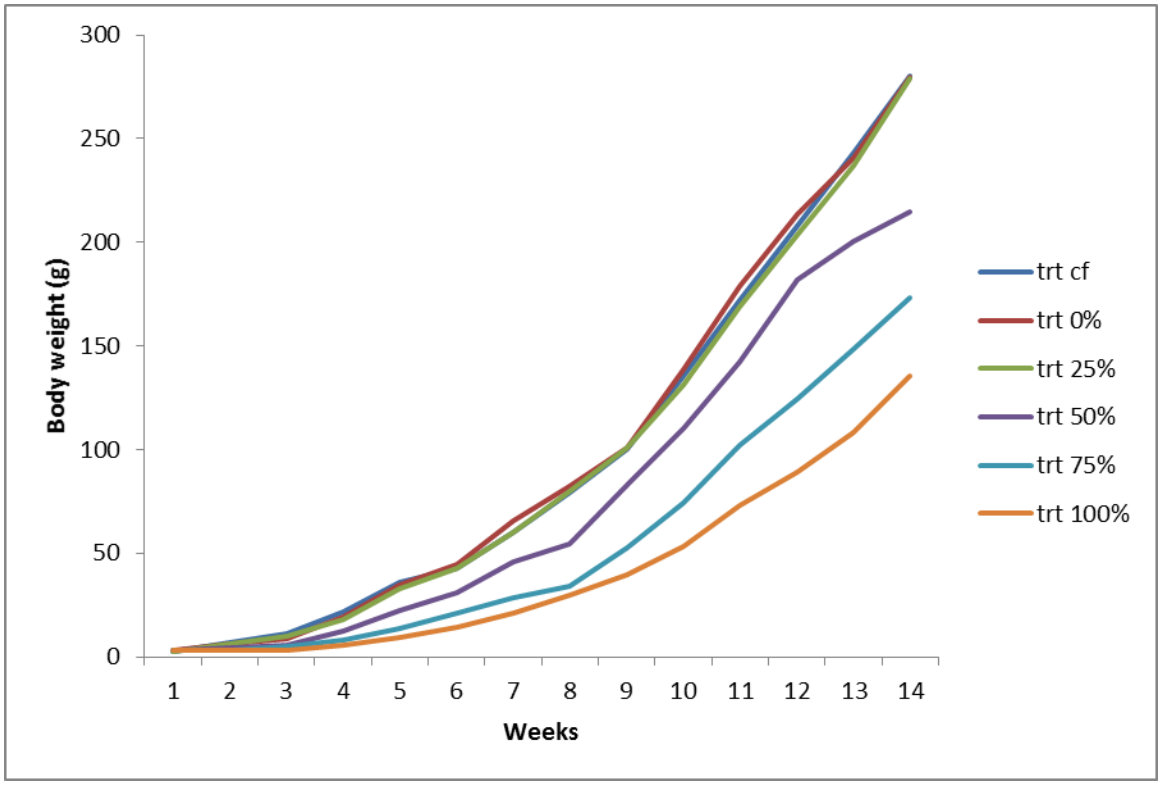


Figure 4.1 Biweekly growth pattern of *Clarias gariepinus* fed graded levels of fermented *Senna obtusifolia* seed meal

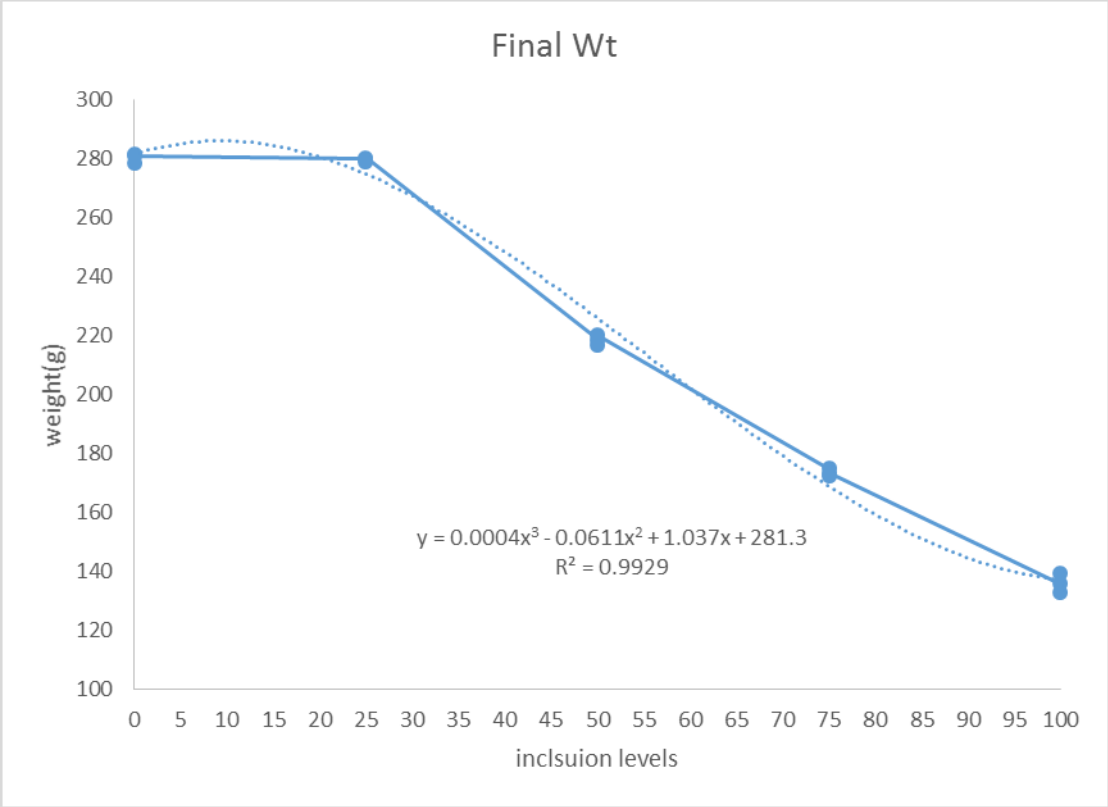


Figure 4.2: Trend line showing the optimum inclusion of fermented Senna obtusifolia seed meal

4.9. Carcass Composition

The carcass composition of *Clarias gariepinus* fed the experimental diets is shown in Table 4.9. The initial carcass compositions of fish were 7.41, 63.27, 5.25, 8.64, and 14.91% for moisture, protein, lipid, ash and NFE respectively. Moisture contents of fish at the end of experiment were 6.51, 6.34, 6.34, 8.76, 9.84% and 10.48% respectively for fish fed, commercial feeds (CF), 0, 25, 50, 75 and 100% fermented SOSM. There were a significant difference ($p<0.05$) in the initial and final carcass composition and among treatments.

The protein contents of the fish carcass were 67.71, 67.41, 67.21, 66.25, 65.52, and 65.18% respectively for fish fed commercial feeds, 0, 25, 50, 75% and 100% fermented SOSM. All treatments had higher lipid contents than the initial lipid composition.

There was no significant variation ($p>0.05$) in the carcass lipid of the CF and control but a significant difference ($p<0.05$) occur in the carcass lipids of the fish fed 25% FSOSM and the control which is significantly higher ($p<0.05$) than the other treatments.

The ash content was highest in fish fed 100% fermented SOSM (12.50±0.86%) but there were no significant differences ($P>0.05$) in fish fed diets containing 50 and 75% FSOSM, the ash content 9.51% in fish fed CF was not significantly different ($p> 0.05$) from fish fed 0 and 25% inclusion of FSOSM. The highest ash content was recorded in fish fed 100% FSOSM but there was no significant difference ($P> 0.05$) in fish fed 50 and 75% FSOSM.

Table 4.9: Carcass compositions of *Clarias gariepinus* fed graded levels of Fermented *Senna obtusifolia* seed meal

Indices	Initial	CF	0%	25%	50%	75%	100%	SEM
Moisture	7.41^{d}	6.51^{e}	6.34^{e}	6.34^{e}	8.76^{c}	9.85^{b}	10.48^{a}	±0.36
Crude protein	63.27^{e}	67.71^{a}	67.41^{ab}	67.21^{b}	66.25^{c}	65.52^{d}	65.18^{d}	±0.33
Lipids	5.25^{e}	7.81^{a}	7.81^{a}	7.68^{b}	6.46^{c}	5.98^{d}	5.84^{d}	±0.22
Ash	8.64^{c}	9.51^{b}	9.67^{b}	9.63^{b}	12.35^{a}	12.48^{a}	12.50^{a}	±0.35
NFE	14.91^{a}	8.88^{d}	9.13^{c}	9.24^{b}	6.77^{e}	6.41^{f}	6.08^{g}	±0.63

Means in the same row with different superscripts differed significantly ($p<0.05$)

CF: Commercial Feeds

4.10. Proximate Composition of the Experimental Diet and Concentration of Chromic Oxide

Table 4.10 shows the proximate composition of the experimental diet and concentration of chromic oxide. The diets are isonitrogenous and isocaloricas there was no significant difference ($p>0.05$) in the protein and carbohydrates contents. There was no significant difference ($p>0.05$) in the chromic oxide concentration in the test diets.

4.11 Proximate Composition of the Faecal Sample

Table 4.11 shows the proximate composition of the feacal samples of the fish fed the control and the test diets. There was no significant difference ($p>0.05$) in the protein content of the control and the$FSOSM_{25.}$Significant difference ($p<0.05$) was recorded in the protein content of feacal matter as the inclusion levels of FSOSM in the feed increased. The crude lipid contents of the feacal samples of the fish fed the control diets was not significantly different ($p> 0.05$) from 0% and 25%, but there was significant difference ($p< 0.05$) from 50%,75% and 100%.

There was a significant difference ($p< 0.05$) in the crude fibre contents of the feaces of the fish fed the control and the other diets. There was also no significant difference ($p>0.05$) in the nitrogen free extract (NFE) contents of the feaces of the fish fed the 25% inclusion of FSOSM, while significant difference ($p<0.05$) was obtained in the fish fed diets 50%,75% and 100% inclusion of FSOSM respectively. The highest chromic oxide concentration of 1.415-1.416 in the feaces was recorded in the feacal samples of fish fed the commercial feed, control and 25% FSOSM inclusion while the lowest was in the feacal samples of fish fed 100% FSOSM. There was significant difference ($p< 0.05$) among the chromic oxide concentration of the feaces of fish fed control diets and test

diets but this variation lacked definite pattern, which is not related to the level of inclusion of FSOSM.

Table 4.10: Proximate Composition of Experimental Diets and Concentration of Chromic oxide

Composition	CF	0%	25%	50%	75%	100%	SEM
Crude protein	42.48^{a}	42.16^{a}	42.15^{a}	42.22^{a}	42.13^{a}	42.24^{a}	±0.05
Moisture	7.81^{c}	7.37^{e}	7.47^{d}	7.89^{bc}	8.37^{a}	7.91^{b}	±0.08
Crude lipid	14.67^{a}	11.47^{b}	11.46^{b}	11.69^{b}	11.82^{b}	11.79^{b}	±0.27
Crude fibre	2.80^{d}	4.91^{a}	4.65^{b}	4.56^{b}	4.40^{c}	4.89^{a}	±0.18
Ash	6.70^{d}	8.21^{b}	8.30^{b}	8.44^{a}	8.18^{c}	8.19^{c}	±0.14
NFE	25.52	25.57	25.85	25.13	25.14	25.98	±0.10
Dry matter	92.47^{a}	92.62^{a}	92.51^{a}	92.25^{a}	91.72^{ab}	92.12^{b}	±0.10
Chromic oxide	0.436 ^{a}	0.436 ^{a}	0.437 ^{a}	0.439 ^{a}	0.439 ^{a}	0.439 ^{a}	±0.00

Means on the same row having the same superscript are not significantly different ($p>0.05$)
CF: Commercial feeds

Table 4.11: Proximate composition of feacal samples of *Clarias gariepinus* fed the experimental diets

Composition	CF	0%	25%	50%	75%	100%	SEM
Crude protein	15.00^{e}	16.04^{d}	16.05^{d}	19.41^{c}	22.36^{b}	24.14^{a}	±0.83
Moisture	10.54^{a}	9.68^{b}	9.38^{b}	9.12^{c}	9.04^{d}	7.81^{e}	±0.22
Crude lipid	6.29^{c}	6.32^{c}	6.34^{c}	6.98^{b}	7.75^{a}	7.49^{ab}	±0.15
Crude fibre	7.82^{cd}	7.38^{ab}	7.47^{bc}	7.89^{a}	7.8^{ab}	7.0^{6d}	±0.08
Ash	11.52^{a}	11.61^{a}	11.53^{a}	6.44^{b}	5.26^{c}	5.38^{c}	±0.72
NFE	48.91^{b}	48.90^{b}	48.51^{bc}	49.50^{a}	48.07^{c}	48.03^{c}	±0.14
Dry matter	89.47^{d}	90.32^{c}	90.62^{c}	90.88^{b}	90.96^{b}	92.19^{a}	±0.21
Chromium	1.417^{a}	1.415^{a}	1.416^{a}	1.218^{b}	1.112^{c}	1.104^{d}	±0.03

Figures on the same row having the same superscript are not significantly different ($p>0.05$)
NFE: Nitrogen free extracts, CF: Commercial feeds.

4.12: Apparent Digestibility Coefficient of the Experimental Diets Fed to *Clarias gariepinus*

Table 4.12 presents the apparent digestibility coefficient of the experimental diets fed to *C. gariepinus* fingerlings. There was no significant difference ($p>0.05$) in the apparent digestibility coefficient of protein of the control and fish fed $FSOSM_{25}$. There was significant difference ($p<0.05$) in the apparent digestibility coefficient of protein as the inclusion levels of FSOSM in the diets increases. There was also no significant difference ($p>0.05$) in the apparent digestibility coefficient of lipids, dry matter and carbohydrates of the control and fish fed 25% FSOSM, but significant difference ($p<0.05$) was obtained in the apparent digestibility coefficient of lipids, dry matter and carbohydrates as the inclusion levels of FSOSM in the diets increased.

4.13. Haematological Effects of Feeding *C. gariepinus* with Graded Levels of Fermented *S. Obtusifolia* Seed Meal

Table 4.13 shows the haematological effects of feeding *Clarias gariepinus* graded levels of fermented *Senna obtusifolia* seed meal.The packed cell volume (PCV) of fishes fed the commercial, control and 25% FSOSM had higher PCV in comparison with those fed diet containing 50% to 100% FSOSM. The fishes fed 25% FSOSM were not significantly different ($p>0.05$) from those that were fed the control diet. The white blood cell (WBC) increased as FSOSM increased in the diet. The WBC of fish group that fed on control and 25% FSOSM were significantly different ($p<0.05$) from those that fed on 50%, 75% and 100% FSOSM. The red blood cells (RBC) decreased as the level of FSOSM increased in the diet. The commercial feed (CF) and diets containing 25% FSOSM had the highest RBC value of 3.23 and 3.10 which were not statistically significant ($p>0.05$) from the value of 3.09 obtained in fish fed diet containing 0% FSOSM. Fishes fed diet containing

Table 4.12: Apparent digestibility coefficient of the feed fed to *Clarias gariepinus*

Indices	CF	0%	25%	50%	75%	100%	SEM
APD	88.85^{a}	88.53^{b}	88.48^{b}	83.25^{c}	79.18^{d}	77.20^{e}	±1.14
ALD	87.38^{a}	82.92^{b}	82.62^{b}	78.81^{c}	74.50^{d}	74.30^{d}	±1.13
ADD	70.58^{a}	69.60^{ab}	69.87^{b}	64.28^{c}	60.55^{d}	60.15^{d}	±1.07
ACD	41.23^{a}	41.29^{a}	41.59^{a}	28.72^{b}	24.54^{c}	23.48^{d}	±2.00

Means on the same row having the same superscript are not significantly different ($p>0.05$)
CF: Commercial feeds
APD: Apparent protein digestibility,
ALD: Apparent lipids digestibility,
ADD: Apparent dry matter digestibility
ACD: Apparent carbohydrates digestibility

Table 4.13: Haematological indices of *Clarias gariepinus* fed graded levels of fermented *Senna obtusifolia* seed meal

Composition	CF	0%	25%	50%	75%	100%	SEM
PCV	38.95^{a}	38.90^{a}	38.20^{a}	34.15^{c}	32.28^{d}	30.32^{e}	±0.83
WBC	4.45^{c}	4.35^{c}	4.38^{c}	4.87^{b}	5.09^{a}	5.10^{a}	±0.22
RBC($10^{6}mm^{3)}$	3.10^{a}	3.09^{a}	3.23^{a}	2.59^{b}	2.23^{c}	1.90^{d}	±0.15
Hb (gdl^{-1})	10.87^{a}	10.72^{a}	10.96^{a}	9.60^{b}	7.98^{c}	7.90^{c}	±0.08
MCHC(gdl^{-1})	27.54^{b}	26.71^{cd}	28.35^{a}	27.33^{bc}	24.81^{e}	26.02^{d}	±0.72
MCH(gdl^{-1})	34.71^{c}	34.21^{c}	36.14^{b}	33.18^{d}	36.54^{b}	42.52^{a}	±0.14
MCV	126.06^{d}	126.17^{d}	125.96^{d}	138.30^{c}	147.16^{b}	162.43^{a}	±0.21
LYM (%)	92.00^{d}	90.00^{c}	90.84^{cd}	93.15^{b}	96.22^{a}	98.00^{a}	±0.03

Means on the same row with the same superscript are not significantly different ($p>0.05$)
PCV: Packed Cell Volume
WBC: White Blood Cell
RBC: Red Blood Cell,
HB: Haemoglobin
MCHC: Mean Corpuscular Haemoglobin Concentration
MCH: Mean Cell Haemoglobin
MCV: Mean Cell Volume
LYM: Lymphocyte

50% to 100% FSOSM had decreased RBC values. The haemoglobin (Hb) of fishes that fed on the commercial, control diets and 25% FSOSM were higher in Hb concentration than those fed diet containing 50% to 100%. The lymphocyte count increased as the level of FSOSM increased in the diet. The highest lymphocyte count of 98.00% was observed in fish fed diet containing 100% FSOSM and lowest value of 90.00% was in the fish fed the control diets. The mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) increased with an increase in FSOSM in the diet.

4.14. Economic Viability of Using Fermented *Senna obtusifolia* Seed Meal

Table 4.14 presents the economic viability of using fermented *Senna obtusifolia* seed meal. Amongst the experimental feeds the commercial diet was most expensive (₦360/kg), while the diet containing 100% FSOSM was the cheapest. The cost of feeding reduced progressively as the inclusion of FSOSM increased. The mean cost of fingerling and feeding for the 24 weeks was ₦195.4 in control diet, ₦121.79 in diets containing in 0% FSOSM, ₦118.49 in 25% FSOSM, ₦107.19 in 50% FSOSM, ₦87.36 in 75% FSOSM and ₦70.26 in 100% FSOSM. The benefit cost analysis indicated that the cost of production of the control commercial diet (₦360) and diet containing 0% FSOSM (₦210.65) had the highest cost throughout the experimental period, while the diet containing 100% FSOSM had the least. The mean sales value (₦196.23) was highest in commercial feed and lowest (₦94) in diets 100% FSOSM. The benefit cost ratio (1.65:1) was highest in diets containing 25% FSOSM and lowest (1:1) in commercial diets.

Table 4.14: **Economic Viability of fermented SOSM in the diets of *Clarias gariepinus***

Parameters	**CF**	**0%**	**25%**	**50%**	**75%**	**100%**
Cost of feed(₦)/Kg	360	210.65	203.36	195.30	181.46	180
Mean feed intake(g)	487.23^{a}	483.20^{b}	484.31^{b}	446.46^{c}	371.19^{d}	279.23^{e}
Mean feeding cost(₦)	175.40^{a}	101.79^{b}	98.49^{c}	87.19^{d}	67.36^{e}	50.26^{f}
Cost of fingerling(₦)	20	20	20	20	20	20
Total expenses(₦)	195.40	121.79^{b}	118.49^{c}	107.19^{d}	87.36^{e}	70.26^{f}
Mean final weight(g)	280.33^{a}	279.68^{a}	279.29^{a}	218.54^{b}	173^{c}	135.6^{d}
Cost of fish (₦)/Kg	700	700	700	700	700	700
Mean yield cost(₦)	196.23^{a}	195.78^{a}	195.50^{a}	152.98^{b}	121.1^{c}	94.92^{d}
Net profit	0.83^{f}	73.99^{b}	77.01^{a}	45.79^{c}	33.74^{d}	24.66^{e}
Benefit cost ratio	1.00^{e}	1.61^{b}	1.65^{a}	1.43^{c}	1.43^{c}	1.34^{d}
Incident cost	0.63^{a}	0.37^{d}	0.36^{d}	0.40^{b}	0.38^{c}	0.38^{c}

CF: Commercial Feeds

4.15. Gross Margin and Net Return Analysis of *Clarias gariepinus* Production

Table 4.15 presents the gross margin and net return analysis of *Clarias gariepinus* production fed the experimental diets. The mean variable total cost (TVC) of the commercial feed was the highest at ₦175.40, while the least was at the fish fed 100% inclusion of FSOSM. The total cost of production ₦195.40 was also highest in the fish fed the commercial feed and the least was at the fish fed 100% inclusion of FSOSM. However, the net return on investment was highest at the fish fed the 25% inclusion while the least was at the fish fed the commercial diets.

The average total cost per kg of fish produced was ₦116.75, while the average total revenue per kilogram of fish raised was ₦159.42. The average value of gross margin was ₦62.67while the net revenue was ₦42.67.

Table 4.15: Gross Margin and Net Return Analysis of *Clarias gariepinus* production.

Parameters	**Cf**	**0%**	**25%**	**50%**	**75%**	**100%**	**Total**
Mean TVC (₦/kg)	175.4	101.79	98.3	87.19	67.36	50.26	96.72
Cost of fingerlings (₦/kg)	20	20	20	20	20	20	20
Total cost(₦/kg)	195.40	121.79	118.49	107.19	87.36	70.26	116.75
Total revenue(₦/kg)	196.23	195.78	195.50	152.98	121.1	94.92	159.42
Gross margin(₦/kg)	20.83	93.99	97.01	65.79	53.74	44.66	62.67
Net return(₦/kg)	0.83	73.99	77.01	45.79	33.74	24.66	42.67

TVC: Total Variable Cost
CF: Commercial feeds

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effects of Different Processing Methods on Nutrients Composition of *Senna obtusifolia*

Amongst the processing methods employed fermentation of *S. obtusifolia* seeds resulted in a higher increase in crude protein and free amino acids, decrease in crude lipid, crude fiber, and least anti-nutritional compounds in comparison to toasting and boiling. For instance, the crude protein content of fermented *Senna obtusifolia* seed meals (FSOSM) was significantly higher than that of the boiled, toasted and raw samples. The increase in nutrient composition of FSOSM could be attributed to the biochemical activities of micro-organism that is responsible for the fermentation process and the release of nutrients. Higher quantity of proximate composition and reduction in anti-nutritional compounds in leaves, seeds and fruits during fermentation has been associated with microbial and biochemical activities Yigzaw *et al*. (2004).

As fermentation days increased, crude protein increased progressively which may confer nutritional advantage on the FSOSM. This increase in protein value through fermentation compare favourably with the values reported for beni seed (Oshodi *et al*., 1999). Increase in protein value with fermentation time has been attributed to net synthesis of protein which results in the production of some amino acids during protein synthesis (Marero *et al*., 1989; Uwagbute *et al*., 2000). The high protein contents could also be attributed to the ability of the micro-organism to secret some extra cellular enzymes which further degraded the pulverised seeds during fermentation (Oseni and Ekperingin, 2007).

Boiling and toasting processes reduced the crude protein of *S. obtusifolia* seeds because as the boiling and toasting time increases progressively, leaching and destruction of nutrients also increased. Heating leads to Maillard reactions and subsequent Amadori rearrangements making the protein and its amino acids significantly unavailable (Olaofe *et al*., 1994). Similar reduction in crude protein with increasing toasting time had been observed for fluted pumpkin seeds, boiled soybean and mung bean (Kylen and McCready, 1975; Fagbemi, 2007).

Fermentation from 48hrs to 96hrs decreased total carbohydrate in SOSM considerably. The apparent decrease in carbohydrate could be attributed to increase activity of amylolytic enzymes which hydrolyze starch and other complex carbohydrates to simple sugars. The sugars then provide energy for the fermenting micro-organisms and carbon skeleton for possible synthesis of other compounds thereby increasing nutrient availability (Kazanas and Fields, 1981). Similar findings were reported by Achinewhu and Isichei (1990), Nnam (1995) and Onweluzo (2009) on fermented fluted pumpkin seeds, cowpea, millet and pigeon pea respectively. In this study, the carbohydrate contents of the toasted and boiled seeds of SOSM increased, this is associated with softening and breaking down of the cellulose making the starch more available (Agianga *et al*., 2010). Fermentation reduces the fibre content of the seed due to activities of hydrolyzing enzymes such as cellulase, and α-galactosidase produced by the micro-organisms leading to the conversion of insoluble fibre to soluble dietary fibre which are used as a source of energy for cellular activities by micro-organisms (Chandrasekara and Shahidi, 2012). Chitra *et al*. (1996) also reported the reduction in dietary fibre contents of

fermented chick pea from 161.1 to 82.4g/kg after fermentation. Similar results were also reported by Oseni *et al*. (2011) when *Jatropha curcas* seeds were fermented.

Boiling and toasting processes was found to increase the fibre contents these increase might be due to conversion of insoluble dietary fibre to short length chains or units (Yuan *et al*., 2005). High fibre contents have been found to bind trace elements, thereby preventing their absorption and consequently leading to deficiencies of iron and zinc (Siddhuraju *et al*., 1996).

The decrease in lipid content of the fermented seed when compared to the raw sample in this study might be attributed to the increased activities of the lipolytic enzymes during fermentation which hydrolyses lipids into fatty acids and glycerol (Chinma *et al*., 2009). Fermentation significantly decreased oil content of African oil bean seed and this was attributed to the production of glycerol, one of the hydrolytic products of lipids, which is not fat soluble (Landers and Rathmann, 1981; Akubor and Chukwu, 1999). The high lipid content of the boiled and toasted seeds could also be linked to the disruption of the cell structures and the testa of the seeds by heat during boiling and toasting causing the fats to melt and be released (Adegunwa *et al*., 2012)

The decrease in ash content of fermented *Senna obtusifolia* seed meal (FSOSM) could be due to losses of dry matter and volatile materials which normally occur during fermentation in addition to destruction of organic matter during incineration at high temperature (Nnam and Obiakor, 2003). Similarly the reduction in the ash content as boiling durations increased could be attributed to leaching of the mineral elements into the water (Onu and Okongwu 2006; Fasasi, 2009).

5.2 Effects of Different Processing Methods on the Anti-nutrient of *Senna obtusifolia* seed

A significant reduction in phytic acid composition with processing time in the fermented samples when compared with raw samples might be associated with hydrolysis of phytate via the action of microbial phytase enzymes to lower inositol phosphates (Sandberg, 1991; Hurrell, 2004). Microbial phytases originate either from the micro flora on the surface of cereals and legumes or from a starter culture inoculate (Sandberg, 1991). Consumption of fermented food has been shown to improve the intestinal balance of beneficial lactic acid bacteria, and the consumption of fermented foods may be another effective way to reduce the potential of dietary phytic acid impairing mineral absorption (Vin, 2009).

Tannin in fermented (96hr) sample was lower than in other treated samples. This suggests that fermentation reduce the level of tannin in the *Senna obtusifolia* seed sample. Tannins have the capability of decreasing the digestibility and palatability of proteins because they form insoluble complexes (Osagie *et al*., 1996). Obizoba and Atii (1991) reported that tannin-protein, tannic acid-starch and tannin-iron complexes are broken down during fermentation to release free nutrients. Since tannins are known to reduce the availability of proteins, carbohydrates and minerals through the formation of indigestible complexes, breakdown of such complexes by fermentation may improve the availability of the nutrients.

The lowest cyanide level as observed could be due to hydrolytic activities of enzymes by the fermenting microorganisms (Montgomery, 1980). Endogenous hydrolytic enzymes that occur in cyanophoric tissues may also have contributed to the hydrolysis of the

cyanogens into non-toxic sugar and cyanohydrins. Cyanide can also be reduced by boiling and fermentation as a result of alkaline condition of the reaction which breaks down cyanohydrin to hydrogen cyanide which in turn is readily reduced by soaking and fermentation (Liener, 1989).

A high reduction in oxalate composition of *Senna obtusifolia* with processing time in comparison with the raw sample was observed in this study. Fermentation caused reduction of 100% oxalate compared with vigorous toasting and boiling.

There is a reduction in the level of saponins in the samples with increasing processing time. For instance, toasting for 10 minutes significantly reduced or degraded the amount of saponins in comparison with the raw sample. Saponins are heterogenous group of naturally occurring foam producing triterpenes or steroidal glycosides that occur in a wide range of plants, including pulses and oilseeds such as kidney beans, chickpea, soybean, groundnut and sunflower (Leiner, 1980; and Jenkins and Atwal, 1994).

5.3 Amino Acid Composition of Differently Processed *Senna obtusifolia* Seeds

Leucine is the most concentrated essential amino acid while glutamic and aspartic acids were the most concentrated non-essential amino acids in the raw seeds of *Senna obtusifolia*. The values of glutamic and aspartic acids made up to 9.79g/100g protein as the most abundant amino acids in seed sample; similar results have been reported by other workers (Olaofe *et al*., 1994; Oshodi *et al*.,1998; Adeyeye,2004). Boiling and toasting reduced the concentration of most the essential amino acids. The limiting amino acids were found to be the sulfur amino acids (methionine + cystine). The lysine and methionine contents of the raw *Senna obtusifolia* seed meal is far lower than protein

required for nutrition of finfish (FAO/WHO,1990) hence, this shows that there will be need for supplementation of the meal containing *S. obtusifolia* seed with lysine and methionine.

The total sulphur amino acids (TSAA) for the processing method is lower than the 3.2g/100gcp recommended for *Clarias gariepinus* by Fagbenro *et al*. (1998) therefore there is the need to supplement the diet with methionine and cystine when using the processed seeds to feed *Clarias gariepinus*. Nutritive value of a protein depends primarily on its capacity to satisfy the needs for nitrogen and essential amino acids (Oshodi *et al*., 1998). The reduction in the concentration of amino acids in the boiled and toasted seed could be associated with denaturing at high temperature which may lead to loss of toxic substances, particularly of the proteinaceous toxins such as trypsin inhibitors and haemaglutinins (Liener, 1980) and as heating progress, protein quality decreases and consequently reduce due to maillard browning reaction which cause lysine to reduce or lost completely (Aremu *et al*., 2010).

Comparatively, the total values of essential and non-essential amino acid profiles of the toasted and boiled samples were lower than the raw sample, but the fermented sample was slightly higher. The increased amino acids of fermented *S. obtusifolia* seed meal sample could be attributed to one of the catabolic process which involves the breakdown of proteins into amines and amino acids during fermentation. Trans-amination and deamination reactions might be responsible for the slight changes in the amino acids of the raw and processed seeds of *Senna obtusifolia* seed meal (Aremu *et al*., 2009).

Heating (boiling and toasting) reduces some of the amino acid compositions of the seeds. These reductive effects of boiling and toasting on protein and amino acid compositions of the seeds may be attributed to Amadori rearrangements which led to the production of glucose phenylosazone from glucose phenylhydrazone as a result of high and prolonged heat treatment (Olaofe *et al*., 1994).

5.4 Effects of Different Processing Methods on the Mineral Contents of *S. obtusifolia* Seeds

The increase in mineral contents as fermentation progressed may be due to breakages in bond as catabolism progressed. Potassium was the predominant macro mineral, then calcium, phosphorus and magnesium in *S. obtusifolia*, and micro elements such as iron and zinc are present in low concentrations. The minerals in raw and fermented *S. obtusifolia* seeds were quite higher than in raw coconut (*Cocus nucifera*) seed flour and oil (Amoo, 2004). The fermented sample was therefore referred to as good source of calcium, magnesium, potassium and phosphorus. Initial soaking and boiling could have led to the decrease of some of the minerals by leaching and subsequent volatility of other elements during heating while increment of some minerals might be associated with the conversion of the insoluble reserve foods by enzymes during fermentation (Rooney *et al*., 1990).

5.5 Growth Performance and Feed Digestibility

In this study, the maximum fermentation time (96hrs) was chosen after a preliminary study of the nutrient composition of the seeds during which it was established that fermented seeds of *S. obtusifolia* for 96hrs had reduced anti-nutrient concentration and better amino acid content than boiled and toasted seeds.

Water quality parameters such as temperature, pH and dissolved oxygen recorded were within the recommended limits for warm-water fishes such as the clariids and tilapines (Boyd and Gross, 2000).The optimum growth of African catfish, *Clarias gariepinus*, requires 28-30^{o}C, 6.5-9.0 pH and not less than 5mg/L dissolved oxygen in the rearing water (Chapman, 2000).

The pelleting qualities of the diet, retention of the nutrient and fish growth have been attributed to the use of appropriate binder in fish feeds (NRC, 1993; Wilson, 1994).The low feed striking time, especially in the diet with high inclusion of fermented *Senna obtusifolia* seed meal indicates low palatability of the diets. The fact that fish fed with experimental diets gained weight shows that protein was converted to muscles, which is an indication of growth.

The fingerlings consumed all the diets but feed intake was higher in groups that received diets with lowest levels (25%) of fermented *S. obtusifolia* seed meal (FSOSM), this could be due to the presence of low molecular weight metabolites such as free amino acids (FAA) which act as feed attractants in fish rations. Free amino acids are often associated with fermentation (Erbas *et al*., 2005). The lower feed intake in the groups fed the 50%, 75% and 100% may be due to poor palatability resulting from residual anti-nutritional compounds such as tannin, saponins, hydrogen cyanide and non-starch polysaccharides that have been reported to have adverse effect the growth performance of young fish (Leenhouwers *et al*., 2009; Alegbeleye *et al*., 2011).

At the end of the experiment, there were no significant differences in growth performance and nutrient utilization between the control groups and those fed diets containing 25% fermented (SOSM). The commercial, 0% and 25% groups had the highest mean weight gain (279 to 280 g), which is significantly superior to the groups that received the 50% and 75%. The group that received 100% FSOSM had the poorest weight gain (132.72g). The higher growth performance observed in the group containing 25% FSOSM might be due to the synergistic effect of combining two biological compounds to have a single and superior effect than when applied individually. This observation is in agreement with suggestions that combination of protein sources is better than single protein source in the diet of fish (Ugwumba *et al*., 2001; Sogbesan *et al*., 2005; Sogbesan and Ugwumba, 2006a).

Similarly, the physiological mechanism in the fish may have compensated for the presence of low amount of anti-nutrients hence the superior growth of the fish fed 25% inclusion of FSOSM. The poor growth performance at the higher (50 to 100%) inclusion of FSOSM may be due to the inability of the fish to cope with the deleterious effects of the anti-nutrients. Hence lower level of inclusion of FSOSM did not affect the growth and nutrient utilization of the fish.

The lowered growth performance of fish fed higher level of inclusion of FSOSM may be attributed to various factors such as reduced bioavailability of minerals, impaired protein digestibility caused by growth inhibiting compounds and possibly formation of phytic acid-protein complexes (Francis *et al*., 2001). Similarly, the inferior growth performance of *Clarias gariepinus* fed 75 and 100% FSOSM may be attributed to inadequate protein

utilization due to residual concentration of other anti-nutrients such as alkaloids, phenols and saponins (Bressani, 1994; Francis *et al*., 2001; Adeniji *et al*., 2007). Furthermore, anti-nutrients such as saponins have also been reported to reduce pancreatic lipase activity, thus, delaying intestinal absorption of dietary fat (Putsztai, 1989; Adewolu and Adamson, 2011).

The inability of *Clarias gariepinus* to effectively utilize high dietary inclusion level of FSOSM beyond 25% could be due to low concentration of the enzyme cellulase, which is absent in most piscivores such as *Malapterurus electricus, Gymnarchus niloticus, Parachanna obscura* but available in *Tilapia* species (Fagbenro *et al*., 2005). In *Clarias gariepinus* which is an omnivore but with higher tendency towards being a carnivore the enzyme cellulase is anticipated in the gastro-intestine in appreciable quantity to effect digestion, but this did not appear to be the case in this study.

In this study, the lowest feed conversion ratio (FCR) value indicated better feed utilization by the fish, which may have accounted for better growth performance of *C. gariepinus* fed 25% FSOSM amongst other diets. Adikwu (2003), reported that the lower the FCR the better the feed utilization by the fish. This result supports the observation made by Shabbir *et al*. (2003) and Jabeen *et al*. (2004) in related studies.

The protein efficiency ratio PER values in this study exhibited significant differences in all treatments except in the control diets and the fish fed the 25% FSOSM. Protein efficiency ratio PER values increased among the experimental fish with respect to the quantity of total feed intake. The higher PER value were obtained at the control and 25%

FSOSM treatments indicates maximum utilization of nutrients in the diets in comparison with higher inclusion levels of FSOSM. Adejumo (2005), reported highest PER value at 20% inclusion level of replacing maize with fermented millet. DeSilva and Anderson (1995), reported that protein efficiency ratio is a measurement of how well the protein sources in a diet could provide the essential amino acids requirement of the fish fed. Protein efficiency ratio (PER) is also known to be regulated by the non-protein energy input of the diet and is a good measure of the protein-sparing effect of lipid and/or carbohydrate (Lie *et al*., 1989; Tibbets *et al*., 2005).

The decrease in apparent net protein utilization (ANPU) when the FSOSM in diets increased might be due to increase in non-starch polysaccharides (NSP) in the diet as the inclusion level of the FSOSM increases. Inclusion of NSPs in the diet of fish is known to reduce amino acid digestibility (Refstie *et al*., 1999). The decrease in nitrogen utilization efficiency following (NSP) rich diet is probably due to increase of Nitrogen secretion either endogenously and/or through intestinal bacteria (Leenhouwers *et al*., 2006).The control diet and inclusion of (25%) FSOSM resulted in a better ANPU compared to other treatments. It may be that the protein deposition is significantly influenced by the ratio of FSOSM and soybean meal in the diets.

Digestibility was directly related to inclusion level of fermented *Senna obtusifolia* seed meal (FSOSM).Crude protein digestibility of FSOSM at 25% inclusion in the diets was high (above 84%) in *Clarias gariepinus*, suggesting that FSOSM might be a good protein source for this species. Higher apparent digestibility coefficient for protein of FSOSM at

25% inclusion than 50%, 75%, and 100% inclusions in the diets implies that a greater amount of protein was available to the fish from the FSOSM at 25% inclusion.

In this study, higher inclusion of FSOSM in the fish diet decreased lipid digestibility. This may be associated with the increase in the non-starch polysaccharides (NSP) content, which reduces absorption of fat into the gastro intestinal tract; another possible reason could be that NSP entrap bile salts, thereby reduce their effectiveness in solubilizing fats (Krogdahl *et al*., 2003; Gatlin *et al*., 2007; Øverland *et al*., 2009).

The dry matter digestibility in this study show a decreasing trend as the inclusion of FSOSM increased in the diet. The fish fed 25% inclusion of FSOSM had better dry matter digestibility than the groups fed 50%, 75%, and 100% inclusions. The reduced dry matter digestibility may be due to the presence of non-starch polysaccharides and low carbohydrates digestibility (Lupatsch *et al*., 1997; Krogdahl *et al*., 2003).

The low carbohydrate apparent digestibility coefficient at the higher inclusion levels of the FSOSM may be attributed to the presence of non-starch polysaccharides (NSP) content and its poor digestibility by *Clarias gariepinus*, it may also be due to the reduction in transit time of the feed as they pass through the gastro intestinal tract due to the presence of dietary fibre, which is part of the carbohydrate component of plant ingredients and most fish cannot effectively utilize it (Storebakken *et al*., 1999; Eusebio *et al*., 2004). These results demonstrates that *C. gariepinus* were efficient in digesting protein, dry matter and lipid from the FSOSM at 25% replacement level but poor in

digesting carbohydrates, while fish fed at higher level of replacement of soybean meal in general exhibited lower digestibility.

Generally, the minimum average weight gain (135.81g) and maximum average weight gain (278.02g) for all the treatments at the end of the six(6) months feeding trials in this study were generally lower than 386g mean body weight of *Clarias gariepinus* reared in a flow through system in a (6) months culture period (Jacob,1998).Observation from this study showed that some shooters in control and 25% inclusion of *S. obstusifolia* seed meal had up to 650g while the greater part of the population weighed less than 300g. Those that were bigger will obviously dominate the smaller ones. This heterogeneity of growth and social dominance based on size has also been observed in other fish species such as *Heterobranchus longifilis* (Ewa-Oboho and Enyenihi, 1999) and *Clarias batrachus* (Sahoo *et al*., 2004).

Similarly, lower mean weight gain of the cultured fish may also be due to inadequate acquisition of food by the smaller sized fish, as a result of differential growth and social dominance of the larger sized fish and or inherent differences in feeding behaviour of the fish (Valente *et al*., 2001; Sundström *et al*., 2003; Martins *et al*., 2006).

Lower mean weights in this study, may also be related to possible effect of inbreeding brought about by repeated use of parent stock of fish of the same lineage (Dunham, *et al*., 2001); other reasons could be due to inherent difference in genetic expression (Sundström *et al*., 2003); slow physiological response of the fish to the feeds (Fagbenro *et al*., 2010); stress due to periodic handling (Conte, 2004) and confinement stress and subsequent inability to swim in a natural fashion (Chizinski *et al*., 2010).

5.6 Carcass composition of *Clarias gariepinus*

The carcass composition of the experimental fishes showed that all the fishes fed the experimental diets had higher carcass protein, lipid and ash contents but lower nitrogen free extract content than the initial fish sample. These values are however, similar for fish fed the commercial diets and those fed 25% inclusion of FSOSM. This shows that there was protein synthesis and increased tissue production in *C. gariepinus* and that fish growth was not due to the increase in weight alone (Koven *et al*., 2001; Fountolaki *et al*., 2003). The moderately high level of carcass fat in fish fed the control and diets with 25% inclusion of FSOSM indicated an enhanced production of lipids in the fish (Udo *et al*., 2012). Increase in lipids have been associated with increase efficiency of metabolism, similar result was also observed in gilthead bream fingerlings (Fountolaki *et al*., 2003).

Fish fed 75-100% inclusion of fermented SOSM, with the least weight gain had the lowest fat and protein content. This is likely, an indication that the fish is in a poor condition having consumed feed with a higher level of residual metabolites. A similar observation of low weight and fat contents had been made in catfish fed cotton seed meal diet containing 671mg of free gossypol/kg (Barros *et al*., 2000)

Similarly, fish fed diets higher in fermented SOSM had higher body moisture content and low lipid. The decrease in lipid content is probably due to poor feed intake which resulted in starvation and in turn led to mobilization of body lipid reserves to meet energy requirements for vital body functions. Similar results have been reported in *Clarias lazera* (Hogendoorn, 1983) and rainbow trout (Reinitz, 1983). The presence of residual saponin and other growth inhibiting compounds may also have contributed to inhibited

pancreatic lipase activity and hence delayed intestinal absorption of dietary fat (Han *et al*., 2000). Carp fed diets containing high amounts of plant proteins such as mustard oilcake, linseed and sesame meal had high body moisture and low lipid (Hossain, 1988; Hossain and Jauncey, 1989). The increase in ash with increased FSOSM inclusion in this study may be linked to starvation. A similar increase in ash content was observed in *Oreochromis mossambicus* after 60 days of starvation (Pandian and Raghuraman, 1972).

5.7. Haematological Response of *Clarias gariepinus*

The haematological parameters of fish in this study were within the recommended physiological ranges reported for *Clarias gariepinus*. The packed cell volume (PCV), red blood cells (RBC) and haemoglobin (Hb) reduced as the level of fermented *S. obtusifolia* seed meal (SOSM) increased in the diet. The packed cell volume PCV of fish in this study is within the range of 20–50% reported by Pietse *et al*. (1981). Reduction in the concentration of the PCV in the blood usually suggests the presence of toxic factor which has adverse effect on blood formation (Oyawoye and Ogunkunle, 1998).

RBC range ($1.90 \times 10^6 mm^{-3}$-$3.23 \times 10^6 mm^{-3}$) in this study is fairly comparable with milk fish ($1.70 \times 10^6 mm^{-3}$-$4.00 \times 10^6 mm^{-3}$) (Bhasker and Rao, 1990) and *Clarias gariepinus* ($2.24 \times 10^6 mm^{-3}$-$2.49 \times 10^6 mm^{-3}$) (Sotolu and Faturoti, 2009). However, the decrease in RBC may be attributed to the higher concentration of residual anti-metabolites in the diets containing higher percentages of fermented *S. obtusifolia* seed meal.

Haemoglobin (Hb) range (7.90-10.96g/100ml) compared well with the value (8.70g/100ml) for *Clarias gariepinus* (Sowunmi, 2003). These values were also higher than (4.46g/100ml) reported for *Heterotis niloticus* (Fagbenro *et al.,* 2000). The increase in the red blood cell and haemoglobin concentration in fish fed lower levels of fermented SOSM may be attributed to the increase in the size of the fish as a result of growth and metabolic activities. Conversely, haemoglobin contents and erythrocyte counts tend to increase with length and age of the fish. The reduction in the Hb concentration in fish fed diets having higher substitutions levels of FSOSM contained lower quality protein, due to residual metabolites such as the anti-nutritional compounds which probably affect blood production. Previous studies have also reported reduced blood hematocrit and haemoglobin in fish fed diets rich in plant protein sources (Blom *et al*., 2001; Yildirim *et al*., 2003; Pham *et al*., 2007; Lim and Lee, 2008, 2009). It has been reported that hematocrit and haemoglobin values vary according to deficiency of essential nutrients, environmental conditions, growth status, and anti-nutritional factors (Garrido *et al*., 1990; Lim and Lee, 2009).

White blood cells (WBC) and lymphocytes count increased as the level of fermented SOSM increased in the diets. High WBC count is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system (Oyawoye and Ogunkunle, 1998). The increase in WBC and lymphocytes as fermented SOSM increased in the diet could be attributed to residual anti-nutrients present in the feed, and also increase in leucopoiesis as a means of combating stressors in the body system of the fish, the primary consequence in stressed fish is suppression of the immune system and

increased susceptibility to disease (Wedemeyer *et al*., 1974; Ezeri, 2001 and Gabriel *et al.,* 2004).

The mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) of fish in this study are at high values in fishes fed with 100% fermented SOSM and are comparable with value ranges reported by previous workers (Adedeji and Adegbile, 2011 and Anyanwu *et al*., 2011). Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) are useful in the diagnosis of anaemia in most animals (Cole, 1986). The observed lower haemoglobin levels and increased MCV, MCH and MCHC as the inclusion level of FSOSM increased in this study may be due to abnormal maturation of the red blood cell (Waagbø, 1999).

5.8 Economic Viability of Fermented *Senna obtusifolia* in Diets of *C. gariepinus*

The total cost of producing fermented SOSM and purchasing other ingredients progressively reduced from N210.65/kg to N180.00/kg in control to 100% fermented SOSM respectively. This indicates a reduction in the cost of diets of *Clarias gariepinus* fed with alternative plant sources such as *Senna obtusifolia*. For instance Abu *et al*. (2010) found a reduction in the cost of the diets of *C. gariepinus* when cassava root meal was used to replace maize meal. Similarly, Alegbeleye *et al*. (2011) reported reduction in the cost of feed when *C. gariepinus* was fed fermented pigeon pea meals at various replacement levels of soybean meal.

Value of fish (₦/kg) was significantly higher in 25% SOSM compared to other inclusion levels and the control. This observation may be due to the experimental diet which was

better utilized at 25% than at other levels, due to the higher values of mean feed intake and mean weight gain. The net returns recorded was highest (₦77.01) at 25% SOSM and least (₦24.66) at 100% SOSM. The possibility of obtaining a significantly higher value of profit index at 25% inclusion level above those of the control and higher inclusion levels clearly indicates that more profit would be generated from fish produced from 25% FSOSM.

The best cost benefit and best net profit that was obtained in diet having 25% FSOSM is an indication that the economic viability of feeding *Clarias gariepinus* with the diet produces better return on investment, this shows that it is cheaper to feed the fish at 25% replacement of soybean meal with fermented *Senna obtusifolia* seed meal (FSOSM) in the diet.

The benefit cost ratio (BCR) value (1.65) shows that catfish production is profitable at 25% inclusion of FSOSM. The value (1.65) simply means that every ₦1.00 invested in the fish production will yield ₦1.62. According to Adegeye and Ditto (1982), this ratio is one of the concepts of discount method of project evaluation. As a rule of thumb, project with benefit ratio greater than one, equal to one, or less than one indicate profit break–even or loss respectively (Olagunju *et.al*.,2007). Since the ratio in the diet is above one, it is therefore considered that replacing soybean meal with fermented SOSM is profitable especially at 25% replacement level.

The gross margin was highest at 25% replacement of soybean meal with fermented SOSM. According to Olagunju *et al*. (2007) gross margin is a good measure of profitability; therefore replacement of soybean meal at 25% is highly profitable

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The various processing methods fermentation, boiling and toasting impacted some chemical changes in the nutritional composition of *Senna obtusifolia* seed meal (SOSM) and resulted in a decrease in anti-nutrients. The fermentation time (96hrs) was recommended as suitable for use after it was established that 96hrs fermentation reduced the anti-nutrients to a greater extent and had better amino acid content compared to the raw, boiled and toasted seeds. The potential for nutritional exploitation was further enhanced by the fact that these seeds would not require prolonged and expensive heat treatment prior to use.

The water qualities were within range recommended for *Clarias gariepinus*. There were no significant differences ($p \geq 0.05$) in nutrient utilization between the control groups and those fed 25% fermented SOSM inclusion. The commercial, 0% and 25% groups had the highest mean weight gain (279 to 280 g), significantly ($P<0.05$) higher to the groups that received the, 50%, 75% while the group that received 100% (SOSM) diet had the lowest mean weight gain (132.72g).

Feed conversion ratios (FCR) of all treatments ranged from 1.75 to 2.18. These values increased slightly as the levels of fermented SOSM increased from 50 to 100%. The best FCR (1.79 ± 0.02) was recorded for fish fed 25% FSOSM which indicated a superior level of utilization of the FSOSM by the fish. There was no significant difference ($p \geq 0.05$) in

the protein intake among the group of fish fed 0 and 25% FSOSM but significant difference ($p<0.05$) was established in fish fed 50, 75 and 100% FSOSM.

Protein efficiency ratio (PER) exhibited significant differences ($P<0.05$) in all treatments except the control and fish fed the 25% fermented SOSM. There were no significant differences ($P\geq0.05$) in apparent net protein utilization (ANPU) among treatments CF, 0% and 25%. The (ANPU) was highest in fish fed 0% fermented SOSM (91.6±0.04) while fish fed 75 and 100 % fermented SOSM were the least (71.4±0.02 and 73.4±0.03) respectively.

There was a significant difference ($P<0.05$) in the effect of the inclusion levels of fermented SOSM on final body carcass composition among treatments.

Protein, lipid, dry matter and carbohydrates digestibility of the experimental diets were 77-88%, 74-87%, 60-70%, and 23-41% respectively. There was no significant difference ($p\geq 0.05$) in the apparent digestibility coefficient of protein, lipid, dry matter and carbohydrates of the control and fish fed 25% fermented SOSM. However, significant variation ($p<0.05$) was obtained in the apparent digestibility coefficient of protein, lipid, dry matter and carbohydrates as the inclusion levels of fermented SOSM in the diets increased from 50-100%.

The packed cell volume (PCV) ranged from 30.05 to 39.08% , red blood cells (RBC) ranged from 1.85 x $10^6 mm^{-3}$ to 3.10 x $10^6 mm^{-3}$ and haemoglobin (Hb) ranged from 7.81 to 10.69g/100ml, and these reduced as the level of *S. obtusifolia* seed increased in the diet and are significantly different ($P<0.05$). White blood cells and lymphocyte count

increased as the level of fermented *S obtusifolia* seed meal increased in the diet and are also significantly different (P<0.05). The MCHC, MCH and MCV observed in this study recorded their highest values in fishes fed with 100% fermented *S obtusifolia* seed.

The highest benefit cost ratio and net profit (1.65 and 77.01) in fish fed 25% fermented SOSM were significantly different (p<0.05) from fish fed. Commercial feed, 0% SOSM, 50%, 75% and 100%. Fermented SOSM can replace soybean meal at 25% inclusion level, due to the highest economic benefit attained at 25% replacement level in the diet of the *C. gariepinus* fingerlings in this study.

6.2 Conclusions

Fermentation of SOSM for 96hrs reduced the anti-nutrient compounds considerably, it had better amino acid content compared to boiling and toasting, which would require prolonged heat treatment, and therefore, increase the cost of energy.

Fermented *Senna obtusifolia* seed meal can substitute Soybean meal up to 25% level in the diets of *Clarias gariepinus* without any negative effects on the growth and feed utilisation.

Fermentation of SOSM and feeding it at 25% level of inclusion had no effect (P>0.05) on apparent digestibility coefficient of protein, lipids and dry matter.

The haematological analysis revealed that 25% substitution of soybean meal with fermented *Senna obtusifolia* seed meal in *Clarias gariepinus* did not have adverse effect on the blood parameters.

As the level of inclusion of FSOSM increases in the diets, the carcass crude protein and lipids decreased while moisture content increased.

The use of fermented *Senna obtusifolia* meal at 25% replacement in feed formulations to partially replace soybean can bring about a reduction in price of fish feeds by 43.6% thereby enhancing profit margin.

6.3 Recommendations

8. It is recommended that *Senna obtusifolia* seed meal be fermented, rather than heat treated in compounding feed for *Clarias gariepinus.*
9. The inclusion of FSOSM in the diet of *C. gariepinus* should not exceed 25% level of substitution with soybean.
10. Other methods of fermentation should be employed this may further enhance the digestibility of the feed.
11. Further studies with fermented *S. obtusifolia* seed meal under natural earthen pond conditions and in re-circulating systems are recommended to elucidate growth performances of *C. gariepinus*.

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APPENDICES

Appendix 1: Analysis of variance for growth performance of *Clarias gariepinus* fed graded levels of fermented *Senna obtusifolia* seed meals

Sources of variation	df	Mean square	P. value	LOS
Initial weight	5	0.00034	0.955	NS
Final weight	5	11840.28	0.0001	**
Mean weight gain	5	11841.52	0.0001	**
Specific growth rate	5	0.0429	0.0001	**
Feed conversion ratio	5	0.0971	0.0001	**
Protein efficiency ratio	5	0.0586	0.0001	**
Apparent net protein utilization	5	318.54	0.0001	**
Survival	5	177.30	0.0001	**
Mean feed intake	5	21220.63	0.0001	**
Protein intake	5	3743.17	0.0001	**

df = degree of freedom, **NS=** Not significant, **P<0.01

Appendix 2: Analysis of variance for proximate carcass of *Clarias gariepinus* fed graded levels of fermented *Senna obtusifolia* seed meals

Sources of variation	df	Mean square	P value	LOS
Moisture	6	9.093	0.0001	**
Crude protein	6	7.372	0.0001	**
Lipid	6	3.502	0.0001	**
Ash	6	8.480	0.0001	**
Nitrogen free extract	6	27.437	0.0001	**

**P<0.01 significant

Appendix 3: Analysis of variance for experimental diets of fed to *Clarias gariepinus* with graded levels of fermented *Senna obtusifolia* seed meals

Sources of variation	df	Mean square	P value	LOS
Moisture	5	0.3857	0.0001	**
Crude protein	5	0.0518	0.5	NS
Lipid	5	7553.0	0.5	NS
Ash	5	1.2432	0.0001	**
Nitrogen free extract	5	0.3623	0.0001	**
Crude fibre	5	1.8661	0.0001	NS
Dry matter	5	0.3430	0.0029	NS
Chromium oxide	5	0.0000	0.0001	**

df = degree of freedom, **NS=** Not significant, **P<0.01

Appendix 4: Analysis of variance for apparent digestibility coefficient of *Clarias gariepinus* fed graded levels of fermented *Senna obtusifolia* seed meals

Sources of variation	df	Mean square	P value	LOS
Apparent protein digestibility	5	80.040	0.0001	**
Apparent lipid digestibility	5	78.850	0.0001	**
Apparent dry matter digestibility	5	69.391	0.0001	**
Apparent carbohydrates digestibility	5	233.59	0.0001	**

df = degree of freedom, **P<0.01

Appendix 5: Analysis of variance for haematological indices of *Clarias gariepinus* fed graded levels of fermented *Senna obtusifolia* seed meals

Sources of variation	df	Mean square	P. value	LOS
Packed cell volume	5	41.45	0.0001	**
White blood cell	5	0.4336	0.0001	**
Red blood cell	5	0.8848	0.0001	**
Haemoglobin	5	6.099	0.0001	**
Mean corpuscular haemoglobin concentration	5	4.665	0.0001	**
Mean corpuscular haemoglobin	5	33.24	0.0001	**
Mean cell volume	5	669.01	0.0001	**
Lymphocyte	5	30.09	0.0001	**

df = degree of freedom, **P<0.01



Appendix 6: *Senna obtusifolia* plant with the fresh fruits



Appendix 7: Dried seeds of *Senna obtusifolia*



Appendix 8: Overnight soaking of *Senna obtusifolia* seeds



Appendix 9: Fermented seeds of *Senna obtusifolia*



Appendix 10: Sundrying of fermented Seeds of *Senna obtusifolia*



Appendix 11: Experimental tanks with aeration system supply



Appendix 12: Five diets containing 0-100% replacement of soybean meal with *Senna obtusifolia* seed.