EFFECTS OF REPLACING SOYA BEAN SEED MEAL WITH FERMENTED *SENNA OCCIDENTALIS*SEED MEAL IN THE DIET OF *HETEROCLARIAS*

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MAY, 2019

DECLARATION

I declare that the work in the dissertation entitled "EFFECTS OF REPLACING SOYA BEAN MEAL (*GLYCINE MAX*) WITH FERMENTED COFFEE SENNA (*SENNA OCCIDENTALIS)* SEED MEAL IN THE DIET OF *HETEROCLARIAS*" has been performed by me in the Department of Biology under the supervision of Professor S. A. Abdullahi and Professor J. Auta. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree at any University or any other institution.

SAFIYYA MUHAMMAD RABIU _____ _____
P16LSBS8029 Signature Date

CERTIFICATION

This dissertationentitled “EFFECTS OF REPLACING SOYA BEAN WITH FERMENTED *SENNA OCCIDENTALIS* SEED MEAL IN THE DIET OF *HETEROCLARIAS*” by SAFIYYA MUHAMMAD RABIU meets the regulations governing the award of the Master of Science (M.Sc. Fisheries) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

An investigation of 84 days feeding trial was carried out to examine the effect of replacing the dietary soybean meal with *Senna occidentalis* seed meal on the growth performance, nutrient utilization, carcass composition, haematological indices and cost benefit of *Heteroclarias* fingerlings. Five iso-nitrogenous diets were formulated with *Senna occidentalis* seed meal replacing 100, 75, 50, 25, and 0% soya bean meal in the diets. The result shows fermented *S. occidentalis* seed had 91.58, 2.155, 14.35, 3.515, 3.64 and 29.7 g/100g dry matter, ash, crude fibre, ether extract, nitrogen free extract and crude protein, respectively while alkaloid, saponin, tannin, phytate, oxalate and flavonoids were in concentrations 5.45, 3.71, 8.50, 2.51, 5.60, 9.8 $mg/g^{-1}$with 12.10, 19.35, 7.61, 20.32, 25.12, 11.71% respectively. Fermentation significantly increased the crude protein content of the seed while the anti-nutritional were all reduced. The results showed significant difference ($p<0.05$) in weight gain, SGR, FCR and PER between treatments. The results revealed that the best diet was the control diet which gave the mean weight (337.13g), standard length gain (15.31cm), total length gain (16.19cm), specific growth rate (1.38 %days), nitrogen metabolism (910.02), survival rate (100%) and net profit (N39.47) compared to other diets. The crude protein of the fish carcass ranged between 50.37 and 55.05 g/100mg compared to the initial of 49.99 g/100mg. The result of the haematology revealed the best result for PCV (41.67%), Hb (13.87g/dl), TRBC (6.93%) and MCHC (30.00%) when the fish was fed with 0% *S. occidentalis*seedmeal. Cost of feed production decreased with increase in inclusion level of fermented *S. occidentalis* seed meal in the diets. The use of *S. occidentalis* seed meal at 0% (control diet) level of replacement gave the best. Thus the use of *S. occidentalis* seed at 0% replacement level of soya bean seed is recommended in the diet of *Heteroclarias* fingerlings.

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ABBREVIATIONS

Abbreviation	Full Meaning
AOAC	Association of Analytical Chemist
BOD	Biological oxygen demand
CM	Centimeter
$CaCl_2$	Calcuim chloride
$CUSO_4$	Copper sulphate
DO	Dissolved oxygen
DL	Deciliter
FAO	Food and Agricultural Organization
G	Gram
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HCT	Haematocrit
Kg	Kilogram
KJ	Kilo joule
$KMNO_4$	Potassium per manganate
L	Liter
M	Miter
Mg	Milligram
Ml	Milliliter
MM	Millimeter
$MnSO_4$	Manganase sulphate
N	Nitrogen
Na_2CO_3	Sodium carbonate

Na_2SO_4	Sodium sulphate
$Na_2S_20_4$` ``	Sodium dithionite or sodium hydrosulfite
NFE	Nitrogen free extract
Pg	Pico gram
pH	Hydrogen ion concentration
Ppm	Part per million
SBM	Soya bean meal
SOSM	*Senna occidentalis* seed meal
Spp	Species
TCA	Tricyclic antidepressant
WFE	World Fishing and Aquaculture
µL	Micro liter

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Fish is an important source of high quality protein in human diet, providing about 16% of the animal protein consumed by the world's population (Adewole and Olaleye, 2014). It accounts for 20% of animal protein consumed in Africa (Dulvy and Allison, 2009) and is also an important source of other nutrients such as vitamins A, B, D and E as well as calcium, iron and iodine (FAO, 2005). Fish has the highest level of easily metabolisable high quality protein, fats, vitamins, calcium, iron and essential amino acids when compared to other sources of animal protein such as poultry and beef (Ayoola, 2010).

The African catfish is the most important fish species cultured in Nigeria (Gbadamosi *et al*., 2006). It belongs to the family Clariidae and genus *Clarias* (Teugels, 1986). They are over 60 species in the genus *Clarias* found throughout Africa (WFE, 2014). The African catfish has a slender body, a flat bony head, and a broad, terminal mouth with four pairs of barbells. Its prominent berbells give it the image of cat-like whiskers (Amisah *et al*., 2009). The African catfish is an excellent species for aquaculture as it is omnivores, grows fast, and tolerates relatively poor water quality (Rad *et al*., 2003). The African catfish is the major species cultured in Nigeria because of its high growth rate, good flesh quality, tolerance to poor water quality, ability to withstand high stocking densities, and good taste (Olagunju *et al*., 2007).

Clarias gariepinus (Burchell, 1822) and *Heterobranchus bidorsalis* (Geoffroy, 1809) are of high economic importance in many countries of the world especially African and Asian continents (Adebayo and Fagbenro 2004; Olaniyi and Omitogun 2013, 2014); and also serve mainly as food

in many homes and hotels (Omitogun *et al*. 2012). Today, *Clarias gariepinus* has become more widely distributed, especially, with recent advances in Aquacultural techniques, such as balanced commercial feeds (FAO, 2012).

Heterobranchus has fast growth (Kori-Siakpere*et al.*, 2006).*Clarias gariepinus*is hardy, can tolerate adverse water quality conditions, grows fast and feeds on a large variety of Agricultural by-products, and can be raised in high densities (WFE, 2014).The African catfish (*Clarias gariepinus)* is appreciated by consumers for the quality of its meat (Pruszynski, 2003) and is mostly smoked and used in soups. It is recognised by its long dorsal and anal fins, which give it a rather eel-like appearance (Amisah *et al*., 2009).

The yearnings of farmers and scientists to have a farmed African catfish that combines the fast growth traits of *Heterobranchus* spp and early maturing traits of *C. gariepinus* led` to the development of a hybrid '*Heteroclarias*' spp (Bartley *et al*., 2000; Kori-Siakpere *et al*., 2006; Solomon and Ezigbho, 2010). These hybrids have been reported to show heterosis which makes it a very good aquacultural candidate (Nwosu *et. al*., 2009). *Heteroclarias* are in high demand by most farmers due to their hardness and fast growth. There seeds are, however, not readily available to meet the needs of large scale fish farmers because unlike tilapia, they do not readily reproduce in captivity (Nwosu *et al*., 2009).

Hybrid catfish is a good (*Heteroclarias*) is a good experimental fish in aquaculture due to its relative fast growth rate, better feed conversion efficiency, resistance to diseases, ability to tolerate harsh environmental conditions and wide acceptance by the populace (Omoruwou and Edema, 2011). The rapid increase in its market demand because of its fleshy and tasty body has

added impetus to the aquaculture sector to augment the deficit in the needed sustainable production and supply (Omoruwou and Edema, 2011).

In aquaculture, fish requires adequate food supply in the right proportions and with proper nutritional contents needed for growth, energy, reproduction, movement, and other activities (Steven and Louis, 2009; Umaru *et al*., 2016). Fish feeds in sustainable fish culture system (intensive), has been reported to account for 40-60% of the total recurrent cost of production (Steven and Louis, 2009) which to a large extent determines the viability and profitability of fish farming enterprise (Umaru *et al*., 2016). Quality fish feeds are used in aquaculture to increase production and maximize profit (Eyo, 2001). Although, few studies have compared the growth response of fish to local and imported feeds (Shapawi *et al*., 2011;Ekanem *et al*., 2012), there is adequacy of information on the comparison of growth response of catfish fed with different imported feeds (Tunde *et al*., 2016).

In order to attain more economically, sustainable, environmentally friendly and viable production, research interest has been directed towards the evaluation and use of non-conventional sources of plant protein (Dienye and Olumuji, 2014). Researchin fish nutrition is aimed at exploring alternative, cheaper protein sources for use as soya bean replacers in aqua feeds (Li *et al*., 2009). The decrease in global production of soya bean clearly demonstrates that the sustainability of this industry will depend on the sustained supply of plant proteins for aqua feeds (Hu *et al*., 2013).

The failure of aquaculture to meet the challenge of closing the widening gap between fish supply and demand in Nigeria, results from a number of factors including lack of quality fish feeds. These, however, can only promote limited growth and further growth is restricted by

insufficiency of nutrients from primary production (Edwards *et al.*, 2000). Better growth is only possible through provision of high quality feed to sustain the increased demand for quality feed (Kumar, 2000; FAO, 2012; Daniel, 2017; Eunice *et al*., 2017). According to Hecht (2007), poor financial circumstances of the farmers within sub-Saharan Africa are one of the main constaints impeding aquaculture.

Several studies have shown that vegetable protein sources have high potentials for supplying fish with required protein needed for their maximum productivity (Nwanna *et al.*, 2008). By-products of banana (Ogunsipe *et al*., 2010; Ekwe *et al*., 2011); cashew (Omosulis *et al*., 2011); Neem seed cake (Hassan *et al*., 2015) and Hassan Cassava leaf meal (Hassan *et al*., 2017) has been successfully tested in animal husbandry and fish culture.

Soya bean meal is the most important protein source used to feed farm animals. It represents two-third of the world output of protein feedstuffs, including all other major oil meals and fish meal (Oil World, 2015). Soybean meal is currently the most commonly used plant protein source in fish feeds production in Nigeria and amounts to 500 g kg-1 of the diet of freshwater omnivorous fish species (Yue and Zhou, 2009). As a result, soybean meal, both imported and locally made, is utilized with the hope to help decreasing the costs of plant feed ingredients, but as it turns out, it is also quite expensive(Yuangsoi *et al*., 2014). Herdsmen, no matter either undersized or oversized farms, are looking for a new cheaper raw material to decrease the cost of plant feed ingredientsthough, however, it might not be as preferable as soybean meal.

This new material should be able to be produced locally in Nigeria, should be inexpensive and should provide high nutrition. Certain plant materials offer the most promising alternative fish feed ingredients and in fact locally produced materials have already been used (Yuangsoi and

Masumoto, 2012).Therefore,research interest in substituting conventional sources of protein and energy with locally available ingredients have been recommended (FAO, 2006).

Many researchers have documented the beneficial effects of fermentation in improving the nutritional quality of feed ingredients (Igbalbul *et al*. 2014; Adebowale and Maliki, 2014). The fermentation process that an African woman employed was behind the dramatic improvement in the protein value of the food (Dirar, 1992). The fermentation of meals of cereals and legumes are known to increases the protein content (El Tinay *et al*., 1979).

Fish haematology is gaining increasing importance in fish culture because of its significance in monitoring the health status of fish (Hrubec *et al.*, 2000; Bahmani *et al*., 2001; De pedro*et al*., 2005). Blood parameters are an important tool for monitoring both nutritional and health status of fish (Hlophe and Mayo, 2014). Haematological characteristics of most fish have been studied with the aim of establishing normal value range and deviation from it may indicate a disturbance in the physiological process (Rainza Paiva *et al*., 2000). Environmental and physiological factors are known to influence fish haematology; these include stress due to capturing, transportation, sampling, age and sex. Haematological components of blood are also valuable in monitoring toxicity especially with feed constituents that affect the formation of blood (Dienye and Olumuji, 2014). RBC, Hb and HCT decreased significantly in *C. gariepinus* fed high Moringa meal levels (Hlophe and and Mayo, 2014). This decrease further indicates nutritional stress (Hlophe and and Mayo, 2014).

According to Qiang *et al*. (2013) when dietary protein levels are low, physiological stress is induced and this damages the liver, leading to reduced RBC and Hb concentration. Similarly, Abdel-Tawwab *et al*. (2010) reported a decrease in RBC and fish feed low protein levels in the

diet. The packed Cell Volume (PCV), red blood cells (RBC) ad haemoglobin were observed to reduce in the diet of *Clarias gariepinus* (Bello *et al*., 2013).

Reduction in the concentration of the PCV in the blood usually suggests the presence of toxic factor which has adverse effect on blood formation (Osuigwe *et al.*, 2005). The decrease in RBC may be ascribed to the higher concentration of anti-metabolites especially phytates (Roberts *et al.*, 2000). Adamu and Audu (2008) reported reduced RBC to be due to gill damage or impaired osmoregulation. The reduction in Hb concentration could imply that diets having higher substitutions contain low quality protein, and this may result in poor transportation of oxygen from the respiratory organs to the peripheral tissue (Roberts *et al.*, 2000).

White blood cells (WBC) are the defense cells of the body. It has been demonstrated that the quantity of WBC has implication in immune responses and the ability of the animal to fight infection (Douglas and Janes, 2010). WBC count showed an increase an increase as the level of Moringa Leaf Meal increased in the diet of *C. gariepinus* (Bello *et al*., 2013; Ochang *et. al*., 2015). High WBC count is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system (Osuigwe *et al.*, 2005). The increase in WBC as plant-based meal increase in a diet imply some form of feed toxicity (Bello *et al*., 2013; Ochang *et. al*., 2015).

1.2 Statement of the Research Problem

Fish feed technology is one of the least developed sectors of aquaculture in Nigeria (Umaru *et al*., 2016). For aquaculture to be highly successful in Nigeria, there is need for good quality and affordable feed (Glencross *et al*., 2007).

Soaring food prices have triggered an increase in hunger worldwide, the competition between human and livestock for the consumption of soybean and the increasing role of soybean in the world as a biodiesel as reported by FAO(2008) and Ingweye *et al*.(2010), have increased its cost and demand and heightened the competition between human, livestock and fish for soybeans, this is also the case of other conventional plant protein sources.

The high cost of imported commercial fish feed is a major constraint to the expansion and growth of aquaculture sector in Nigeria and therefore, concerted effort is needed to seek suitable alternative fish feed ingredient.

1.3 Justification

It is necessary to reduce the dependence on soybean and groundnut by partial replacement with less popular wild legume seeds. However, the over-dependence has already caused a hike in the price of soybean meal; therefore, utilization of other inexpensive plant protein source would be beneficial in reducing the feed cost (Yue and Zhou, 2009).

Seeds of *S. occidentalis* are good source for alternative plant proteins. The chemical composition as revealed by Augustine *et al*. (2013) indicated that the seed meal has promising nutritional value but also contains some anti-nutritional factors such as tannins, oxalates, phytates and saponins which will limit its utilization with adverse consequences on animal performance. Proximate composition results showed high dry matter (92.50%), crude protein (29.54%) and crude fiber (10.18%), but low ether extract, nitrogen free extract, ash and calorific values (Augustine *et al*., 2014).

1.4 Objectives of the Study

The objectives of the study are to:

i. determine the proximate composition, anti-nutritional factors and macro-elementsof*Senna occidentalis* seed.

ii. investigatethe growth response and feed utilization of hybrid African catfish fingerlingsfed *S.occidentalis*replacing soya bean meal.

iii.determinethe effects of replacing soyabean meal with *Senna occidentalis* on some haematological parametersofhybrid African catfish fingerlings.

iv.determine the cost effectiveness of replacing soybean meal with *Senna occidentalis* seed meal in the diet of hybrid African catfish fingerlings.

1.5 Hypotheses

i. There is no significantdifference between the proximate composition, anti-nutritional factorsand macro element fermented*S. occidentalis*meal.

ii. There is no significant difference ($p>0.05$) between the growth response andfeed utilization of *Heteroclarias* fed fermented *S. occidentalis* seed meal replacing soyabean.

iii. There is no significant difference ($p>0.05$) between some haematological parameters of *Heteroclarias* fed on fermented *S. occidentalis*meal based diet substituting soya bean.

iv. The cost effectiveness of substituting soya bean meal with fermented*S. occidentalis* seed meal in the diet of *Heteroclarias*does not differ ($p>0.05$).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Proximate Composition of *Senna occidentalis* and Related Species

The crude protein of *S.occidentalis* have been reported to be 29.54% by Augustine *et al*. (2014), 2.75% by Aja *et al*. (2017) and 14.88%(Abdelseed*et al*.,2011). The proximate composition of *Senna occidentalis* for ash, crude fiber, ether extract, dry matter, crude protein, Nitrogen Free Extract was 5.7, 13.80, 4.03, 92.5, 19.62, 49.80, respectively (Augustine*et al*., 2016) and 6.65, 13.71, 3.35, 92.40, 17.45, 37.18, respectively and energy (kcal/kg)(Augustine*et al*.,2018).

Proximate composition of *Senna obstifolia*results showed high dry matter (92.50%), crude protein (29.54%) and crude fiber (10.18%), but low ether extract, nitrogen free extract, ash and calorific values (Augustine *et al*., 2014).

2.2 MineralComposition of *Senna* Species

Senna obstifolia seeds are abundant in calcium (960 mg/100 g), potassium (1,200 mg/100 g), phosphorus (810 mg/100 g), sodium (600 mg/100 g), magnesium (640 mg/100 g), iron (234.60 mg/100 g), zinc (53.12 mg/100 g) and copper (10.48 mg/100 g) but low in molybdenum, cobalt, chromium, selenium, sulphur and fluorine (Ingweye *et al*., 2010). According to Alli Smith (2009), he reported from his study that Iron (Fe), Magnesium (Mg), manganese (Mn), potassium (K), calcium (Ca), sodium (Na), copper (Cu), lead and phosphorus (Zn) had values of 112.00, 876.00, 35.10, 812.00, 932.00, 612.00, 0.84, 0.34 and 10.84 ppm respectively, in *Senna siamea* leaves. According to Abdulwaliyu *et al*. (2018), *Senna alata* contains K (1356 mg/100mg), Zn (2.02 mg/100mg), Mg (134.0mg/100mg), Fe (14.50mg/100mg) and Ca (288.50mg/100mg).

2.3Description and Habitat of *S. occidentalis*

Senna occidentalis L. is a leguminous plant belonging to the family Fabaceae. It is an annual or perennial plant (Kapoor, 2010), grown as an ornamental plant (Shankar, 2003). It is also a low branching perennial shrub and grows to about three (3) cm feet high (Sadiq *et al*., 2012). It is a pan-tropical plant species that is characterized by alternate compound leaves(Augustine *et al*., 2016).

The pod is about 12.5cm x 0.7cm containing 23-30 seeds(Augustine *et al*., 2016). The flower is ovate yellow *Senna* has compound leaves with narrow linear-to-oval-shaped dark green leaflets. In the spring and summer it has yellow pea-like flowers which are followed by brown pods which contain brown seeds. Leaves, fruit and flowers of *Senna* are used for medicinal purpose (Malviya and Sharma, 2013).This plant is called in different regional/vernacular languages like Nigeria/Hausa (Raidore), Hindi (Badikasondi, Chakunda, Kasonda), English (Coffee senna, Negro coffee, Rubbish cassia, Stinking weed, Foetid cassia) etc.

2.4Geographical Source and Distribution of *Senna* species

Senna occidentalis is a plant native more to Africa (It can be found in so many states in Nigeria including Zaria, Kaduna state, Kano, Katsina etc.), Asia, and America, and belong to the family Fabaceae/Leguminosae, subfamily Caesalpinoidae and Genus *Senna*(Augustine *et al*., 2016). It grows in low lying coastal area, river banks, abundant in waste places and other moist places like uncultivated fields, up to 1000-1400 meters (Harshal *et al.,* 2011).



Plate I: seeds of *Senna occidentalis*



Plate II: *Senna occidentalis* plant containing the leaves, seed pods, stems and flowers

2.5Economic Importance of *Senna occidentalis*

Senna species are commonly used as ornamentals, foods (beverages); and many have important medicinal properties and are used in both traditional and modern medicine (Monkheang *et al*., 2011). Potentially manageable or useful resources may present actual health and agricultural cost due to improper management or utilization (Mebrahtom *et al*., 2014).

Senna occidentalis is regarded as 'Edible weeds of Agriculture or 'Famine food'in Nigeria.The leaves of *S. occidentalis*(Linnaeus) were reported (Adeyemo *et al*., 2014) to contain protein, carbohydrate, fiber, lipids, vitamins, moisture, caloric value and low levels of toxic agents, whose levels could be reduced on processing before consumption, and therefore concluded that, the plant can contribute significantly to the nutrient requirements of man and may ameliorate some nutrition related illnesses.

2.5.1Medicinal uses

Both the leaves and the seeds are used in herbal medicine (Augustine *et al*., 2016).Its infusion is given against the white dischargefor humans and animals in Nigeria(Willcox *et al*., 2012). In Mali, it is used as ingredient in a malarial treatment formulae based on a traditional recipe comprising of leaf of *Senna occidentalis*, leaves of *Lippia chevalieri* and flower heads of *Spilanthes oleraces* (Willcox *et al*., 2012). Extracts of *Senna occidentalis* roots and black pepper is useful in filarial treatments in fish (Arvind and Shamshun, 2007). In the Malyagiri hills, a decoction is made from 15 leaves each of *S. occidentalis*, *Glycosmis pentaphylla* and *Vitex negundo* and used for bathing the new born as vaccine against skin diseases (Yadav *et al*., 2010). In traditional medicine, seed powder (half a tea spoon) is used to cure fever while two table

spoonfuls of leaf juice mixed with honey cures cough. For intestinal gas half a cup of leaf extract is taken twice daily and paste of leaf is applied for skin diseases(Yadav *et al*., 2010).

It is used in several traditional medicines to cure various diseases (Kapoor, 2010). In Brazil, hydroalcoholic extract of *S. occidentalis* stem and leaf has been marketed by Pharmaceutical Laboratory (LAPERLI) with commercial name of *Cassia virgínica* and has been indicated for the treatment of flu, fever, erysipelas, febrifuge and as analgesic,hepatoprotective and diuretic (Silva *et al.,* 2011).

2.6Anti-Nutritional Factors Present in *Senna* Species

Anti-nutritional factors are a chemical compounds synthesized in natural feedstuffs by the normal metabolism of fish species (Soetan and Oyewole, 2009). Anti-nutritional factors are subs6ances which either by themselves or through their metabolic products, interfere with feed utilization and affect the health and production of fish or which reduce nutrient intake, digestion, absorption and utilization (Akande *et al.*, 2010).Also, anti-nutrients or anti-nutritional factors are chemicals which have been evolved by plants for their own defense, among other biological functions and reduce the maximum utilization of nutrients especially proteins, vitamins and minerals, thus preventing optimal exploitation of the nutrients present ina food and decreasing the nutritive value. Some of these plant chemicals have been shown to be deleterious to health or evidently advantageous to fish health if consumed at appropriate amounts (Ugwu and Oranye, 2006).

Many plant components and seeds of legumes and other plant sources contain in their raw state wide varieties of anti-nutrients which are potentially toxic to fish (D'Mello, 2000). Some of these chemicals are known as ''secondary metabolites'' and they have been shown to be highly

biologically active (Habtamu and Nigussie, 2014) and Most of these secondary metabolites elicit very harmful biological responses, while some are widely applied in nutrition and as pharmacologically active agents (Soetan, 2008).

Anti-nutritional factors (ANFs) are compounds which reduce the nutrient utilization and/or food intake of plants or plant products used as human foods or animal feeds and they play a vital role in determining the use of plants for humans and animals (Soetan and Oyewole , 2009). The toxicity due to the consumption of various forages is very common among the farm animals. The anti-nutritional factors present in the forages are mainly responsible for this (Smitha*et al*., 2013). Anti-nutritional factors may be divided into two major categories. They are: (1). Proteins (such as lectins and protease inhibitors) which are sensitive to normal processing temperatures. (2). Other substances which are stable or resistant to these temperatures and the major ones includes: toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins (phytohaemagglutinins), protease inhibitors, chlorogenic acid and amylase inhibitors (Akande *et al*., 2010). More often than not, a single plant may contain two or more toxic compounds, generally drawn from the two categories, which add to the difficulties of detoxification.

According to Augustine *et al.* (2018), *S. occidentalis* gave the values of oxalates, tannins, phenols, phytates, saponins as 2.96, 3.16, 8.35, 4.16 and 3.50 mg/100g, respectively. Augustine *et al.* (2014) revealed that values for Tannins, Alkaloids, Saponin, Oxalates and Phenols are 193.12, 252.55, 167.62, 68.85, 137.85 (mg/100g) for *S. obstifolia* seed meal while 269.23, 251.00, 176.50, 79.05, 220.33 mg/100g) for *S. occidentalis*seed meal respectively. The results revealed that except for oxalates *S. occidentalis* has more concentration of Tannins, Alkaloids,

Saponin and Phenols. This clearly showed that *S. occidentalis* may have more toxic potentials, lower digestibility and utilization of dietary nutrients.

Senna obstifolia have been reported to have 193.12mg of Tannin, 167.62mg of Saponin while *S. occidentalis* contains 269.23mg of Tannin and 176mg of Saponin(Augustine *et al*., 2014). According to him both *Senna* sp showed high concentrations of anti-nutritional factors which could be harmful to livestock including fish and so on. The concentration of anti-nutrients in the seeds of *S. obstifolia* recorded high values - 260, 185, 388.50, 83.25mg/ 100g for alkaloid, saponin, tannin and oxalate, respectively, while phytate, hydrocynic acid, phytohaemaglutinin levels were low (Ingweye *et al*., 2010), while*Cassia tora*was 7.20g/mg for raw, 6.71g/mg in fermented (Adamu*et al*., 2013). The high levels of most antinutrients indicate the potential for interfering with the utilization of the nutrients by the animals. This therefore creates a need for detoxification of the seeds through processing before using in fish feeds (Ingweye *et al*., 2010).

The oxalate level within the tolerable limit for man is 10-20kg (Francis *et al*., 2001). For phytic acid, 0.74 mg/ 100g was reported by Balogun (2013) and Francis *et al*. (2001) recommended tolerable limits below 5g/kg. Saponin level of 0.3mg/100g was recommended Balogun(2013) and tolerance limits of below 1g/kg of diet in commonly cultured fish (Francis *et al*., 2001).

Balogun (2013) reported 0.68, 8.75, 12.08, 11.39 and 2.74 mg/100g for Hydrocyanide, tannin, oxalate, phytic acid and saponins respectively, the raw *Bauhinia monandra* seed. The results obtained from the anti-nutritional analysis of *S. alata* revealed 2.00 g/100g alkaloids, 4.52 g/100g oxalate, 1.86 g/100mg phytic acid and saponins were not detected (Abdulwaliyu *et al*., 2018). However, 7.84, 21.69, 15.07 mg/100g were recorded for oxalate, cyanide and phytate in

the leaf of *Senna alata* while 9.9, 13.04, 12.44 mg/100g were recorded for oxalate, cyanide and phytate in the root bark of *Senna alata,* respectively (Abubakar *et al*., 2015).

Osheke and Akinyemi (2015) also reported phytic acid (%), tannin (%), oxalate (%), cyanogenic glycoside (mg/ 100 g) and phytate (mg/100g) as 0.99, 0.14, 3.72, 7.22 and 0.28, respectively for *Senna occidentalis*. Phytic acid is an important storage form of phosphorus in plant, it is insoluble and it has 12 replaceable hydrogen atoms with metals such as calcium, iron, zinc and magnesium (Osheke and Akinyemi, 2015). Tannins are known to inhibit active enzymes and hence, the presence of even low levels of tannins is not desirable from the nutitiional point of view (Vadivel and Pugalenthi, 2008). Oxalates can bind tocan bin d to calcium present in food thereby rendering calcium unavailable for normal physiological and biochemical role such as the maintenance of strong bone, teeth and as clotting factor in the blood (Ladeji *et al*., 2004). Oxalate may be present as oxalic acid or as insoluble calcium oxalate which when present in high concentration in diet may increase the risk of renal calcium absorption (Osagie, 1998).

Cyanogenic glycoside on hydrolysis yield toxic hydrocyanide acid (HCN). The cyanide ions inhibit several enzymes systems; depress growth through interference with certain essential amino acids and utilization of associated nutrients. *S. occidentalis* was found with the presence of cyanogenic glycoside but having lesser quantity far below the fatal dose (50mg/kg) (Sarjekar *et al*., 1994). The phytate molecule forms insoluble complexes; thereby making minerals unavailable for absorption. It is also negatively charged at physiological pH and is reported to bind with essential, nutritionally important divalent cations such as iron, zinc, magnesium, calcium etc. phytate molecules inhibits the digestion of protein and starch and formed complexes with them (Oatway *et al*., 2001).

2.7Methods to Reduce the level of Anti-Nutritional Substances in Feedstuffs

Various methods have been attempted to de-activate tannins in a wide range of browse species, grain seeds and agro-industrial by-products (Makkar, 2000). These methods have included mechanical or physical techniques (e.g. wilting, processing, ensiling, etc.), inoculation with tannin resistant bacteria and chemical techniques (treatment with alkalis, organic solvents, precipitants, etc.). The use of polyethylene glycol for which tannins have higher affinity than for proteins, is by far the most used reagent to neutralize these secondary compounds (Muller, 2001).

Balogun (2013) reported the results of *Bauhinia monandra* being subjected to several methods of processing: Boiling – Hydrocyanide, tannin, oxalate, phytic acid and saponins were 0.64, 6.24, 8.95 and 0.55 mg/100g when boiled for 10 minutes; 0.53, 6.23, 7.63, 4.01 and 0.54 mg/100g when boiled for 20 minutes; 0.23, 6.23, 6.73, 3.03 and 0.51mg/100g when boiled for 30 minutes; 0.22, 4.35, 2.67, 3.01 and 0.44 mg/100g; 0.68, 8.75, 12.08, 11.39 and 2.74 mg/100g for the raw seed, respectively.

Toasting - Hydrocyanide, tannin, oxalate, phytic acid and saponins results were 0.64, 8.71, 11.21, 10.01 and 0.61 mg/100g when toasted for 10 minutes; 0.53, 5.75, 10.02, 10.00 and 0.58 mg/100g when toasted for 20 minutes; 0,52, 8.73, 10.01, 10.01 and 0.55mg/100gwhen toasted for 30 minutes; 0.42,6.23, 8.74, 10.00 and 0.53%mg/100g when toasted for 40 minutes; 0.68, 8.75, 12.08, 11.39 and 2.74mg/100g for the raw seed, repectively.

Soaking - Hydrocyanide, tannin, oxalate, phytic acid and saponins values were 0.42, 6.25, 6.71, 3.55 and 0.51mg/100g when soaked for 24 hours; 0.21, 2.82, 2.35, 3.44 and 0.43mg/100gwhen

soaked for 48 hours; 0.16, 3.21, 2.18, 3.13 and 0.40 mg/100gwhen soaked for 72 hours; 0.03, 2.88, 1.94, 0.75 and 0.31mg/100g when soaked for 96 hours; 0.68, 8.75, 12.08, 11.39 and 2.74mg/100g for the raw seed, respectively.

Adekanmi *et al*. (2009) reported that soaking in water reduced tannin *Senna* sp by 15% while soaking at 60°C for 7hoursreducted tannin by 61%.Phytic acid content of *Bauhinia* seed reduced from 11.30mg/100g in raw seed to 0.75mg/100g in soaked seed; boiled seed (3.01 mg/100g) and toasted seed (10.00 mg/100g), respectively (Balogun, 2013). In general, amongst various processing methods (boiling, toasting and soaking) employed, the soaking method was found to reduce the levels of various anti-nutritional substances (Balogun, 2013).

Tamburawa (2010) documented that boiling best reduced the amount of Locust been seed meal from 0.71 mg/100mg (raw) to the bearest minimum with increased duration of soaking. Tamburawa (2010) reported that soaking reduced the level of tannins to the bearest minimum with increased duration of processing time, from 1.08 in raw locust bean seed meal to 0.28 when soaked for 1 day, 0.25 (2 days) and 0.17 (3 days). He also reported that oxalate from 1.78mg/100g. In the raw locust been seed meal when subjected to soaking for 1 day to 1.25, 2 days (0.67mg/100g) and 3 days (0.46mg/100g) while soaking and subsequesnt fermentation for 3 days reduced the oxalate level 0.43mg/100g. However, the finding of Tamburawa (2010) indicated that toasting reduced the level of oxalate to 0.41mg/100g.According to Tamburawa (2010) boiling also progressively and substantially reduced the level of oxalate to the bearest minimum with increased duration of processing time; in 1 hour (0.22mg/100g, 2 hours (0.21mg/100g), 3 hours (0.24mg/100g), 4 hours (0.22mg/100g).

Balogun (2013) reported that oxalate level of *Bauhinia* seed was substantially and significantly reduced from 12.08 mg/100g in raw to 1.94 mg/100g in the soaked seed sample followed by the boiled seed (2.67mg/100g) with least reduction in toasted seed sample (8.74mg/100g).

Augustine *et al.* (2018) reported that soaking of *S.occidentalis*result gave the results of: oxalates, tannins, phenols, phytates, saponins contained 2.79, 2.97, 7.65, 3.88 and 3.25 mg/100g with % reduction of 5.4, 6.01, 8.38, 6.73 and 7.14 at 10 hours; 2.03, 2.65, 7.20, 3.76 and 3.18 mg/100g with % reduction of 31.41, 16.13, 13.77, 9.61 and 12.86 at 15 hours, respectively.; 1.65, , 2.5, 6.55, 2.87,3.05 mg/100g with % reduction of 44. 25, 28.79, 21.55, 31.01 and 12.86 at 25 hours respectively; 1.46, 1.94, 6.22, 2.48 and 2.99 mg/100gwith % reduction of 46.36, 38.61, 25.51 and 14.57 at 25 hours, respectively. Similar significant reduction of various anti-nutritional compounds during soaking treatment was reported for several under-utilized legiouminous materials such as *Crotolaria retusa* L. (Yashim *et al*., 2009), Locust bean meal seed (Olaniyi*et al*., 2009; Tamburawa, 2010) and *Mucuna Pruriens* Var (Vidavidel and Pungalenthgi, 2007).

According to Oladape *et al*. (2018), 204.33, 487.28, 31.50, 172.11, 42.53, 18.06, 127.02, 12.10 and 0.05 mg/100g were found for alkaloid, flavonoid, glycosides, oxalate, phenol, phytate, saponin, tannin, terpenoid and caffeine when *S. occidentalis* seeds were roasted at 190˚C for 10 minutes, respectively. Similarly, 166.76, 568.43, 463.51, 17.17, 161.06, 58.33, 16.03, 115.33, 14.18, 0.04 mg/100g were found for alkaloid, flavonoid, glycosides, oxalate, phenol, phytate, saponin, tannin, terpenoid and caffeine when *S. occidentalis* seeds were roasted at 210˚C for 15 minutes, respectively. He also reported that 147.60, 754.92, 426.61, 25.68, 152.81, 121.00, 15.20, 174.21, 14.76, 0.05 mg/100g were found for alkaloid, flavonoid, glycosides, oxalate, phenol, phytate, saponin, tannin, terpenoid and caffeine when *S. occidentalis* seeds were roasted at 230˚C for 20 minutes, respectively.

Similarly, Augustine *et al*. (2018) reported the result of *Senna obstifolia*leaves subjected to bio-process as follows: Shade-dried - oxalates, tannins, phenols, phytates and saponins with 1.38, 1.85, 15.03, 3.70 and 3.81%, respectively with all having 0.00% reduction level.

Ensiled - oxalates, tannins, phenols, phytates and saponins with 0.27, 0.27, 6.52, 0.96 and 0.96% with eachhaving % reduction of 80.43, 85.56, 58.49, 74.05 and 74.05%, respectively. Also, Boiled+ Fermented *Senna obstifolia* leaves - oxalates, tannins, phenols, phytates and saponins with 0.21, 0.25, 4.23, 0.64 and 0.84% with each having % reduction of 84.78, 86.49, 67.49, 82.70 and 77.95 %, respectively.

Germination followed by dehulling reduces phytic acid and tannin by 47–52% and 43–52%, respectively (Ghavidel and Prakash, 2006). According to Abu (2005) fermentation reduced phytate in Locust bean seed (*Parkia filicoidea*).

Fermentation was also found to decrease trypsin inhibitor activity (TIA), amylase inhibitory activity, Phtytic acid and Tannins (Osman, 2004; Ejigui *et al*., 2005; Eltayeb *et al*., 2007; Abdel-Haleem *et al*., 2008). In view of the above, it has become imperative to detoxify the seeds before feeding to animals.

2.8Effect of Replacing Soybean Meal with Some Plant Protein Sources

Most published research on the use of plant protein as a substitute of soya bean meal in fish feed has focused on the inclusion of palm kernel meal (Ng and Chen, 2002). Cotton seed meal (Yuo and Zhou, 2008) and Faba beans (Azaza *et. al*., 2009) with the goal to increase inclusion of sustainable plant-based diet for fish and all results show that dietary protein source from plant origins did not positivelyaffect growth and survival of fish.

The result of the study conducted by Hassan *et al*. (2017) revealed that inclusion levels of water melon seed meal replacing soya bean beyond 75% can not be tolerated by *Clarias gariepinus,* and fiber content beyond 5% as negate the recommendations of Sawaya *et al*. (1986) who stated that water melon seed should not be included at levels higher than 20%, because these levels brings up the fibre content to the ration over 10%, which reduce feed intake. The lipid content increase in their study is likely due to the fact that both soya beans and watermelon seeds are oil seeds (Abbas, 2007). Manjappa *et al*. (2011) opined that better nutrients utilization in fish carcass fed high lipids diets is related to both the dietary protein level and availability of non-protein energy sources.

It has been reported by Alatise *et al*. (2014), it was inferred that inclusion of Jatropha kernel meal in the meal to replace soya bean will improve growth yield of *Clarias gariepinus*. Though, the Jatropha kernel meal can be included upto 50% since the fish showed good apetite for all the treatment diets. Growth performance of *Clarias gariepinus* increases with increase in inclusion level of boiled Jatropha kernel meal in the diet. Inclusion of Jatropha kernel meal in the meal does not have detrimental effect on *Clarias gariepinus* as revealed by the survival rate. The 30% boiled Jatropha kernel meal replacement with soya bean meal is optimal for *Clarias gariepinus*growth performance.Fakunle *et al*.(2013) also reported that 30% boiled Jatropha kernel meal can replace soyabean meal in the diet*Clarias gariepinus*.

Moringa seed cake have beenreported to replace soybean meal at level of not over 500g/kg in bocourti's catfish diet and could support the growth, adversely affected digestibility, haematological, serum biochemistry parameters and histopathological change in bocourti's catfish (Yuangsoi *et al*., 2014). Yuangsoi *et al.* (2014) reported that the replacement of protein in soybean meal with Moringa seed cake in African catfish feed did not lead to mortality and

slightly lower growth performance of fish fed more than 750g/kg of soybean meal protein replacement.

Hashem *et al*. (2017) reported that dried Moringa seed meal could replace soybean meal upto 75% in clariid without any negative influence on the growth, beyond which growth was significantly depressed.

Up to 25% peanut meal can be used as a protein source alternative to cottonseed meal or soybean meal in Channel Catfish diets without adversely affecting growth, feed efficiency, and body composition. (Menghe and Penelope, 2016).All soybean meal could be replaced by two or three moderate- and high-protein alternative feedstuffs without significantly affecting growth performance, processing yield, and fillet proximate composition of pond-raised Channel Catfish during food fish grow out (Menghe *et al*., 2016).

Yue and Zhou (2008) reported that up to 33.76% cotton seed meal can be used to replace 60% of Soybean meal in diets for juvenile hybrid clariid. Omosowone and Ogunrinde (2018) revealed in from study that full fat*Telfairia occidentalis* seed meal can replace soya bean meal in the diet of *Heteroclarias* at a minimal level of 25%.

Jimoh *et al*. (2013) reported that it is possible to replace soyabean meal in the diet of *Clarias gariepinus* fingernus with cooked *Luffa cylindrica* seed meal, with optimum growth responseat a 15% replacement level.Ndirmbita *et al*. (2018) revealed that *Faidherbia albida* seed meal is not suitable for replacing soyabean meal in *Clarias gariepinus* diets because all most the growth indices inclusing survival rate decrease with increase in levels of *Faidherbia albida* seed meal in the diets.

2.9 Proximate Composition of African Catfish Carcass

According to Bello *et al*. (2013) and Dienye and Olumuji (2014), moisture content, crude lipid, crude protein, crude fibre, total ash and NFE to be 5.97-6.67, 5.20-6.02, 59.40 - 62.47, 0.03-0.04, 5.50-6.31 and 19.49-23.55%, respectively for *C. gariepinus* fed dietary levels of *Moringa oliefera* leaf meal. Similarly, Ochang *et al.* (2015) reported the carcass composition of *C. gariepinus*fed dietary *Moringa oliefera* leaf meal based diet for eight weeks as carbohydrate (2.33-2.53%), protein (17.66-21.00%), fat (1.86-3.15%), ash (2.06-3.35%) fibre (0.32-0.38%) and moisture (73.56-73.93).

2.10Haematological Indices of Fish Species

Akinrotimi *et al*. (2011) reported the reference range of African catfish - haemoglobin (10.02-18.64 g/dL), Red Blood Cell (3.051-8.64 $x10^2$/L), Packed Cell Volume (32.64-45.74%), White Blood Cell (18.66-25.61$x10^9$/L), Mean Corpuscular Volume (72.11-91.34f1), Mean Corpuscular Haemoglobin (30.21-46.74pg), Mean Corpuscular Haemoglobin Concentration (38.21-46.74 g/Dl), Thrombocytes (92.10-158.74 $x10^9$/L), Lympocytes (51.14-70.16%), Neutrophils (27.64-40.14%) and Monocytes (1.86-4.10%).

According to Diyaware *et al.* (2010), PCV (27.87%), the Hb (g/dL), WBC ($x10^3$/µL), RBC($x10^3$/µL), MCV (fl), MCH (pg), MCHC (g/dL), Plateletes ($x10^3$/µL), Thrombocytes ($x10^3$/µL) and Lymphocytes (%) in *Heteroclarias* were 9.63, 193.70, 2.46, 113.07, 39.10, 34.57, 16.67, 190.40 and 98.30, respectively.

Adesina (2017) reported the values ranges of PCV (%), Hb (gm/100ml), WBC ($x10^9$ /ml), RBC($x10^{12}$ /µL), MCV (µg/ml), MCH (µg/ml), MCHC (gm/ml), Plateletes ($x10^9$/ml), Neutrophils (%), Monocytes (%), Lymphocytes (%) and Total Protein (g/100ml) as26.67-38.0,

9.6-12.70, 8.33-12.53, 8.11 -10.21, 34.67-37.0, 11.0-12.0, 32.0-32.67, 10.0-14.0, 24.67-42.33, 0.5-2.0, 57.67-75.0 and 2.6-4.17, respectively for *C. gariepinus* juveniles fed graded levels of mechanically extracted sunflower seed meal-based diets.

The PCV have been reported to be 20-35 %(Erondu *et al*. 1993), 22.40 % (Bhaskar and Rao, 1989), 38.40 %(Kori-Siakpere and Ubogu 2008) and27.58-35.50 %(Musa and Omoregie, 1999), 36.0 %(Adeyemo, 2007).Agbabiaka *et al*. (2013) recorded 38.0 to 44.7 % for *C. gariepinus* fingerlings fed graded levels of tiger-nut based diet.

Haemoglobin values were reported as 10.63g/dL Osuigwe *et al*. (2005) for controlled juvenile hybrid between *H. longifilis* x *C. gariepinus*, 15.31g/dL by Kori-Siakpere and Ubogu (2008) documented for juvenilehybrid, 13.00g/dL recorded from *C. gariepinus*(Ogunji *et al*. 2005), 27.00g/dL(Sunomonu and Oyelola, 2008), 16.0- 18.43 g/dL (Onyia*et al*., 2013), 870 g/100ml for *C. gariepinus (*Sowunmi, 2003), 7.90-8.90 g/100ml(Dienye and Olumuji, 2014), 4.46g/100ml (Fagbenro *et al.*,2000).

The results forWBC were documented as $18.8x10^3$/µL (Ogunji *et al*., 2005), $37.78x10^3$/µL for wild adult *C. gariepinus*(Gabriel *et al.*,2001), $8.42x10^3$/µL by for juvenile *Heteroclarias* were recorded by (Osuigwe *et al*., 2005). Bunmi (2010) observed $49.73x10^3$/µL for wild *Clariabranchus* (*C. gariepinus*x *H.bidorsalis),* $16.51x10^3$/µLfor adult *C. anguillaris*, 9.04 $x10^3$/µL for *C. macromystax* in North east Nigeria and 22.23 ± 2.52 x 10^9/ml recorded for *Clarias batrachus* (Maheswaran*et al*., 2008).

For the RBC, 1.63 x $10^{12}x10^3$/µLwas reported (Kori-Siakpere and Ubogu, 2008), 1.43 x $10^{12}x10^3$/µL (Osuigwe *et al*., 2005) and 1.77x 10^6 mm^3(Maheswaran *et al*., 2008) for *Clarias batrachus* and1.9 x 10^{12}/l found in *C. gariepinus* juveniles (Ayoola,2011)

The MCV (fl) values observed were 240.18 recorded for juvenile hybrid African catfish (*Heteroclarias*)(Kori-Siakpere and Ubogu, 2008), 200.93 for *C. gariepinus* fingerlings(Gbore, 2006), 113.93 to 138.07 for juvenile intergeneric hybrid catfishes (Diyaware *et al*., 2013) and 92.62 for *C. gariepinus* fingerlings (Ochang *et al*., 2007).

Earliar reports on MCH (pg) values were 24.24 *C. gariepinus*juveniles by Omitoyin(2007), 24.24 for *C. gariepinus* juveniles by Omitoyin (2006), 33.10 (Ochang *et al*., 2007). Kori-Siakpere and Ubogu (2008) reported value higher than 35.10 for *Heteroclarias*juvenile and Gbore(2006) reported 51.50 for hybrid catfish.

MCHC (g/dL) values recommended for healthy fish by Bhaskar and Rao (1989) was ≥39.90, 33.67 to 39.03 for juvenile intergeneric hybrid catfishes (Diyaware *et al*., 2013), 35.27 for *Clariabranchus* and very close to 35.47 for *Heteroclarias*juvenile (Kori-Siakpere and Ubogu, 2008).

Some Plateletes ($x10^3$/µL) values earlier reported are 132 for juvenile *C. gariepinus*reported by Sunomonu and Oyelola (2008) and 175.92 for *Sarotherodon melanotheron* adult (Akinrotimi *et al*., 2007).Lymphocytes (%) were reported as 33.00 for juvenile *C. gariepinus* (Adeyemo,2007)and 82.8 for juvenile *C. gariepinus*reported by Yaji and Auta (2007).

2.11 Haematological Parameters of Fish Fed Plant Leaves and Seed Based Meal

The Mean Corpuscular Haemoglobin Concentration, Mean Corpuscular Haemoglobin and Mean Corpuscular Volume according to Bello *et al.* (2013) recorded their highest values in fish fed with 50% *Moringa oliefera* leaf meal based diet (replacing soyabean meal) and are comparable with value ranges reported by many workers (Adedeji *et al*., 2000; Adedeji and Adegbile 2011;

Anyawu *et al*., 2011). Hematological assays might give a useful guide on the physiological condition of fish (Haghighi and Rohani, 2013). Toxocological test show that 10% substitution of Soya bean with *M. oliefera* leaf meal in catfish (*C. gariepinus*) diet would not have any adverse effect on the blood and serum enzyme (Bello *et al.*, 2013).

2.12The Cost Effectiveness of Replacing Soybean Meal with Some Plant Protein Sources

Yusuf(2014) reported economic analysis of including *Senna obtusifolia*in the diet of African catfish showed no significant difference (P≥0.05) but the higher the inclusion level above 20% the longer the duration for culturing the fish to marketable size. Increasing the inclusion level of boiled *S. obtusifolia* seed in diet will correspond increased the expenditure; this can probably be due to growth performance of fish.

2.13 Water Quality Parameters Monitored for Clariids

Diyaware *et al.* (2013) reported temperature as 29.43- 90.30˚C, pH 7.49-7.85 while dissolved oxygen between 4.36-5.14 mg/l in ponds where *C. anguilaris, Clariabranchus, H. bidorsalis* and *Heteroclarias*juveniles were reared. Sinyangwe *et al*. (2017) reported water temperature range between 25.65-28.51 and 6.74-7.08 mg/l DO for normal growth of African catfish fry (*C. gariepinus).* Barton *et al*. (2002)recommends that temperatures should be within 23˚C-30˚C corroborating with FAO (1996). Optimum temperature for maximum growth of African catfish is 28˚C and DO levels is 6.0-9.0 mg/l (Hecht 2013; Zehra and Khan, 2012). Similarly, temperature, DO and pH range for hybrid catfish were reported as 25.00-32.00, 4.50-5.60 and 6.90-7.20, respectively (Diyaware *et al*. 2009).According to Diyaware *et al*. (2011), mean temperature, DO and pH were 25˚C, 5.5mg/l and 7.0, respectively for *C. gariepinus* fingerlings.

Onyia *etal*. (2013) reported temperature range as 25-25.5˚C, while pH, DO and conductivity were between 7-9.15, 5-5.88 mg/L and 31-34.10, respectively for *C.gariepinus*.

2.14Description of *Heteroclarias*

The filet of *Heteroclarias* is white in comparison with the pink/reddish colour of *C. gariepinus* filets and contain 30% more fat than *C. gariepinus* filets, which improves the taste. The gonads of hybrids are almost absent and not active. The *Heteroclarias* fingerlings show a wide variation in growth and severe cannibalism noticed, especially when frequent grading is negleclected. The *Heteroclarias* is easily stressed (FAO, 2019).Body elongate.Head large, depressed and bony with small eyes. Narrow and angular occipital process, gill openings wide, air-breeding labyrinthic organ arising from gill arches, first gill arch with 24 to 110 gillrakers and cleithrum pointed, narrow with longitudinal rigdes and with sharpness. Mouth terminal, large. Four pairs of barbels present.Long dorsal and anal fins; without dorsal fin spine and adipose fin. Anterior edge of pectoral spine serrated. Caudal fin rounded (FAO, 2019). They are rugged and diseaseresistantand they consume less feed when compared to clarias species. They attain bigger sizes than *Clarias* spp, and they weigh much higher at harvest.

2.15Feeding Habits of *A*frican catfish (*Heteroclarias*)

Heteroclarias feeds on insects and fish material more than any other food item. Changes in food composition and feeding habits of fish in relation to the size and age of the fish and season are biological phenomena which are common to many species of fish in the tropics and temperate areas of the world. The success of *Heteroclarias* can be attributed to its ability to colonize the variety of habitats created by the formation of the lake (Enyidi, 2012) and its ability to utilize a

wide range of food.

2.16Nutritional Requirements of *Heteroclarias*

De-Silva and Anderson (2007) opined that the quality of a feed is a function of how well that feed meets the nutrient requirements of a fish. Thus, the need for fish feed importation into the country which gave rise to different brands presently available in Nigerian market.

2.16.1 Carbohydrates

Atypical African catfish feed contains 25 percent or more soluble (digestible) carbohydrates plus 3 to 6 % more carbohydrates that are generally present as crude fiber (mainly cellulose). The gross energy and digestible energy requirement for African catfish is around 19 kJ/kg and 14kJ/kg respectively (Dekker*et al*., 1986). Uys and Hecht (1985) 21% carbohydrate requirement was reported to be 21% by Uys and Hecht (1985) for larvae, 15-35% by Ali(2001) and Ali and Jauncey (2005c) and 26-32% (Pantazis, 1999) for grow out. Metabolize energy reported by Machiels and Henken (1985) as 13min kJ/gfor grow out, digestible energy 12.7 min kJ/g by Yilmaz (2006) for grow out, gross energy 21.2min kJ/g byAli and Jauncey (2005a)for grow out, protein to energy ratio (mg/kJ) 20.5 by Ali and Jauncey (2005c) for grow out. Lipid to carbohydrate ratio (g/g) was reported as 2.47 (lipid 13%, carbohydrate 33.42%) by Ali (2001) for grow out.

Catfish cannot digest crude fiber well, so it should be kept at as low a level as possible; thus it is desirable in catfish feeds because indigestible materials may pollute the water. Commercial catfish feeds typically contain less than 5 percent crude fiber. (Meng and Edwin, 2009)

2.16.2 Lipid

The use of lipids (fats and oils) in catfish feeds is desirable because lipids are a highly digestible source of concentrated energy (containing about 2.25 times as much energy as does an equivalent amount of carbohydrate), supply essential fatty acids, serve as a vehicle for absorption of fat-soluble vitamins, increase feed palatability, and serve as precursors for steroid hormones and other compounds. In their storage form,lipids affect the flavor for fish as well as help maintain neutral buoyancy. The type and amount of lipid used incatfish diets are based on essential fatty acid requirements, economics, constraints of feed manufacture, andquality of fish flesh desired (Meng and Edwin, 2009).

Essential fatty acids (EFAs) are fatty acids that cannot be synthesized in the animal's body; thus, they must be provided in the diet. EFAs are classified based on their chemical structure and are designated as either omega-3 (n3) or omega-6 (n6) fatty acids. In general, fish appear to require n3 fatty acids while land animalsappear to require n6 fatty acids. This generalization does not always hold. Certain fish (including some speciesof tilapia and carp) apparently require both n3 and n6 fatty ac ids. EFA requirements for catfish and most otherwarmwater fish have not been precisely defined, but catfish apparently require a small amount of n3 fatty acids. It appears that 1 to 2% dietary linolenic acid (18:3 n3) is as good as 0.5 to 0.75% highly unsaturated fatty acids for normal growth, because catfish apparently elongate and desaturate linolenic acid to synthesize highly unsaturated fatty acids. The EFA requirement can be supplied by marine fish oil such as menhaden oil. Naturalpond food organisms may also be a source of EFA. For example, plankton contain relatively high levels of EFA.Catfish appear to have the ability to synthesize most of their fatty acids; thus, nutritionally there may be no "best" level of dietary lipid except that needed to provide EFA. Generally, weight gain and feed efficiency are

depressed in aquatic species when fed diets containing 15% or more lipid (Meng and Edwin, 2009).

Catfish have been fed diets containing up to 16% lipid without conclusive evidence as to which level is best for optimum growth. Even so, there is likely an optimum level of lipid to be used in catfish feeds with respect to protein sparing, product quality, and constraints of feed manufacture (Meng and Edwin, 2009).

Since lipid is a concentrated source of energy and can spare the more expensive protein, some lipid should be included in catfish diets. However, too much dietary lipid may result in excessive fat deposition in the visceral cavity and tissues that may adversely affect yield, product quality, and storage of processed products. Also, high-lipid feeds are difficult to pellet. If needed, supplemental lipid can be sprayed on to the finished feed pellet. Lipid levels in commercial catfish growout feeds rarely exceed 5 to 6%. About 3 to 4% of the lipid is inherent in the feed ingredients with the remaining 1 to 2% being sprayed on to the finished pellets. Spraying feed pellets with lipid increases dietary energy and aids in the reduction of "fines" (Meng and Edwin, 2009).

A mixture of vegetable and animal lipids has been *Edwardsiella ictaluri* used in commercial fish feeds. These were recommended over marine fish oils because high levels of fish oil may impart "fishy" flavors to the fish flesh. In addition, there is evidence that levels of menhaden oil as low as 2% of the diet reduce survival of catfish exposed to the bacterial pathogen. This is likely caused by the immunosuppressive effect of highly unsaturated n3 fatty acids. Catfish feeds manufactured in Mississippi are generally sprayed with catfish oil, which is a local product

extracted from catfish offal. In some cases, menhaden oil or a mixture of catfish oil and menhaden oil is used (Meng and Edwin, 2009).

Uys and Hecht reported 9% for larval stage; 8.2% by Ali and Jauncey (2005a) and 13% by Ali (2001).

2.16.3 Protein and Amino Acids

Protein comprises about 70% of the dry weight of fish muscle. A continual supply of protein is needed throughout life for maintenance and growth(Meng and Edwin, 2009). Most of the studies on protein requirements of fish have been based on weight gain and feed efficiency. Data from those studies indicate that the dietary protein requirement for catfish ranges from about 25 to 50%(Meng and Edwin, 2009).

FAO (2003) reported that the African catfish's propensity towards carnivores feeding habit suggests that the fish has high dietary protein requirement up to 40-50% crude protein on a dry matter basis. The minimum dietary requirement for larvae is 55%as reported by Uys and Hetcht (1985), fingerlings 40-42%(Uys, 1989) and 50% for nursery (0.5-10g), for hybrid between *C. gariepinus* and *H. bidorsalis* (Adebayo and Alasoadura, 2001). Diyaware *et al*. (2009) suggested 50% crude protein (cp) as dietary requirement of hybrid African catfish (*H. bidorsalis* x *C. anguillaris*).Sinyangwe *et al*. (2017) reported that *C. gariepinus*performed better when fed with 52% cp diet. For grow out (10-1000g), they need not less than 40-42% (Uys, 1989), 40% (Micheals and Hanken, 1985; Micheals and Hanken, 1987), 43 % (Ali, 2001; Ali and Jauncey, 2005b; Ali and Jauncey, 2005c). Least coasted and/or appetite protein feed requirement was repoted as 35% (Ali, 2001) and 38% reported Uysby (1989) for grow out fish. Argenine requirement for nursery (0.5-10g) was 4.5% (Fagbenro *et al.*, 1999), 4.45-4.50% Singh and

Khan(2007) for hybrids between *C. gariepinus* and *C. macrocephalus*.Histidine for nursery was 1.0-1.05% (Khan and Abidi, 2009) and grow out 1.39 (Pantazis, 1999). Isoleusine for grow out was 1.56% (Pantazis, 1999). Leucine for grow out was 4.87 (Pantazis, 1999). Lysine for nursery was 5.7% (Fagbenro *et al.*, 1998) and for grow out was 4.49 (Pantazis, 1999). Methionine for laevae was 2.5% (Uys and Hecht, 1985) and 3.2 for grow out (Fagbenro *et. al*, 1998). Phenylalanine for grow out was 4.56% (Pantazis, 1999). Threonine for grow out was 2.04 % (Pantazis, 1999). Tryptophan for nursery as reported by Fagbenro and Nwanna (2003) was 1.1 and 2.57 for grow out (Pantazis, 1999). Valine was 2.08 for grow out (Pantazis, 1999).

2.16.4 Vitamins

Concentrations as low as 25 ppm vitamin C have been shown to enhance survival of African catfish during challenge with the bacterium *Edwardsiella ictaluri*. There is evidence that the vitamin C requirement of catfish is as low as 15 ppm(Meng and Edwin, 2009).

It was reported by Wilson and Moreau (1996) that vitamins A and D contains 1000-2000 IU/kg and 500-1000IU/kg, respectively in nursery phase. He also reported that vitamin E, thiamine, riboflavin, pyridoxine, pantothenic, niacin, folic acid and choline all contained 25-50, 1, 9, 3, 10-15, 33.1, 1.2 and 400 min mg/kg in nursery phase. Similarly, Mohamed *et al*. (2004) reported that biotin (requirement determined for *Clarias batrachus)* was 2.49 min mg/kg for grow out. Ascorbic acid (min mg/kg) was 150 (Merchie *et al*., 1997) and 500 (Kuczynski, 2002) for larvae while11-60 (Wilson and Moreau, 1996) and 50 (Adewolu and Aro, 2009) for nursery.

2.16.5 Minerals

Among macro-minerals, phosphorus is particular important in fish feeds because fish require a relatively largequantity of the mineral in the diet. Feedstuffs, especially those of plant origin, are

poor sources of biological available phosphorus, and fish do not obtain significant amount of phosphorus from pond water. As a result, catfish feeds are usually supplemented with phosphorus. Di-calcium and de-fluorinated phosphates are commonly used as phosphorus supplements in catfish feeds.

According to Wilson and Moreau (1996), Calcium, phosphorus, magnesium, potassium has 0.45, 0.45, 0.04, 0.26 (%), respectively for nursery phase while iron copper, manganese, zinc and selenium has 30, 5, ≤2.4, 20 and 0.25 mg/kg dry diet, respectively.Uys (1989) also reported 1.5 and 0.5 % for calcium and phosphorus respectively for grow out phase.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1Collection and Processing of *Senna occidentalis* Seeds

Senna occidentalis seeds were obtained from the wild (from abandoned farm lands, road side and refuse dump areas)in Zaria. Zaria is located between Latitude 11^{o}C´N and Longitude 07^{o}38´E, at an altitude of 686 metres above sea level. It lies within the Guinea Savanna zone, and has 3 distinct seasons including harmattan (Nov- Feb), hot (Mar -May) and rainy (Jun- Oct) (Ayo *et al*., 1998). Its annual rainfall, average temperature and relative humidity are 1055millimetres, 24.55˚C and 43.6%, respectively as reported by Institute of Agricultural Research(IAR, 2009). Samples of plant collected were taken to the Herbarium of the Department of Botany, Ahmadu Bello University Zaria, for proper identification.

3.1.1Fermentation of *Senna occidentalis* seed

Sample was prepared according to the method of Shlini and Siddalinga Marthy (2015). Then fermented and raw samples were been tested.

Seeds of *Senna occidentalis*were threshed mechanically with a mortar and pestle, then soaked for 14 hours, (Shlini and Siddalinga Marthy, 2015), washed thoroughly to remove seed testa and bad ones. Then seeds were fermented for 72 hours by placing them in an airtight container (Plastic container of about 8 liter size, containing about 7 liters of water), air-dried, weighed and oven dried at 60˚C in a paper bag for 24 hours followed by cooling. The dried seeds were pulverized using a laboratory blender and sieved using a 0.5 mm mesh sieve (Ingweye *et al.,* 2010). The flour was stored in screw-capped bottles at room temperature for further analyses.

3.2 Experimental Fish

The fish was a cross between male *Heterobranchus bidorsalis* and female *Clarias gariepinuss* (*Heteroclarias*) obtained from Kateez Integrated farm, Zaria.The fish was a cross between *Heterobranchus bidorsalis* and *Clarias gariepinuss.* The number of fish was 180 and their whole weight was 1780g and their average length was 9.62cm and average standard length was 8.47cm.

3.3 Experimental Design

3.3.1Replacement levels

Various replacement levels were designed. The designation of the diets was diet I/1 (control), II/2, III/3, IV/4 and V/5 as 0, 25, 50, 75 and 100% respectively.

3.3.2 Replications

The number of replications was three (3).

3.3.3 The fish sample size and rearing facility

Ten plastic aquaria of 20cm were used (two aquaria/experimental diet) in a static culture system. One hundred and eighty fish were obtained. The fishes were acclimatized for 14 days.After 14 days of acclimatization, the average body weight of 10 juveniles of *C. gariepinus* was measured and transferred into each of the Plastic experimental tanks using a scoop net.

3.3.4Design

The design used for the experiment was Completely Randomized Design (CRD)

3.3.5 Feeding frequency

Feeding frequency recommended by Marimuthu *et al.* (2010) was adopted. The fishes were fed twice daily (in the morning and evening) with their various experimental feeds at 3 percent body weight.The water was changed twice in month in order to avoid contamination of the water by the uneaten feed and faeces.Feeding response was monitored recorded.

3.3.6 Culture period

The fish were reared for was 84 days (12 weeks) days culture.

3.3.7 Data collected

The data collected during the experiment such were final weight (g), final length (cm), final standard length, two weeks data for growth patterns, total feed consumed/applied, cost of replacing soya bean with SOSM (cost of soya bean, cost of *Senna occidentalis*, cost of processing, cost of other ingredients), water quality parameters.

3.3.8 Growth and nutrient utilization parameters

The growth parameters for the estimation of each of the treatments include, weight gain, length gain, standard length gain, specific growth rate, feed conversion ratio, protein efficiency ratio, net protein utilization, survival rate, nutrient metabolism.The above parameters were estimated as follows:

3.3.8.1*The daily feeding ratio*: was measured at every two weeks using the Electronic weighing balance to measure Mean Weight (g), Ruler for Standard Lenth (SL) (cm) and Total Length (TL) (cm).

3.3.8.2 *Weight Gain (g/day)* - following the methods of Sawhney and Gandotra, (2010); Eyo and Ekanem (2011); Mustapha *et al*. (2014)

Weight gained=Final weight (g) - Initial weight (g)

3.3.8.3 *Specific Growth Rate (SGR)* - following the use by Aderolu and Sogbesan (2010); Sawhney and Gandotra (2010); Eyo and Ekanem (2011); Mustapha *et al*. (2014)

$$SGR = \frac{\text{Log } (Wt_2) - \text{Log } (Wt_1)}{t_2 - t_1} \times \frac{100}{1}$$

where,

Log (Wt_1) =Natural Log of the weight of the animal at the initial stage (t_1)

Log (Wt_2) = Natural Log of the weight of the animal at the final stage (t_2)

t_2-t_1 = Time taken(in days)

3.3.8.3 *Survival Rate (SR)* - following the method of Sawhney and Gandotra, (2010); Eyo and Ekanem (2011); Mustapha *et al*. (2014)

$$SR = \frac{\text{Initial number of fish stocked} - \text{mortality}}{\text{Initial number of fish}} \times 100$$

3.3.8.4 *Food Conversion Ratio (FCR)* - following the method of Sawhney and Gandotra, (2010); Eyo and Ekanem (2011); Mustapha *et al*. (2014)

This is the amount of unit weight of food that specimens were able to convert to unit muscle.

$$FCR = \frac{\text{Total weight of diet fed (g)}}{\text{Total weight of fish (g)}}$$

3.3.8.5 *Net protein Utilization (NPU)*- following the use by Balogun *et al.* (2004)

$$\frac{\text{Final body protein - initial body protein}}{\text{Protein consumed + total dietary protein}} \times \frac{100}{1} (\%)$$

3.3.8.6 *Protein Efficiency Ratio (PER)* - following the method of Sawhney and Gandotra (2010); Eyo and Ekanem (2011); Mustapha *et al*. (2014)

$$\frac{\text{Weight gain (g)}}{\text{Protein intake of fish (g)}}$$

3.3.8.8 *Protein intake*

PI = Feed intake X Percentage (%) Protein in diet

3.3.8.9 *Nitrogen metabolism (Nm)* - using the formula of

$$\frac{(0.5)(b-a)h}{2}$$ (Dabrowski, 1997)

3.4 Experimental Diet

The experimental diet was *Senna occidentalis*. Pearson Square Method was used to compound a feed of 45% crude protein content (Pearson, 1991) that will suit the protein need of the *Heteroclarias.*

3.4.1. Processing of experimenal diet

The feed was milled and added appropriately to replace soya bean using various replacement. levels. The ingredients were mixed together in each case and water added together accordingly as shown on table 3.1 (Akinwande *et al.*, 2002).A hand pelletizer was used in pelleting the feed, sun dried and packed in separate containers.

Table 3.1: Composition (kg) of Experimental Diets Used for Feeding Trial (isonitrogeneous diet)

Ingredients	**Levels of *S. occidentalis* seed meal replacing soya beans (%)**				
	0	25	50	75	100
Soya bean	40.930	30.680	20.450	10.230	0
S. occidentalis	0	10.23	20.45	30.68	40.9
Fish meal	20.45	20.45	20.45	20.45	20.45
Yellow maize	28.64	28.64	28.64	28.64	28.64
Bone meal	1	1	1	1	1
Palm oil	3.5	3.5	3.5	3.5	3.5
Salt	0.8	0.8	0.8	0.8	0.8
Vitamin premix	0.7	0.7	0.7	0.7	0.7
Methionine	2	2	2	2	2
Lysine	2	2	2	2	2
Total	100	100	100	100	100

ME (kg/cal) = 800.79

3.5Chemical Analyses of*Senna occidentalis* Seed Meal (SOSM)

3.5.1 Determination of Dry Matter (DM)

Moisture content was determined using the method of Association of Official Analytical Chemists (AOAC, 2009). A clean crucible was dried to a constant weight in an oven at 105˚C, cooled in a desiccator and weighed (W_1). Two grams of the Senna occidentalis seed mealflour was weighed into the crucible (W_2) and dried in the oven at 105˚C. The crucible and its contents were cooled to room temperature in a desiccator and weighed. The procedure was continued several times until a constant weight was obtained (W_3). Three replications were used. The moisture content was calculated thus:

% Moisture content = W_2-W_3/W_2-W_1 X 100

3.5.2 Determination of Ash Content (AC)

Ash was determined using the method of AOAC (2009). Two grams of the finely ground SOSM powder was weighed (W_2) into a previously weighed, clean and empty crucible (W_1). The sample was then ignited and cooled in a desiccator before being taken to the furnace. After maintaining the sample at 550˚C in a muffle furnace for eight hours, the crucible and its residual ash were removed from the furnace and then allowed to cool to room temperature in a desiccator before being weighed (W_3). Three repilications were made. The ash content was calculated thus:

% Ash = W_2-W_3/W_2-W_1 X 100

3.5.3 Determination of Ether Extract (EE)

Ether extract was determined using the method of AOAC (2009). Two hundered cm^3 of petroleum ether (40-60˚C) was transferred into a clean dry 250 ml round bottom flask fitted with

soxhlet extraction unit after some boiling chips were added. Fat free extraction thimble containing 20 g of the sample of SOSM flour was then placed into the soxhlet extraction unit usin forceps. Cold water circulation was put on. The heating mantle was switched on and heating rate adjusted to a temperature between 40 and 60℃ until the solvent was refluxing at a steady rate. Extraction was carried out for six hours after which the heating mantle was switched off. By means of a rotary evaporator, the solvent was recovered and the oil left behind was quantified. Three replications were used.

The extractable lipid of the sample was estimated using the following formula:

% Lipid= Weight of lipid extracted / Weight of direct sample X 100

3.5.4 Determination of Crude Fibre (CF) content

Crude fibre was determined using the method of AOAC (2009). Two grams of the finely grounded SOSM powder was placed into a round bottom flask. Then 100 ml of a 0.023 moules of Sulphuric Acid (H_2SO_4) was added and the mixture was boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. This was then quantitatively transferred into the conical flask and 100 ml of 0.312 moules Sodium Hydroxide solution was added and the mixture boiled again under reflux for 30 minutes before being quickly filtered under suction. The insoluble residue was washed until it was base free, dried to constant weight in an oven set at 100℃, cooled in a desiccator and reweighed (C_3).

The crude fibre content was estimated using the following formula:

% Crude fibre = $C_2 – C_3$/W X 100

The loss in weight on ashing (incineration) = $C_2 – C_3$, Weight of original sample = W

3.5.5 Determination of Crude Protein (CP)

Crude protein was determined accordingthe method of AOAC (2009) using Kjeldahl method. Two grams (2g) of the SOSM powder was weighed into 100 ml Kjeldahl digestion flask. Then 1g of catalyst mixture (Na_2SO_4 + anhydrous $CuSO_4$, ratio 1:10) was added to speed up the reaction. Twenty five(25) ml of concentrated Sulphuric acid was also added into the flask. The content in the Kjeldahl digestion flask was then heated slowly at first in a Kjeldahl digestion unit until fretting subsided and then more vigorously (with occasional rotation of the flask to ensure even digestion and to prevent overheating of the content) until the green digest was obtained. The solution was transferred into a 100 ml volumetric flask and diluted to which indicate the value of the mark with distilled water. Then ten ml aliquot of the diluted solution (or digest) was pipetted into a Kjeldahl distillation flask and ten ml of 40% Sodium hydroxide solution was added. The solution was steam distilled and the liberated ammonia was trapped in a 250 ml conical flask containing 10 ml of 4% Boric Acid and a drop of mixed indicator (methyl red and methyl blue in a of ratio 2:1). Distillation was continued until the pink colour of the indicator turned greenish. The content of the conical flask was titrated with 0.1 moules Hydrogen chloride(HCI) and endpoint was indicated by a change from greenish to pink colour. The volume of the acid used for each distillate (as well as the blank) was recorded. Three replications were made.

The % total N per sample was estimated using the following formula:

$\%N = V_1 – V_0 \times M \times 14 \times 100 / 2 \times 1000 \times 10 \times 1 \times 100$

Where,V_0 = Volume of HCl required for the blank, V_1= Volume of HCI required for 10 ml sample solution, M = Concentration of HCl (0.1 M), 14 = Atomic weight of nitrogen, 100 = Total volume of digest (ml), 10 = Volume of digest distilled (ml), 2= Weight of sample taken in (gram)

1000= Conversion factor to decimeter

The crude protein was estimated as: 6.25 X %N

3.5.6 Determination of Nitrogen Free Extractscontent (NFE)

The AOAC (2009) method was used. Carbohydrate as Nitrogen Free Extract (NFE) was estimated as: NFE = 100 – (Crude protein + ether extract + Ash + Moisture + Crude fibre).

3.5.7 Determination of Anti-nutritional Factors (ANFs)

3.5.7.1 *Determination of oxalate of Senna occidentalis seed meal*

It was determined by the method of AOAC (2009). One gram of the sample was placed a 200ml volumetric flask, 150ml of distilled water and 10ml of 6m HCl were added. The mixture was warmed in a water bath at 70˚C for 4 minutes. The supernatant was then diluted to 200ml using distilled water. Three 50ml aliquots of the supernatants were evaporated 25ml; the brown precipitate was filtered off and washed. The combined solution and washings were titrated usingconcentrated ammonia solution drop by drop until salmon pink colour of methyl orange changed to faint yellow.

The solution was heated on water bath to 90˚C and the oxalate was precipitated with 10ml of 5% calcium chloride ($CaCl_2$) solution. The solution was allowed to stand overnight and then was centrifuged. The precipitate was washed into a beaker with hot 25% H_2SO_4 dilute to 125mls with

distilled water and after warming to 90˚C, it was titrated against 0.05M $KMnO_4$. The oxalate content was estimated using the following formula:

1ml of 0.05M $KMnO_4$ = 2.2mg oxalate

3.5.7.2 *Determination of Phytic acid / Phytate of Senna occidentalsi seed meal*

Phytate phosphorus was determined by the method of AOAC (2009). One gram of the defatted sample was extracted for 1 hour with 100ml of 3% TCA with occasional swirling by hand. The suspension was centrifuged and a definite volume (10ml) of the supernatant was precipitated with 4ml of ferric chloride solution (containing 2mg of ferric ion ml"1 in 3% TCA). The precipitate of ferric Phytate was converted to ferric hydroxide with 4ml 1.5M NaOH. The ferric hydroxide was dissolved in hot 40ml 3.2M HNO_3 and transferred to100ml volumetric flask. The volume was made up with distil water.

Five milliliters (5ml) of the aliquot was transferred to another 200ml volumetric flask and diluted to approximately 70mL to the contents,20ml of 1.5 moule (Potassium thiocyanate) KSCN was added and volume make up with water. The iron was determined calorimetrically using 1.5M KSCN. The absorbance was read at 480nm against 1.5M KSCN as reagent blank.

Standard Iron: This was prepared by dissolving 0.5g of iron III chloride ($FeCl_3$) in distilled water and the volume made up to 1 litre. This stock solution contained 3.4 x 105 ppm of iron.

Working Standard: 1ml of the stock solution was pipetted into 100ml volumetric flask and diluted to mark with, distilled water, there by obtaining the concentration of 3ppm. Absorbance was measured at480nm with 1.5M KSCN. The phytate (%) was estimated using the following formula: C unknown $=A_{unknown\ x}C_{standard\ /}\ A_{standard}$

Phytic acid content was calculated on the assumption that it contained 28.20% phosphorus by weight. The mole of iron to Phytate phosphorus is 1:1.

Therefore C unknown = C iron = C phytate-p; *Phytic acid = C phytate-p – 28% C phytate-p*

3.5.7.3 *Determination of Tannin*

The method of AOAC (2009) was used 1.0g ground sample was weighed into a conical flask and l00ml of distilled water added. This was boiled gently on a hot plate for one hour and filtrated through a Whatman filter paper (No.44) into a 10ml volumetric flask. The extract on the paper was washed with distilled water and extract diluted to volume.Pipette 50ml of distilled water and into 10ml of dilute extract(aliquot volume) into a 100ml conical flask followed by, 5ml Folin-Denis reagent and 10ml of saturated Na_2CO_3 solution. Dilute to volume with distilled water. After mixing the solution is allowed to standard for 30 minutes in a water bath (25˚C). Optical density is measured at 700mm and the absorbance compared on a standard tannic acid (tannin) curve.

The tannic acid standard curve was prepared by dissolving 0.2g of tannic acid in distilled to 200ml (1mg/ml)., Varying concentration (0.1-l.0mg/ml) of standard tannic acid solutions are pipetted into 10 different 100ml conical flask. 5ml Folin Denis reagent and 10 ml saturate Na_2CO_3 solution was pipetted into the test tubes. Then make up to the 100ml mark with distilled water. The solution is left to stand for 30mins and the optical density was measured at 700mm. A plot of optical, density tannic acid concentration was made; with the line passing through the origin. % Tannin content was estimated as follows:

Tannin content C(mg/100g)= C (mg) $_x$ extract volume $_x$ 100/Aliguot volume $_x$ Wt of sample(g),

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

3.5.7.4 *Determination of Saponin*

The method of AOAC (2009) was used, 5.0g ground was weighed into a thimble and transferred into the soxhlet extractor chamber filled with a condenser. Some quantity of acetone reflux was poured into the flask. The sample was exhaustively extracted, at its lipid for 3 hours and the solvent distilled off. This was the first extraction.

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

% Saponin = (wt of saponin) / (Wt of sample) 100

3.5.7.5 *Determination of Flavonoids*

AOAC (2009) method was used. Each sample (0.30 g) weighed into a beaker was extracted with 30 cm^3 of distilled water for 2 hours and filtered with Whatman filter paper number 42 (125 mm). To 10 cm^3 of the aqueous filtrate of each wood extract was added 5 cm^3 of 1.0 M dilute ammonia solution followed by the addition of 5 cm^3 of concentrated tetraoxosulphate (VI) acid. Appearance of yellow colouration which disappeared on standing shows the presence of flavonoids.

2.5.7.6 *Determination of Alkaloids*

The method of AOAC (2009) was used. Extraction of component from 2 grams of each wood powder sample was carried out using 5% tetraoxosulphate (VI) acid (H_2SO_4) (20 cm^3) in 50% ethanol by boiling for 2 minutes and filtered through Whatman filter paper number 42 (125 mm). The filtrate was made alkaline using 5 cm^3 of 28% ammonia solution (NH_3) in a separating funnel. Equal volume of chloroform (5.0 cm^3) was used in further solution extraction in which chloroform solution was extracted with two 5 cm^3 portions of 1.0 M dilute tetraoxosulphate (VI) acid. This final acid extract was then used to carry out the following test: 0.5 cm^3 of Dragendorff's reagent (Bismuth potassium iodide solution) was mixed with 2 cm3 of acid extract and precipitated orange colour infers the presence of alkaloid.

3.5.8Determination of minerals

AOAC (2009) procedure was adopted. Working solution for digestion was prepared using Nitric acid 600ml, perchloric acid 80ml and sulphoric acid 20ml. Apparatus: 100ml beaker, crucible tongue, Digestion system (temperature at 450°C), measuring cylinder and grinding or milling machine.

Some 0.3g of the ground samples of *Senna occidentalis* were weighed into 100ml beaker and 30ml of the working solution was added. Sample was digested in a digesting system at 450°C, until the fuming become pure white and no carbon or particles is longer seen, then samples were made using up to 50ml using distill water. AA-6200 Atomic Absorption spectrometer (AAS) (Company – Shimadzu) was used to estimate the mineral composition of the sample in part per million (ppm).

3.6Carcass Composition

The proximate composition of fish carcass was carried out before and after the feeding trial according to the recommended methods of AOAC (2009). The analysis was repeated three (3) times.

3.7Extraction of Blood from Fish

Blood samples of about 2 milliliters was collected via the caudal peduncle pincture (AQUALEX, 2004) with the aid of a 2ml thermodynamic plastic syringe and needle, and the blood was dispensed into ethylene diamine tetra-acetic acid (EDTA) anticoagulant bottle for haematological studies.

3.8Haematological Studies

Haematogy was done using Sysmex Hematological analyser, Coagulation Systems. Packed cell volume (haematocrit), haemoglobin (Hb) concentration, Red blood cells (RBC), White blood cells (WBC), Mean corpuscular haemoglobin (MCH) and Mean cell volume (MCV) were analysed using the analyzing machine (Sysmex Hematology Systems, Coagulation Systems) (Dacie and Lewis,2011).

MCHC = Haemoglobin (Hb) Concentration / PCV [Haematocrit (Ht)]

MCV= Hb/EC (Erythrocytes) X 100

MHC = Hb/RBC X 100

Using the method of Onyia *et al*. (2015), the parameters were all calculated.

3.9Cost Benefit Value

The recommended methods of Konyeme *et al*. (2005) was used to calculate the cost benefit value of replacing soya bean meal with *S. occidentalis*seed meal as follows:

Expenditure=cost of feeding (₦) + cost of fingerlings stocked (₦)

Net Profit= Total cost of fish cropped (₦) - Total expenditure (₦)

Profit Index=Value of fish/Cost of feed

Benefit Cost Ratio=Cost of feed(₦)/Value of fish (₦)

3.10 Water Quality Parameters

The water quality parameters monitored were temperature (˚C) using mercury in glass thermometer, pH using a Digital pH meter Model S358236HANNA and Dissolved Oxygen concentration (mg/l) by titration.

The DO was measured using Winkler (2018) method. Water was poured into a 300ml BOD bottle. 2ml $MnSO_4$ and 2ml alkali-iodide azide reagent was added. Then,blocked with care to exude air bubbles.It was then mixed gently by inverting the bottle a number of times until a clear supernatant was obtained.It was then allowed to settle for two minutes after which 2ml conc. H_2SO_4 was added by allowing the acid run down the neck of the bottle. It was stoppered again and mixed by gentle inversion until dissolution was complete.One hundred mills (100ml) of the prepared solution wastransferred into a conical flask. Titrated with 0.0125N of $Na_2S_2O_3.5H_2O$ solution to a pale straw/yellow color.1ml of freshly prepared starch solution was added and the color becomes blue. Titration was continued by adding the thiosulphate drop wise until the blue color disappeared.

3.11Data Analysis

Proximate, mineraland anti-nutritional factors were subjected to student's T-test since there were only two treatments (raw and fermented senna) data obtained from growth, carcass, haematology were subjected to one way analysis of variance (ANOVA).Differences between the treatment means were determined using new Duncan's multiple range tests(DMRT, 1955) with the aid of SPSS 10 at 95% confidence interval ($p \leq 0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 Chemical Composition of *Senna occidentalis*

4.1.1 Proximate composition of *S. occidentalis* seed

The result of proximate composition of *S. occidentalis* seeds meal is shown in (Table 4.1). The raw seeds meal had higher values in Dry Matter (92.87 g/100g), Crude Fiber (7.6 g/100g), Ether Extract (4.6 g/100g) and Nitrogen Free Extract while fermented seeds had higher values for Ash (2.155 g/100g) and crude protein contents (29.7 g/100g). There was significant difference ($P<0.05$) betweenthe dry matter, crude protein, ash, nitrogen free extract and crude fiber in the raw and fermented sample. Fermentation significantly increased the crude protein, ash content of seeds to 22.12 g/100g and 3.48 g/100g, respectively.

4.1.2 Anti-nutritional factors concentration of *S. occidentalis* seed

Generally, the levels of anti-nutritional factors were lower in fermented (processed) *S. occidentalis*.The anti-nutrient composition of *S. occidentalis* is presented in table 4.2. Anti-nutritional factors such as alkaloids, flavonoids, oxalate, phytate, saponin and tannin were detected in the raw seeds. The alkaloid, flavonoid, saponins, tannins, phytates and oxalates content in the raw *S. occidentalis* were higher than those observed in the fermented *S. occidentalis* andtheir percentage reductions are 12.10, 11.71, 19.35, 7.61, 20.32 and 25.13.

Table 4.1:Mean Proximate Composition of Raw and Fermented *S. occidentalis* Seed

Nutrients(g/100g Dry Matter)	*Senna occidentalis* seed meal	
	Raw	Fermented
Dry Matter	92.87±0.04[a]	91.58±0.11[b]
Ash	2.08±0.00[b]	2.155±0.02[a]
Ether Extract	14.92±0.01[a]	14.35±0.12[b]
Crude Fiber	7.6±0.14[a]	3.515±0.0[b]
Nitrogen Free Extract	4.6±0.00[a]	3.64±0.02[b]
Crude Protein	22.64±0.01[b]	29.07±0.07[a]

Means with same superscript along the same column are not significantly different (P>0.05)

Table 4.2: Anti-nutritional Factors of Raw and Fermented *S. occidentalis* Seed

Antinutrients (mg/100g)	*Senna occidentalis* seed meal		
	Raw	Fermented	FAO values
Alkaloids	6.20±0.01[a]	5.45±0.07[b]	-
Tannins	9.20±0.01[a]	8.50±0.01[b]	(0.309mg/100g)
Saponins	4.60±0.01[a]	3.71±0.01[b]	-
Flavonoids	11.10±0.01[a]	9.80±0.01[b]	-
Phytate	3.15±0.06[a]	2.51±0.01[b]	(568.14mg/100g)
Oxalate	7.48±0.04[a]	5.60±0.01[b]	-

Means with same superscript across each row are not significantly different(P>0.05)

4.1.3Mineralvalues of raw and fermented *S. occidentalis* seed meal

Selected mineral composition assessed for*S. occidentalis* is shown in table 4.3. Generally, there was an increase in all the micro-nutrients in the processed (fermented) *S. occidentalis,* exceptfor the iron which dropped in the fermented stuff.The value of calcium increased from 405.55 g/100mg in the raw *S.occidentalis*to 490.1 g/100mg in the fermented *S.occidentalis*. The value of calcium increased from 2.75 g/100mg in the raw *S.occidentalis* to 3.1 g/100mg in the fermented *S.occidentalis.* Magnesium increased from 660.675 mg/100mg in the raw seed to 776.625 g/100mg in the fermented seed of *S.occidentalis* and is the most abundant of all the minerals found in the *Senna occidentalis* seed.

The value of potassium recorded in this study increased from 110 g/100mg in the raw *S.occidentalis* to 112 g/100mg in the fermented *S.occidentalis.* The value of Irondecreased from 245.350 g/100mg in the raw *S.occidentalis* to 234.100 g/100mg in the fermented *S.occidentalis.* Sodium increased from 50 mg/100mg in the raw seed of *S.occidentalis* to 68 g/100mg in the fermented *S.occidentalis*seed. The value of zinc inceased from 8.65 g/100mg in the raw seed to 11.525 g/100mg in the fermented *S.occidentalis* seed. Mg is the most abundant of all, followed by Ca, Fe (though dereased after fermentation), K, Na, Zn, and Cu wasthe least abundant.

4.1.4 Proximate composition of feed

Table 4.4 shows the proximate composition (g/100g Dry Matter)of compounded feed containing *Senna occidentalis* seed meal. Ash content and crude protein decreased with increased incorporation of *Senna occidentalis* seed meal-based diet. The control (0%)replacement level of *S. occidentalis* seed meal had the highest value of crude protein (46.80), followed by 25% (46.73), 50% (46.71),75%

Table 4.3: Mean (± SEM) Mineral Composition of Raw and Fermented *S. occidentalis*meal

Minerals	Raw *S. occidentalis* seed (g/100mg)	Fermented *S. occidentalis* seed (g/100mg)	P Value
Calcium (Ca)	405.55	490.1	*92.79
Copper (Cu)	2.753	.1	*0.77
Potassium (K)	1 1	0 1 1 2	*1.00
Magnesium (Mg)	660.675	776.625	*109.62
Sodium (Na)	5	06 8	*92.79
Iron (Fe)	245.350	*234.100	18.64
Zinc (Zn)	8.65±0.99	11.525	*0.11

There is significant difference in mineral composition between raw and fermented *S.occidentals* seeds ($p \geq 0.05$)

Table 4.4:Mean (±SD) Proximate Composition of the Experimental Diet Containing *S. occidentalis* Replacing Soya bean

	Levels of *Senna occidentalis* seed meal replacing soya bean					
Nutrients	Raw	Diet 1 (0%)	Diet 2 (25%)	Diet 3 (50%)	Diet4 (75%)	Diet 5(100%)
DM (%)	92.87±0.04^{a}	90.47±0.05^{c}	91.69±0.01^{b}	92.85±0.35^{a}	87.54±0.57^{d}	90.18±1.57^{c,d}
Ash (%)	2.08±0.00^{b}	9.76±0.05^{a,b}	9.82±0.17^{a}	8.22±0.18^{b}	5.37±0.39^{c}	5.88±0.14^{d}
EE (%)	14.92±0.01^{a}	21.11±0.04^{b}	15.46±0.06^{e}	23.09±0.01^{a}	17.40±0.46^{d}	18.20±1.86^{c}
CF (%)	7.6±0.14^{a}	3.68±0.01^{b}	2.48±0.19^{a}	2.67±0.01^{a}	2.66±0.07^{a}	3.45±0.38^{b}
CP (%)	4.6±0.00^{a}	46.80±0.02^{c}	46.73±0.03^{c}	46.71±0.01^{c}	46.45±0.15^{b}	46.09±0.16^{a}
NFE	22.64±0.01^{b}	-	-	-	-	-

Means with same superscript across each row are not significantly different (P>0.05)

KEY: DM = Dry Matter EE = Ether Extract CP = Crude Protein CF = Crude Fiber

(46.45) and diet with 100% had the lowest (46.09), crude protein of 25% diet (46.80) gave the highest and best crude protein among the experimental diet. The Dry matter in the experimental diets of 50% (92.85) inclusion level had the highest value followed by 25% (91.69), 0% (90.47), 100% (90.18) and 75% (87.54). The diet with 25% inclusion level had the highest value (9.82) of ash followed by 0% (9.76), 50% (8.22), 100% (5.88) and least was diet with 75% (98.37) inclusion level. Diet with 50% (23.09) inclusion level had the highest value of ether extract followed by 0% (21.11), 100% (18.20), 75% (17.40) and then 25% (15.46) had the least. Diet with 0% (3.68) inclusion level had the highest value of crude fiber, followed by 100 (3.45), 50% (6.47), 75% (2.66) and then 25% (2.48). The DM, CP, EE, CF and ash contents of all the feeds compounded differ significantly among the diets.

4.2 Utilization of *Senna occidentalis* Seed Meal

4.2.1 Growth response of *Heteroclarias* fed *Senna occidentalis* seed meal replacing soya bean

Table 4.5 shows the growth response and nutrient utilization of *Hereroclarias* fed fermented *S. occidentalis* seed meal replacing soya bean. There was a decrease in final weight, mean weight gain, mean length gain, specific growth rate, FCR, PEED and Nm with increase in the replacement levels of SOSM among the five diets. The highest mean final weight (346.50g) was observed in fish fed 0% fermented *Senna occidentalis* seed meal that is 100% soya bean (control), followed by those fed SOSM replacing SBM 25% (330.50g), 75% (323.67g), 50% (315.00g) and fish fed 100% (212.37g) fermented *Senna occidentalis* had the lowest. Among the experimental diets, 25% (330.50g) inclusion level gave the best result with respect to the mean weight gain. The Fish fed control diet (0%) had the highest mean weight gain (337.13g),

Table 4.5: Growth Performance and Nutrient Utilization of Fish Fed Varying Inclusion Levels of *Sennaoccidentalis*Seed Meal Replacing Soya bean

Replacement Levels	Levels of *Senna occidentalis* seed mealreplacing soya bean				
	DIET 1 (0%)	DIET 2 (25%)	DIET 3 (50%)	DIET 4 (75%)	DIET 5 (100%)
Initial Weight (g)	9.37±0.95[a]	9.2±0.95[a]	9.33±0.06 [a]	9.10±1.85[a]	9.33±0.49[a]
Final Weight (g)	346.50±7.41[a]	330.50±1.80[b]	323.67±11.06[c]	315.00±3.16[d]	212.56±0.11.10[e]
Feed Intake (g)	132.06±32.61[a]	88.55±16.93[c]	90.78±24.84[b]	75.95 ±37.28[d]	41.63±5.21[e]
Mean Weight Gain(g)	337.13±8.33[a]	321.30±1.35[b]	307.8±2.25[b],[c]	305.67±3.21[d]	203.03±1.27[e]
Initial Length(cm)	9.99±1.10[a]	9.85±0.74[a]	9.83±0.28[a]	9.20±1.74[a]	9.22±1.67[a]
Final Length (cm)	26.18±0.37[a]	23.73±0.70[b]	21.53±0.15[c]	19.93±1.55[d]	19.06±0.15[e]
Mean Length Gain (cm)	16.19±1.46[a]	13.88±1.36[b]	12.88±2.79[c]	9.34±0.30[e]	10.55±0.70[d]
ISL (cm)	8.27±1.30[a]	8.69±0.60[a]	8.86±0.81[a]	7.85±1.34[b]	8.69±0.26[a]
FSL (cm)	23.58±0.45[a]	21.92±1.04[b]	18.20±0.52[e]	20.63±2.24[c]	19.23±0.85[d]
SLG (cm)	15.31±1.51[a]	13.23±1.63[b]	9.34±0.30[e]	12.88±2.79[c]	10.55±0.70[d]
SGR (%BW/day)	1.38±0.06[a]	1.37±0.05[a]	1.37±0.11[a]	0.34±0.04[b]	1.34±0.01[a]
FCR (%BW/day)	0.39±0.96[b]	0.28±0.05[b]	0.29±0.08[b]	5.20±0.84[a]	0.25±0.12[b]
CF (%BW/day)	1.93±0.12[c.d]	2.48±0.21[c]	0.91±0.07[d]	3.17±0.08[b]	4.06±0.84[a]
PFED (g)	61.80±12.27[a]	41.39±7.93[a,b]	19.19±2.41[b]	42.18±11.66[a,b]	35.47±17.42[c]
PER (%BW/day)	5.68±1.36[c]	7.98±1.68[b]	7.64±1.84[b]	0.43±0.07[d]	9.84+±3.74[a]
NPU (%BW/day)	19.07±4.46[e]	25.56±3.89[c]	20.97±10.13[d]	34.61±17.45[a]	27.88±14.55[b]
SR (%BW/day)	100.00±0.00[a]	93.33±11.55[b]	83.33±5.77[c]	93.33±11.55[b]	100.00±0.00[a]
Nm(%BW/day)	910.02±144.41[a]	488.06±27.51[b]	199.84±61.41[d]	418.12±228.43[c]	186.00±19.61[e]

Means with same superscript acrosseach row are not significantly different (P>0.05)

KEY: ISL-Initial Standard Length, FSL- Final Standard Length, SLG- Standard Length Gain SGR- Specific Growth Rate FCR – Feed Conversion Ratio, CF – Crude Protein Fed, P F- Protein Fed, PER – Protein Efficiency Ratio, NPU – Net Protein Utilization, SR – Survival Rate Nm – Nutrient Metabolism

followed by fish fed with 25% (321.30g), 75% (307.8g), 50% (305.67g), and fish fed 100% (203.03g) fermented *Senna occidentalis,* respectively. The 25% feed gave the best result among the experimental diets.The better final standard length per fish (23.58cm) was recorded for diet with 0% inclusion level. The mean standard length gain per fish ranged between (9.34-15.31cm) in diet 3 and 1 respectively.

The highest mean final length per fish of fish (26.18cm) was observed in *Heteroclarias* fed diet with 0% inclusion level followed by fish fed 25% (23.67cm), 75% (22.87cm), 100% (19.06cm) and fish fed 50% (18.93cm) had the least. The final length of 25% (23.67cm) gave the best result among the fish fed experimental feeds. The mean length gain per fish ranged between (9.84 - 16.19cm) in diets 1 and 5 respectively. Specific Growth Rate was higher in diet 1 containing 0% (1.38% days) *Senna occidentalis* seed meal followed by 75% (1.37.011%days) and 25% (1.37.005%days), 100% (1.34%days) and lowest in diet containing 50% (0.34%days) *Senna occidentalis* seed meal.

Feed utilization efficiency parametersas presented in Table 4.5 revealed that the value of FCR (5.20) was highest in diet containing 75% *Senna occidentalis* seed meal followed by 0% (0.39), 50% (0.29), 25% (0.28) and lowest (0.25) in diet containing 100% *Senna occidentalis* seed meal. PER was highest (9.95) in diet containing 100% *Senna occidentalis* seed meal followed by 25% (7.98), 50% (7.90), 75% (0.43) and lowest (0.27) in diet containing 100% *Senna occidentalis* seed meal. Among the experimental diets, the value of FCR (5.20); PER (0.43.007), was the highest, indicating that the best nutrient utilization was recorded in diet 2. NPU was highest in the diet containing 75% (34.61) followed by 100% (25.56), 25% (25.56), 50% (20.98) *Senna occidentalis* seed meal and lowest (19.07) in diet containing 0% *Senna occidentalis* seed meal. Among the experimental diets, the values of SR recorded were best in 0% and 100% (100%

survival) followed by 25% and 75% (93.33%) and lowest (83.33%) in diet containing 100% *Senna occidentalis* seed meal. Nm was highest (910.02) in diet containing 0% *Senna occidentalis* seed meal, followed by 25% (488.06), 75% (418.12), 50% (199.83) and the lowest in diet containing 100% (186.00) *Senna occidentalis* seed meal. Among the experimental diets, the values of NPU (20.98) and Nm (488.06) were best in 75% and 25% respectively. The values of CF (0.79) and PF (42.18) were the best obtained.

The final weight, feed intake, final length, mean weight gain, mean length gain, FSL, SLG,SGR, CF, PFED, PER,SR and Nm differed significantly ($p>0.05$) from the control diet.The FCR of the diets only differ significantly in diet containing 75% *Senna occidentalis* seed meal.

4.2.2 Carcass composition of fish fed different dietary levels of *Senna occidentalis*

Table 4.6 showsthe carcass composition of *Heteroclarias* fed Senna occidentalis seed meal replacing soya bean meal. The highest (55.05 g/100g dry matter) carcass Crude Protein recorded in fish fed with 0% level of *Senna occidentalis* replacing soya bean meal, 25% (54.21 g/100g), 75% (52.09 g/100g), 50% (51.89 g/100g) and 100% (50.37 g/100g) all increased from the initial value of (49.99 g/100g). Ether Extract range between (16.65-26.17 g / 100g dry matter). Fish fed 25% (17.59 g/100g) inclusion level had the highest and best ash level among the experimental diets including the initial value (14.42 g/100g dry matter), followed by 75% (14.68 g/100g) (also > initial value of 14.42 g/100g), 0% (12.89 g/100g), 50% (12.84 g/100g) and then 100% (9.26 g/100g) had the least value. The dry matter content range between (91.05-93.49 g/100g).CF of fish range between 0.64 g/100g in diet having 100% inclusion level and 1.9 g/100g in fish of 0% inclusion level.

Table 4.6: Carcass composition of *Heteroclarias*Fingerlings Fed *S. occidentalis* Seed Meal Replacing Soya bean.

Nutrients	Levels of *Senna occidentalis* seed meal replacing soya bean					
	Initial	DIET 1 (0%)	DIET 2 (25%)	DIET 3 (50%)	DIET 4 (75%)	DIET 5 (100%)
DM	91.59±0.36[a]	92.40±0.13[a]	93.49±0.02[a]	92.09±0.01[a]	91.05±0.05[a]	91.37±42.17[a]
ASH	14.42±0.02[c]	12.89±0.06[d]	17.59±0.01[a]	12.84±0.01[d]	14.68±0.02[b]	9.26±0.05[e]
EE	18.91±0.02[c]	26.17±0.04[a]	16.65±0.01[d]	26.15±0.01[a]	22.82±0.03[b]	23.31±17.30[b]
CF	0.80±0.01[b]	1.90±0.02[a]	0.18±0.01e	0.55±0.01[d]	0.79±0.01[b]	0.64±0.02[c]
CP	49.99±0.95[a]	55.05±2.74[a]	54.21±3.12[a]	51.89±1.67[a]	52.09±3.60	50.37±3.81[a]

Means with same superscript acrosseach row are not significantly different (P>0.05)

KEY: DM = Dry Matter EE = Ether Extract CP = Crude Protein CF = Crude Fiber

Based on table 4.6, as far as Crude Protein, Ether Extract (Lipid) and Dry Matter are concern, there is no higher or best mean values at all. All of them bear 'a' as a superscript; meaning they do not differ much significantly ($P<0.05$), however, the Ash Content and Crude Fiber significantly differ at ($P<0.05$) among the experimental diets.

4.3 Haematological Parameters of *Heteroclarias* Fed *S. occidentalis* Seed Meal Replacing Soya bean Meal

Table 4.7 shows the haematological parameters of *Heteroclarias* fed *Senna occidentalis* seed meal replacing soya bean meal for 12 weeks. Higher mean values of Packed Cell Volume (PCV), Haemoglobin (Hb) and Total Protein(TP) were observed in fish fed the control diet. However the PCV values were lower in fish fed 75 and 100%*S. occidentalis* replacement level. There were no significant ($p>0.05$) difference between the PCV and Hb values of fish fed the control diet compared to those fed 25 and 50% *S. occidentalis* replacement level.

Similarly, no significant variations ($p>0.05$) were observed between TP values of fish fed 25-100% *S. occidentalis* replacing soya bean meal.

Higher mean value of Total White Blood Cell Count (TWBC) was observed in fish fed 50% *S. occidentalis* replacement level while significant variations ($p>0.05$) were observed between fish in the initial (coppens), fish fed the control, 25 and 100% *S. occidentalis* replacing soya bean meal. However, higher values of Total Red Blood Count were observed in fish fed the controland 25% for Hetero and Lympho. Moreover, significant variations ($p>0.05$) were observed between Lympho values of fish fed all the dietary levels of *S. occidentalis* replacing soya bean meal.

Table 4.7: Mean(±SD) Haematological Parameters of *Heteroclarias* Fed *S. occidentalis* Seed Meal Replacing *Senna occidentalis* Seed Meal

Blood indices	Levels of *Senna occidentalis* seed meal replacing soya bean					
	Initial	DIET 1(0%)	DIET 2 (25%)	DIET 3 (50%)	DIET 4 (75%)	DIET 5 (100%)
PCV (%)	42.40±0.69^{a}	41.67±6.43^{b}	35.67±2.08^{d}	34.33±586^{e}	38.33±2.52^{c}	30.00±5.66^{f}
Hb (g/dl)	13.67±0.5^{b}	13.87±2.14^{a}	11.83±0.68^{d}	11.40±1.93^{e}	12.73±0.81^{c}	9.95±1.91^{f}
TP (g/dl)	5.00±0.00^{a,b}	4.03±0.06^{a}	3.40±0.53^{a}	4.00±0.40^{a}	4.10±0.26^{a}	4.40±2.26^{a}
TWBC (x10^{9}/ml)	10.20±0.00^{e}	14.10±2.01^{c}	14.73±1.10^{c}	18.40±2.42^{b}	13.83±3.25^{d}	20.00±1.41^{a}
TRBC (x10^{12}/ml)	7.00±0.00^{a}	6.93±1.02^{a,b}	5.97±0.29^{b,c}	5.63±1.01^{b,c}	6.47±0.50^{a,b}	4.95±0.78^{d}
HETERO (%)	25.00±0.00^{a}	6.67±4.51^{d}	7.33±4.16^{d}	9.00±1.73^{c}	13.67±5.69^{b}	9.00±1.41^{c}
LYMPHO (%)	64.00±0.00e	90.33±3.51^{a}	91.00±4.36^{a}	86.33±1.53^{c}	84.00±3.46^{d}	89.00±4.24^{b}
MCVH (%)	60.00±0.00^{a}	60.00±0.00^{a}	59.67±0.58^{a}	61.00±1.73^{a}	59.33±0.58^{a}	60.50±2.12^{a}
MCH (pg/dl)	20.0±0.00^{a}	20.0±0.00^{a}	19.67±0.58^{a}	20.33±0.58^{a}	19.67±0.58^{a}	20.50±0.71^{a}
MCHC (g/dl)	33.00±0.00^{a}	33.00±0.00^{a}	33.00±0.00^{a}	33.00±0.00^{a}	33.00±0.00^{a}	33.00±0.00^{a}

Means with same superscript acrosseach row are not significantly different (P>0.05)

KEY: PCV - Packed cell volume, Hb – Haemoglobin Concentration, TP- Total Protein, TWBC – Total White Blood Cell Count, TRBC - Total Red Blood Cell Count, MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentration, Lymho – Lymphocytes, Hetero - Heterophils

Based on table 4.7, as far as Mean Corpuscular Volume, Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin Concentration are concern, there is no higher or best mean values at all. All of them bear 'a' as a superscript; meaning they do not differ much significantly ($P<0.05$. TWBC, TRBC, Hetero, Lympho all differ significantly ($P<0.05$) but MCV, MCH and MCHC differ significantly among the experimental diets. Packed cell volume (PCV) decreased from initial value of 42. 40% and ranged between 30%-41.6% among the fish fed experimental diets. Haemoglobin (Hb) content increased from an initial value of 13.67 to 13.87% (best) in fish fed diet 1 but decreased in fish fed 4 (12.73 g/dl) diet 2 (11.83 g/dl), 3 (11.40 g/dl) and diet 5 had the lowest (9.95 g/dl).

Total protein (TP) decreased from initial value of 5.00 and range between (3.4 - 4.4 g/dl) among the the fish fed experimental diets. Total White Blood Cell Count increased from 10.20 initial value and range between 10.1 in diet 1 and 20.0 in diet 5 of 100% *S. occidentali*s. Total Red Blood Cell Count decreased from initial value of 7.00 and ranged from 4.95 - 6.93 in fish fed diet containing 100% *S. occidentali*s and 6.93 in fish fed diet containing 0% *S. occidentali*s respectively. Hetero decreased from initial value of 25% and ranges between 6.67% in fish fed diet containing 0% *S. occidentali*s and 13.67% in fish fed diet containing 75% *S. occidentali*s. Lympho increased from initial value of 64% and ranges between 84% in fish fed diet containing 75% *S. occidentali*s and 91.00% in fish fed diet containing 25% *S. occidentali*s. MCH (%) range between 59.33 in fish fed diet containing 75% *S. occidentali*s to 61.00 in fish fed diet containing 50% *S. occidentali*s with initial value was 60.00. MCH (pg/dl) range between (19.67 and 20.50pg/dl) in diet 2 and 5 respectively; with initial value of 20.00. MCHC (g/dl) is 31.00 for all inclusions.The values for PCV (38.33 of diet 4) Hb (12.73 of diet 40), TP (4.40 of diet 5), TWBC (20.00 of diet 3), TRBC (6.47 of diet 4), Hetero (13.67% of diet 4), Lympho (91% of diet

2), MCV (61.00% of diet 3), MCH (20.50pg/dl of diet 5) gave the highest and best results for haematologic indices.

4.4 Water Quality Parameters of Culture Medium

The water quality parameters monitored is shown in table 4.7. The hydrogen pH ranged between 7.35-7.46during the experiment. The temperature was between 27.21 and 28.80°C, electrical conductivity,249.5-300.44 µs, total dissolved solids was between 128.31 and 152.28ppt and dissolved oxygen was between 4.24 and 4.46 mg/l. However, electrical conductivity, Total Dissolved Solids and Dissolved Oxygen differ ($p<0.05$) significantly among the entire treatments.

4.5 Cost Benefit Indices

The cost benefit analysis of the *Senna occidentalis* is shown in table 4.8. Cost of feed, net profit, profit index, expenditure, cost benefit ratio and cost/kg differed significantly ($P<0.05$) among the diets.Among the replacement levels, cost of feed (₦75.51) for each fish, expenditure (₦575.51) and cost/kg (₦555.07) were highest 0% and gave the best net profit (₦39.47 per fish), profit index (0.55) and cost benefit ratio (1.87).

Table 4.9:Water Quality Parameters of Culture Medium

S. occidentalis replacement level (%)					
Water parameters	DIET 1(0%)	DIET 2 (25%)	DIET 3 (50%)	DIET 4 (75%)	DIET 5 (100%)
pH	7.35±0.08[a]	7.40±0.10[a]	7.44±0.02[a]	7.37±0.07[a]	7.46±0.00[a]
T (0°C)	28.71±0.15[a]	27.21±2.64[a]	28.66±0.11[a]	28.80±0.29[a]	28.64±0.22[a]
EC (µs)	300.44±7.73[a]	278.39±10.15[b]	263.00±5.05[c,b]	249.50±11.35[c]	267.25±11.19[b,c]
TDS (ppt)	152.28±2.31[a]	138.50±5.29[b]	131.00±2.20[c]	128.31±2.44[c]	129.25±0.59[c]
DO (mg/l)	4.30±0.08[a]	4.38±0.0[a,b]	4.37±0.12[a,b]	4.24±0.08[a,b]	4.46±0.14[b]

Means with same superscript acrosseach row are not significantly different (P>0.05)

KEY: pH- Hydogen ion Concentration T – Temperature EC –Electrical Conductivity TDS – Total Dissolved Solids DO – Dissolved Oxygen

Table 4.10: Cost Benefit Analysis of Fish Fed Varying Inclusion Levels of *S. occidentatalis*

Cost-benefit indices	*Senna occidentalis* replacement levels				
	DIET 1 (0%)	DIET 2 (25%)	DIET 3 (50%)	DIET 4 (75%)	DIET 5 (100%)
Cost of feed(₦)	73.51±18.09^{a}	46.14±8.64^{b}	20.81±2.61^{d}	41.83±11.38^{c}	21.24±10.46^{d}
Net Profit (₦)	39.47±6.26^{a}	21.30±1.35^{b}	8.07±0.85^{e}	18.13±9.91^{c}	8.67±2.66^{d}
Profit Index	0.55±0.12^{a}	0.48±0.12^{a}	0.39±0.07^{a}	0.45±0.28^{a}	0.45±0.18^{a}
Benefit ratio	1.87±0.46^{b}	2.19±0.54^{a}	2.60±0.42^{a}	2.73±1.28^{a}	2.48±0.90^{a}
Expenditure (₦)	573.51±18.09^{a}	546.14±8.64^{b}	520.81±2.61^{d}	541.83±11.38^{c}	521.24±10.46^{d}
Cost/kg Diet(₦)	555.07±0.05^{a}	524.16±0.00^{b}	500.00±0.00^{c}	462.81±0.00^{d}	340.10±106.27^{e}

Means with same superscript across each row are not significantly different (P>0.05)

KEY: Kg- Kilogram ₦– Naira

CHAPTER FIVE

5.0 DISCUSSION

5.1 Chemical analyses of *Senna occidentalis* Seed Meal

5.1.1Proximate composition of *Senna occidentalis*seed meal

The result of this study indicates that the crude protein increased by 28% (from 22.64-29.07g/100g)after the fermentation of*S. occidentalis*, this confirms that fermentation has positive effect on the seeds of this plant. Augustine *et al*.(2014) reported a crude protein value of 29.54%after the fermentation of *S. obstifolia* seed meal. The crude protein in this study differs from 2.75% reported by Aja *et al*. (2017) and 14.88 % by Abdelseed*et al*. (2011).Elias *et al.* (2002) and Inyang and Zakari (2008) reported that fermentation significantly increased protein content of pearl millet, while El-Hag *et al*.(2002) observed a decrease in fermented pearl millet. The increase in protein may be attributed to increase in number of lactic acid bacteria during fermentation(Anthony and Babatunde, 2014).

In most fermented high protein products, the extent of increase in most important factors is the changes in texture and flavor (Achi, 2005). Soluble low molecular weight peptides and amino acids that contribute to flavor are produced through the enzymatic breakdown of proteins (Ouoba *et al*., 2003) as a result of fermentation. Differences observed in the proximate composition of the raw and fermented seed could be due to the variety of interspecies level (Ingweye *et al*., 2010).

The dry matter content of *Senna occidentalis* in this study was highandthis is a clear indication that the seeds may have less storage problems and reduce the cost of handling.

This finding is similar to those obtained from most raw seeds of legumes like *Milletia purpuresus, Phaseolus aurens*and *Vigina sinensis* (Khattab *et al*., 2009).Umar (2006) and Aminu (2011)reported high dry matter in coffee senna and cotton seed.

The ash content increased slightly after fermentation of *Senna occidentalis* seed, from 2.08g/100g in the raw to 2.16g/100g (though not significantat P≤0.05) in the fermented seed of *S. occidentalis*. The ash content indicates that the seed meal may be a potential source of vital dietary mineral elements. Augustine*et al.* (2016) revealed that the ash content ranged between 5.70% in the unfermented to 6.82% in the fermented seeds of *Senna obstifolia*whileUwagbute *et al.*(2000) and Anthony and Babatunde (2014)reportedan increase in ash content of cowpea as the fermentation period increases. This may be linked to the ability of fermentation to loosen the bonds within the elements resulting to an increased availability of minerals (Adams, 1990).

The ether extract was observed to reduce slightly after the fermentation process. This decrease might be due to the increase in the activities of lypolytic enzymes during fermentation which hydrolyse fat components into fatty acid and glycerol (Chinma *et al*., 2009). Chang and Miles (2004) and Anthony and Babatunde (2014)reportedthat break down of fatty acids is responsible for the aroma, taste, odour and texture of fermented feed ingredient. The values obtained in this study is higher than 8.00% obtained for *S. occidentalis* (Ogundipe *et al*., 2003)

The crude fibre of *S. occidentalis* in this study reduced by about 50%,andsuch reduction might be due to the enzymatic breakdown of the fiber during fermentation by lactic acid bacteria which utilized them as carbon source and converted them to microbial biomass thereby reducing the fiber content (Rainbault, 2001; Magdi,2011).

5.1.2 Anti-nutrients of *Senna occidentalis* seed meal

The anti-nutritional factors obtained in this study decreased in the fermented *Senna occidentalis* seeds compared to the raw ones. The decrease may be due to the metabolic microbial activity during fermentation. Ali *et al.*(2009)reported that tannin act as carbon source for microorganism and as an inducer of the endogenous synthesis of enzymes. Patricia *et al.* (2012) reported that tannin acyl hydrolyses the ester bond of tannins. Heat stable compounds in cereals and legumes such as tannins and hydrates are easily removed after fermentation (Osman, 2004).

The decrease in phytic acid content may be attributed to microbial phytase and endogenous phytate present.Similar observations were made by Anthony and Babatunde (2014) and Igbabul *et al* (2014) during the fermentation of mahogany beans and millet.The phytate content in this study varieswith 5-6 of phytic acid/kg(Ritcher *et al*., 2003). However, this finding was also dissimilar to those observed for pearl millet (El Hag *et al*., 2002; Abdel-Rahaman *et al*., 2005; Eltayeb *et al*., 2007), sorghum (Osman, 2004; Kayode *et al*., 2007; Abdel-Haleem *et al*., 2008), rice (Liange*et al*., 2008) and maize (Abedel-Hady *et al*., 2005; Ejigui *et al*., 2005). According to Abu (2005) fermentation reduced phytate in Locust bean seed (*Parkia filicoidea*)by 17.77%.

Also, phytate reduces the bioavailability of minerals and protein digestibility by the formation of phytic acid - protein complexes. That complex damages the pyloric ceacum and depressing the absorption of nutrients (Francis *et al*., 2002).The reduction of phytates in fermented feed isattributed to hydrolysis of phytates into lower inositol phosphates (Real *et al*., 2007 and Abdelseed *et al*., 2011).

In this study, there was significant reduction of alkaloids in the seeds of *S. occidentalis*. The low level of alkaloids in the fermented sample could be due to the extended hours spent during soaking of the seed sample to remove the seed coat and fermentation process.Alkaloids could be removed from plant seed by aqueous extraction and treatment (Kumar *et al*., 2012).

Similarly, lower saponin level in*S. occidentalis* in this study was due to the effect of fermentation that reduced this compound. Ozovehe (2013) reported that saponins in lower dietary level, increased growth of tilapia but when the concentration of saponin was increased, it gave negative effect and lower growth performance.

The concentration of oxalate in this study is lower than the raw seeds of *S. occidentalis* and this reveals that fermentation has desirable effect on the seeds. This is lower than 83.25 mg/ 100g for the seeds of *S. obstifolia*(Ingweye *et al*.,2010). Oxalate is an anti-nutrient which under normal conditions is confined to separate compartments. However, when it is processed and/or digested, it comes into contact with the nutrients in the gastrointestinal tract. When released, oxalic acid binds with nutrient, rendering them inaccessible to the body. If feed with excessive amounts of oxalic acid is consumed regularly, nutritional deficiencies are likely to occur, as well as severe irritation in the lining of the gut (Habtamu and Nigusse, 2014).

Too much of soluble oxalate in the body prevents the absorption of soluble calcium ions as the oxalate binds the calcium ions to form insoluble calciumoxalate complexes. As a result of this, people with the tendency to form kidney stones are advised to avoid oxalate-rich foods (Adeniyi *et al*., 2009).

5.1.3 Mineral content of *Senna occidentalis* seed

The most abundant mineral in *S. occidentalis* seed is magnesium,which is higher thanthe concentration found in Nigerian cowpea, *M. obanensis* and mung beans (Umeron *et al*., 2005 and Chinma *et al*., 2008). Ingweye *et al*. (2010) reported a lower magnesium value (640 mg/100 g) in *Senna occidentalis*. Mg in the body sustains lower irritability of neuromuscular system and activates enzymes like phosphate which require ATP (Banerjee, 2004).

Potassium concentration in this study increased when the seeds were fermentated and this is an indication that fermentation increases the availability of this element in the seeds of *S. occidentalis*. The value obtained in this studyis lower than 1200g/ 100mg obtained by Ingweye *etal*. (2010) in *S. occidentalis*. Members of the bean family are rich in K (Vasadevan and Sreekumari, 2007). Potassium is necessary for nerve transmission, maintenance of osmotic pressure and acid base equilibrium, activation of certain enzymes, and uptake of certain amino acids as well as carbohydrate and protein metabolism in African catfish (Banerjee, 2004).

The seeds of *S. occidentalis*contains high Calcuim value (405.55 mg/100g) which increased to (490.1mg/100g) after fermentation. This indicates that fermentation enhanced the calcium content of the seeds. The values recorded in this study are high in comparison with 13.9, 47.4, 84 mg/100mg reported for raw *Lathyrus maritimus* (beach pea), *M. obanensis* (Odudu) and mung bean (*P. aurens*) seeds respectively (Umoren *et al*., 2008). The values are low compared to 585.00 mg/100g for raw Nigerian cowpea (Umoren *et al*., 2008) and 960 mg/100g for raw *Senna obstifolia* (Ingweye *et al*., 2010). Calcuim is necessary for

teeth and bone formation, blood clothing, the working of muscles, reputation of heart beat and maintenance of acid base equilibrium in the body of animals and it is said to be abundant in legumes in African catfish(Banarjee, 2004).

The value of iron in this study shows that the seeds are negatively affected, because decrease was observed after fermentation. This is similar to 234.60 mg/100 g reportedfor *Senna obstifolia*(Ingweye *et al*., 2010). However, the ironcontent was higher than 11.5 g/100mg for soya bean and mung beans (Vasudevan and Sreekumari, 2007).

The Sodium content in this study (50 g/100g) shows significantincrease. Feeding fish feed that is rich in *S. occidentalis* has the potential to maintain is lower than 600 mg/100gfor *Senna obstifolia*(Ingweye *et al*., 2010). The value recorded for zinc in this study (8.65 g/100g) is lower than 53.12 mg/100g for *Senna obstifolia*(Ingweye *et al*., 2010). The value recorded for copper in this study (2.75 g/100g) is lower 2.75 mg/100g for *Senna obstifolia*(Ingweye *et al*., 2010).

5.1.4 Proximate composition of experimental diet

The highdry matter recorded in this study shows that the experimentalfeedhas longer shelf life and can easily be stored and preserved without any difficulty. Though there was no significant difference ($P>0.05$) in the dry matter of the experimental diets 1 and 5 and in crude protein content of experimental diet and it is up to the level required by African catfish (40-45%) for good growth. This is an indication that diet containing *S. occidentalis* can favour the good growth of *Heteroclarias*.

The ether extract in this study ishigh and significantly different ($p<0.05$) among the diets. Adebayo and Aladejare (2015)reported that growth of fish is suppressed when the ether

extract value is high and this normally lead poor feed intake.Only slight difference was observed in Crude fibre (CF) valuesamong the diets and ranged between 3.68-2.48%. The digestibility of the feed wil be positively enhanced due to the lower value recorded. Robinson *et al.* (2006)reported the CF of 3-4% could be accepted by *Clarias gariepinus* fingerlings without any adverse effect.

5.2 Fish Performance

5.2.1 Fish growth and feed utilization of *heteroclarias* fed graded levels of fermented *Senna occidentalis* seed

The findings in this study shows decrease in growth parameters as the level of *Senna occidentalis* increased. This could be due to the presence of antinutrients. Hussain *et al*. (2016) reported that increase in *Moringa oliefera* in feed decrease the growth of fish, which may be due to negative effect of some anti-nutrients (in Moringa). The result of this study shows similarity with the findings of Samkelisiwe and Ngonidzashe (2014) and of Richter *et al*. (2003) that higher substitution of *M. oliefera* with fish meal had an impact on lowering the growth performance because of the presence of anti-nutrients such as phenol, tannins, phytates and saponins.According to Eusebio *et al*. (2004) the presence of anti-nutrients may hinder the digestibility and utilization of dietary nutrients. Furthermore, Espe *et al*. (2006) and Olsvik *et al*. (2011) stated that plant based feed may reduce the fish growth due to reduction in feed intake.

The decrease in values of specificgrowth rate (SGR) in the treatments could be due to difference in the *Senna occidentalis* seed meal inclusion level, which decreased at increasing level of *Senna occidentalis* seed meal in the diet. This compared by 2.79, 2.25, 2.41, 2.51 and 1.97 for Aqua, Coppens, Dunante, Pira and Rana respectively, reported by

Tunde *et al*. (2016). Dienye and Olumuji (2014) reported that anti-nutritional factors contained in *M. oliefera* leaf meal based diet were probably responsible for retarded growth response of the fish.

Thehigh FCR observed in this study could be due to the fibre content in various inclusion levels of the feed, and this means the fish did not utilize the feed effectively. Dienye and Olumuji (2014) reported that high FCR may be due to high fiber content of *M. oliefera* leaf meal in the diet, and which resulted in poor digestiblity of the diet (Gatlin, 2010; Aderolu and Sogbesan 2010). Falayi (2009) reported a lower FCR which therefore implies efficient food utilization by that animal. Similarly, Enyidi and Mgbenka (2012) reported that young *Clarias gariepinus* growth rate was impaired by high inclusion levels of plant material as replacement for fish meal in the diets.

The values of weight gain and Specific Growth Rate indicates that the control diet gave the best growth performance. Amongst the experimental diets, 25% inclusion level of *S. occidentalis* gave the best results for SGR and mean weight gain.

The survival rate obtained in this study was high. This is an indication that the *Senna occidentalis* seed meal may not be toxic to the fish. The highest mortality recorded in Treatment C (83%) may be due to stress associated with handling, as most of it occurred after samplings which tend to reduce oxygen level towards the end of the production period. The values obtained are much greater than 53.33 and 49.45 % reported by Sinyangwe *et al*. (2017) in the end of production of *C. gariepinus*.

5.2.2 Carcass composition of *Heteroclarias* fed *Senna occidentalis* seed meal replacing soya bean

The carcasscomposition of *Heteroclarias* fed *Senna occidentalis* seed meal replacing soya bean shows no variation in the crude protein after the feeding trials.This implies that the

dietary crude protein has no negative effect on the experimental fish. Abo- State *et al.* (2014) reported that different inclusion levels of Moringa did not affect any of carcass parameters except CPwhich increased significantly ($P<0.05$) between treatments while ash content declined with increasing dietary content.

This is an evidence that there was synthesis and increased tissue protein production in this study. In contrast, Yusuf *et al*. (2016) reported that growth of fish was due to increase in weight only. Auta *et al*. (2007) reported increased in crude protein when they used boiled castor seed meal to feed fingerling of *C. gariepinus*. The result of crude fibre obtained in this study shows that after the feeding trial trial, the crude fibre decreased. This clearly shows that the crude protein was efficiently utilized without hindreance by high level of CF anddigestibility was enhanced. In contrast, Gatlin (2010) reported that the amount of crude fibre in fish feed is usually less than seven percent of the diet to limit the amount of undigested materials entering the culture system.

In this study, the increase in Ether extract after the feeding trial shows that the feed was utilized by the fish. According to the Madalla *et al*. (2013) lipid content may decrease due to poor feed intake which results in starvation and mobilization of body lip reserves to meet energy requirements for vital body functions. Glencross (2003) and Ganzon-Naret (2014) observed increasing trend of crude fat or lipid after addition of Moringa seed in fish feed to replace fish meal protein and canola meal in sea bream diets.

5.3 Haematological Indices of *Heteroclarias* Fed *Senna occidentalis* Seed Meal Replacing Soya Bean Meal

In the current study, decrease was observed in the values of packed cell volume when the fish was fed experimental diets. This could be due to anaemia resulting from shrunken red blood cells. The values recorded in this study for Packed Cell Volume recorded are not in agreement with 20.67-31.67% documented by Adesina *et al.* (2017) and 36.0% reported by Adeyemo (2007). Akinrotimi (2011) reported 32.64-45.74%, 20-35%(Erondu *et al*., 1993), 22.40% (Bhaskar and Rao 1989), 38.40% by Kori-Siakpere and Ubogu (2008), 27.58-35.50% by Musa and Omoregie (1999) and 27.87%(Diyaware *et al*., 2010). Similarly,Dienye and Olumuji (2014)reported 21.00 to 32.00 % which fell within the range of 20 and 50% reported by Pietse *et al*. (1981) and PCV values above 50% are rarely reported (Etim *et al*., 1999).Reduction in concentration of the PCV in the blood usually suggests the presence of toxic factor which has adverse effect on blood formation (Osuigwe *et al*., 2005). Piotr *et al.* (2014) stated that an increase in PCV can result from increase in RBC (due to acute stress and spleen evacuation), erythrocyte swelling (due to lower pH) etc. Adamu and Audu (2008) reported that a significant decrease in PCV may be attributed to gill damage or impaired osmoregulation causing anaemia and haemodilution.

The Haemoglobin concenrationin this study was high and no significant difference between initial (those fed imported diet before the experimental diets) and the ones fed the control and diet 1. This could be due to reduction in oxygen concentration in the experimental tanks. Conflictingoxygen values were reported in different experimental situations. For instance, Kori-Siakpere and Ubogu (2008) reported 15.31mg/l for juvenile hybrid, 9.63 g/dL x 10^3,13.00mg/l recorded from *C. gariepinus*(Ogunji *et al*., 2005), 27.00mg/l

(Sunomonu and Oyelola, 2008), 18.43 and 16.g/dL x 10^3 (Onyia, 2013), 6.80- 6.90g/100 ml reported for *C. gariepinus* fed sunflower seed meal (Adesina *et al*., 2017), 8.70g/dl for *Clarias gariepinus* (Sowunmi, 2003) and also compared favourably with 9.60 g/100ml (Omitoyin, 2006) for African catfish juveniles fed poultry litter and 10.62mg/l(Osuigwe *et al.*,2005) who fed *C. gariepinus* with jackbean meal-based diets. The present values, however, are higher than 4.46g/100ml reported for *Heterotis niloticus* (Fagbenro *et al*., 2000), 7.90 to 8.90g/ml reported for *C. gariepinus* fed dietary levels of *Moringa oliefera* leaf meal (Dienye and Olumuji, 2014), . The reduction of the Hb concentration could simply imply that the diets having higher substitutions contained low quality protein, and this may have resulted in poor transportation of oxygen from the respiratory organs to the peripheral tissue (Roberts *et al*., 2000). According to Qiang *et al*. (2013), when dietary protein level is are low, physiological stress is induced and this damages the liver, leading to reduced RBC and Hb concentration. Similarly, Abdel-Tawwab *et al*. (2010) reported a decrease in RBC and Hb in fish fed low protein levels in the diet.

Non-variability in total protein as observed in all the fish fed all the diets in the current study shows no significant difference. The observed values of the total protein content of *S. occidentalis* inclusion levels suggest low antinutrients content in the diet and has no deleterous effect on the fish. Yadav *et al*. (2003) also reported a decrease in total protein content in *Channa punctatus* induced with stem-bark extract of *Croton tiglium.*On the contrary, the total protein values (5.00 ± 0.00 – 3.40 ± 0.00 g/100ml) obtained in the current study were much lower compared with 40.19 ± 7.45 g/100ml (Ayoola, 2011), 2.6-4.17(g/100ml) recorded by Akinrotimi (2011) while Olasunkanmi (2011) reported higher total protein levels in *C. gariepinus* fed diets containing raw *Mucuna* meal.

The decrese in white blood cell (WBC) counts of *Heteroclarias* fed *S. occidentalis*seed meal replacing soya bean meal in this study after the feeding trials, could be due to common stress and toxicity. The values in this study are lower than $22.33 \pm 2.52 \times 10^9$ /ml to $31.65 \pm 95.37\ 9 \times 10^9$ /ml recorded for *Clarias batrachus* (Maheswaran *et al*., 2008) and 193.70 $x10^3$/µLby Diyaware *et al*. (2010) but superior to 16.13×10^3 - $16.39 \times 10^3 mm^{-3}$ for *C. gariepinus* (Sotolu and Faturoti, 2011).White blood cells are known to play an important role in the immune system and responses of living organisms. Low WBC count in the fish could be attributed to a reduction in the number of lymphocytes. Alkahem (1994)reported reduced WBC counts in *Oreochromis niloticus* and associated them with the effects oftoxicants and also to a generalised stress response resulting from increased pituitary-interrenal activity. Alkahem (1994) linked reduced WBC count with a reduction in the number ofcirculating thrombocytes and lymphocytes due to a diminution in the delivery of lymphocytes to the circulatory system through a reduced lymphocyte production and a rapid destruction of cells which leads to an increased rate of peripheral removal oflymphocytes.

However, Ajani (2006) and Kori-Siakpere *et al*. (2009)stated that high WBC count means a release of more cells to maintain homeostasis while low WBC count is a common stress response. Therefore, increasing or decreasing numbers of WBCs are normal physiological reactions to toxicants and these show the response of the immune system under toxic conditions. Many researchers have reported low values of leukocyte counts in the blood of fish exposed to different pollutants and attributed them to the reduction in the number of circulating lymphocytes and thrombocytes (Koprucu *et al*., 2006).

White blood cells and lymphocytes are the defence cells of the body. Douglas and Jane (2010) suggested that WBC has implication in immune responses and the ability of the animal to fight infection. High WBC count is usually associated with microbial infection or the presence of foreign body or antigen in the circulatory system (Osuigwe *et al*., 2005).

Red blood cells (RBCs) values obtained in the currentstudy varied significantly with the initial value in all the experimental diets.This could be due to the inability of the erythropoiteic to release new RBCs to improve the carrying capacity of fish blood with resultant higher values of erythrocyte count. The values are higher than 1.24 x 10^{12}/ ml - 1.88 x10^{12} / ml reported by Smith and Kaplan (1952), 2.46 x10^{3}/µL by Diyaware *et al*. (2010) and 1.63 x 10^{12} reported by Kori-Siakpere and Ubogu (2008).These values were also superior to 1.9 x 10^{12} / l reported for *Clarias gariepinus* juveniles (Ayoola, 2011).Erythrocyte count greater than 1.00 x 10^{6} mm^{-3} is considered high and indicative of high oxygen-carrying capacity of the blood, which is characteristic of fish capable of aerial respiration and with high metabollic activity (Jimoh *et. al*., 2012).

Heterophils values in this study were found to bereduced significantly from the initial value (25%), before the feeding trial. However, the reduced heterophil counts indicates absence of bacterial or any pathogennic infection. This result is lower than 24.67-42.33% reported by Akinrotimi(2011). Increase in the total heterophil count has been reported to be a sign of bacterial infection or as a result of stress (Adesina *et al*., 2017).

TheMean Corpuscular Volume (MCV) showed no statistical variation and suggests that MCV was not affected by dietary treatments. Mean Corpuscular Volume (MCV) indicates the status or size of the RBCs and reflects a normal or an abnormal cell division during the production of red blood cells (erythropoiesis). The values are lower than 79.20 –

105.32µg/ml reported for *Heteroclarias*(Anyanwu *et al*., 2011); also lower than 87.50 to 210.00µg/ml reported by Dienye and Olumuji (2014), 240.18 recorded for juvenile hybrid African catfish (*Heteroclarias*) (Kori-Siakpere and Ubogu, 2008), and 200.93 for *C. gariepinus* fingerlings (Gbore, 2006), 113.07µg/ml by (Diyaware *et al*., 2010), 113.93 to 138.07 for juvenile intergeneric hybrid catfishes (Diyaware *et al*., 2013) and 92.62 for *C. gariepinus* fingerlings (Ochang *et al*., 2007). Increase in MCV may be due to swelling of the erythrocytes resulting in macrocytic anaemia, while reduced MCV could be linked to shrinkage of RBCs either due to hypoxia or microcytic anaemia which is responsible for the shrinkage of RBCs(Adesina, 2008;Alwan *et al*., 2009).

The values of the mean corpuscular haemoglobin (MCH) obtained in this study (19.67 to 20.50 pg/dl) suggests that MCV was not affected by dietary treatments. The values are comparableto 20.82 to 26.60pg/dl reported by Anyanwu *et al* (2011) for *Heteroclarias* fed *Carica papaya* leaf meal-meal,24.24 pg *C. gariepinus*juveniles (Omitoyin, 2007), 24.24 pg for *C. gariepinus* juveniles (Omitoyin, 2006), 33.10 pg (Ochang *et al*., 2007). Kori-Siakpere and Ubogu (2008) reported value higher than 35.10 pg for *Heteroclarias*juvenile while (Gbore, 2006) reported 51.50 pg. However, it differs withthe findings of Olasunkanmi (2011) inwhich reported a significant increase was observed in the final MCH values in *C. gariepinus*after treating with raw mucuna seed meal-based diet.

There was no significant difference ($p>0.05$) between Mean corpuscular haemoglobin concentration (MCHC) values in this study, suggesting that MCHC was not affected by dietary treatments. The values were fairly comparable with 33.97% reported by Adeyemo (2007) and range of values (28.75 and 37.62%) reported for fish fed *M. oliefera* leaf meal-based diet (Dienye and Olumuji, 2014).Adedeji and Adegbile(2011), similarly reported

30.70% for *C. gariepinus* from Asejire dam which was lower than 33.67 to 39.03 reported by Bhaskar and Rao (1989) and Diyaware *et al*. (2013) for juvenile intergeneric hybrid catfishes. However,Diyaware *et al*. (2010) reported 34.57 pg/dL for *Clariabranchus* and 35.475% for *Heteroclarias*juvenile (Kori-Siakpere and Ubogu, 2008).

The results obtained, however,disagree with Olasunkanmi (2011) who reported that the final MCHC values in *C. gariepinus* fed raw mucuna seed meal based diets dropped below the initial value but were not statistically different among the treatments.The MCHCis a good indicator of red blood cell swelling (Wepener *et al*., 1992) and a quantitative measurement of mean amount of haemoglobin per erythrocyte in biological organisms (Moses, 2007).

The values of lymphocytes obtained in this studyincreased after the commencement of the feeding trial.This implies that the fish developed immunity leading to the increment of production of lymphocytes to combat any foreign body (or chemicals). Olasunkanmi (2011) observed no significant difference ($p>0.05$) between the initial and final lymphocyte values in *C. gariepinus* fed diets containing 10%, 20% and 30% raw mucuna meals. The values of lymphocytes in this study are far higher than 33.00% recorded for juvenile *C. gariepinus* (Adeyemo, 2007). White blood cells (WBC) and lymphocytes are the defense cells of the body, particularly in immune responses and the ability of the animal to fight infection (Douglas and Jane, 2010).

5.4 Water Quality Parameters of Culture Medium

In this study, water quality values were generally satisfactory for the growth and health of the experimental fish species. No significant different difference was observed in pH and

temperature. The temperature range during the study is within the tolerable range for the culture of African catfishand particularly *Clarias gariepinus* (Swann,2006).

The concentration of pH in all the treatments were mainly alkaline and are within the tolerable range (6.0-9.0) for the culture of catfish, although high level may have influenced elevation of some of the water quality parameters,but pH of 6.3 – 9.6 mg/l gave the best growth in cultured tropical fish species (Boyd, 1982).The dissolved oxygen in this study was low, this may be due to organic matter such as unconsumed feed and faecal matterthat may have been acted upon by agents (like bacteria) that aids deterioration and decay, which may affect the health of fish and facilitate the spread of disease. Dissolved oxygen is by far the most important chemical parameter in aquaculture (Momoh and Solomon, 2017), and oxygen concentration below optimum hampers good growth of catfish (Oyewole *et al*., 2006).

The temperature range of water in this study is within the optimum recommended range for culture of *Clarias gariepinus*(Boyd, 2007). There was no significant difference ($P \geq 0.05$) in temperature and pH values in the various plastic aquaria.

5.5 Cost Benefit Values of *Senna occidentalis* Seed Meal in the Diet of *Heteroclarias*

The total cost of producing *Senna occidentalis* seed meal and purchasing other ingredients systematically reduced from ₦524.16 to ₦340.10 from 25% to 100%, while it was ₦555.07 in the control which had no *Senna occidentalis* seed ingredient. This clearly shows a reduction in the cost of the diets of *Heteroclarias* when fed with alternative plant sources such as *Senna occidentalis* seed. This could be due to reduced growth of fish as *Senna occidentalis* seed meal was increased in the diets and as so those with higher inclusion of

the seed meal were not utilized by the fish, which means it will take the fish longer time to attain marketable sizes. Konyeme *et al*. (2005) and Aderolu and Sogbesan (2010) reported similar findings.

Similarly, Abu *et al*. (2005) reported a reduction in the cost of diets of Heteroclarias fed cassava feed meal. The net profit is considerably higher (₦39.47) in the control when compared to 25% (₦21.30), 50% (₦18.13), 75% (₦8.07) and 100% (₦8.67) inclusion levels. Therefore, the highest accruable profit was at 0% and the chance of obtaining a more variable difference of higher value of net profit at 0% indicates that, more profit would not be feasible from fish produced at more than 0% inclusion level. These findings may be associated with better growth and nutrient utilization of *Heteroclarias* feed diet that has no *Senna occidentalis* seed meal in it. The best cost benefit obtained in diet containing 0% inclusion of *Senna occidentalis* is an indication that the economic viability of *Heteroclarias* is cheaper at 0% replacement of soyabean meal.

The control diet also gave the cost-benefit ratio greater than1 (1.87).Yakubu *et al.* (2014) stated that benefit cost ratio that is greater than 1 is viable. Olagunju *et al*. (2007) when cost ratio is greater than one or equal to one indicates profit, but less than one shows lack of viability or unprofitability of the venture. Since the ratio in the diet is greater than one, therefore it is considered that replacing soyabean with *Senna occidentalis* at 0% is a profitable venture.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION ANDRECOMMENDATION

6.1Summary

In this study,fermentation (120 hours) increased the protein contentof *Senna occidentalis*from 22.64 to 29.07g/100g. It reduced the alkaloid, tannin, saponin, flavonoids, phytate and oxalate by 16.13, 98.82, 19.35, 11.71, 20.32 and 25.13%, respectively. The fermented seed meal was used to compound feed for *Heteroclarias* fingerlings reared for 12 weeks and it replaced soya bean meal at varying inclusion levels (0% as control, 25%, 50%, 75% and 100%). Of all inclusion levels, 0% (without *S. occidentalis* seed meal) gave the best growth performance and utilization; mean weight (337.13g), standard length gain (15.31cm), mean length gain (16.19cm), specific growth rate (1.38%days) and net profit (₦39.49) compared with the other diets. Carcass protein of *Heteroclarias* was best in fish fed 0% (control) level of *S. occidentalis* seed meal replacing soya bean.The result of the haematology showed significant difference in PCV (41.67%), Hb (13.87g/dl), TRBC (6.93%) and MCHC (33.00%) when the fish was fed 0% *S. occidentalis* seedmeal, and was the best.

6.2Conclusions

The proximate composition of *S. occidentalis* seeds shows that fermentation significantly increased the value of crude protein by 6.43 g/100mg dry matter. Thus fermentation of *S. occidentalis* improved the nutrient composition of the meal.Fermentation has reduced the antinutritional factors in the *S. occidentalis* seed meal. *S. occidentalis* can not be used to replace soya bean in the diet of hybrid African catfish (H x C)

6.3 Recommendations

Heteroclarias feed formulators should maintain 0% level of *S. occidentalis* seed meal replacing soya bean meal for an effective utilization and growth in feed for *Heteroclarias*.

Other processing methods such as boiling, toasting and soaking for the removal of anti-nutritional factors should be used to evaluate the most effective method for the use in wild legumes seeds.

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