#### **Research Article**



# **Substitution of saturated with unsaturated lipid extracts affects the fatty acids compositions and hematology in African catfish,** *Clarias gariepinus*

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### **ABSTRACT**

The high cost of fish oil and fishmeal has necessitated the search for alternative ingredients. Palm oil and sesame oil are plausible alternatives to fish oil. Palm oil is saturated while sesame oil is unsaturated. Five experimental diets were made with varying inclusion of crude sesame and palm oil extract. The inclusion percentages of crude sesame extract (CSE) and crude palm extract (CPE), CSE: CPE, were as follows; Feed 1, 25:5; Feed 2, 20:10; Feed 3, 15:15; Feed 4, 10:5; Feed 5, 5:25 and Feed 6(commercial feed). African catfish fingerlings were stocked in aquaria tanks in three replication. The fish were fed to satiation for 70 days with the feeds. After 70 days of feeding experiment, results showed that alanine amino transferase and aspartate amino transferase were both lowest for catfish fed feed F1, F2 and F3. The catfish had significantly higher digestibility for crude sesame extract (CSE) (93.34±0.22 %) than for crude palm extract, (CPE)  $78.98 \pm 0.11$ %. Consequently, treatment Feed F1 had the highest digestibility of 91.32 ± 0.07%, followed by feed F2, 82.25  $\pm$  0.04%. The least digested feeds were Feed 4, (20% CPE) and feed 5, (25% CPE), 70.12  $\pm$ 0.06%and 70.23 ± 0.09% respectively. The fatty acids of CPE are dominated by saturated fatty acids (SFAs), which constitute about 49.9±0.08% of the total fatty acids. Among the SFAs hexadecanoic acid or palmitic acid, comprising 46.0 ±0.02% of the total fatty acid. Oleic acid, C18:1 accounts for  $43.4 \pm 0.08$ %. The fatty acid of CSE is dominated by monounsaturated C18:1 linoleic acid. Oleic acid forms about 45.44±0.01% of the total lipid of sesame crude extract. Sesame extract also has 39.48±0.14 %, C18:2 polyunsaturated fatty acids linoleic acid. Catfish Fatty acids content resembles the fatty acid content of the feeds. The ∑SFA shows that catfish fed with feed F1 had the lowest somatic SFA of 48.58 %. The catfish fed feed 1 had more PUFA than the MUFA or SFA. Incorporation of sesame seed oil in the diets of *C. gariepinus*produces fish high in PUFA.

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# **INTRODUCTION**

Lipids are essential ingredients in fish feed, for the normal growth of fish, provision of energy, and essential fatty acids (Kim et al., (2012). Fish is a very good source of long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) eicosapentaenoic acid (C20:5 n-3; EPA) and docosahexaenoic acid (C22:6 n-3; DHA) (Shahidi and Miraliakikkbari 2004, 2005; Yang et al., 2016). Long-chain fatty acids like omega 3-PUFA, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) have very important roles in the prevention of cardiovascular diseases, neurodegenerative diseases, Alzheimer and cancer (Shahidi and Miraliakikkbari 2004, 2005). The American Heart Association recommends eating fish at least twice a week to prevent heart diseases (Kris-Etherton, 2002). The much-needed fatty acids from fish originate from their food (Parrish et al., 2012; Bailey et al., 2012). Fish oil is the main oil used for fish feed manufacturing (Enyidi et al., 2014; Ido and Kaneta, 2020). Substitution of fish oil with 50% to 60% vegetable can negatively impact the fatty acid composition of fish (Pratoomyot et al., 2008; Petropoulos et al., 2009) Fish also contains some amounts of monounsaturated fatty acids (MUFA) that also originate from fish food. Consequently, the inclusion of lipids in fish feed is very essential in enhancing the fish quality and in protein sparing, by reducing the quantity of protein utilized by the fish for energy production (Enyidi et al., 2014; Fatma and Ahmed 2020). Conversely, some carnivorous fishes have been reported to efficiently utilize protein for energy production, more other energy sources like lipids and carbohydrates. In a previous research it was noted that the spawning rate of catfish *Rhamdia quelen* fed diets containing fish oil was 78.65  $\pm$  3.60% while it was 77.15  $\pm$  3.97% for those fed palm oil, a mixture of fish and palm oil gave  $65.46 \pm 4.57\%$  hatching rate suggesting that feed affects hatching rate (Hilbig et al., 2019). Aside from reproduction, highly unsaturated n-3 fatty acids (n-3 HUFA) or long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), particularly 20:5 n-3 eicosapentaenoic acid (EPA) and 22:6 n-3 (docosahexaenoic acid [DHA]) are necessary for normal fish growth (Sargeant et al., 2003). They also act in regulating responses to folliclestimulating hormones, gonadal maturation, and the reproductive cycle steroid hormones which influence the gonad development, (Hilbig et al., 2019). Sesame seeds contain abundant unsaturated fatty acids: oleic (35.9–42.3%) and linoleic (41.5–47.9%) acids, forming 80% of total fatty acids; less than 20% are saturated fatty acids, mainly palmitic (7.9–12%) and stearic acids (4.8–6.1%) (Enyidi et al., 2014). The composition of the sesame seed oil is subject to physiological, ecological, and cultural factors (Uzun et al., 2002). However, in general, sesame oil contains oleic (35.9-47%), linoleic (35.6-47.6), palmitic (8.7-13.8%), stearic (2.1-6.4%), as well as arachidic acids (0.1-0.7%) (Uzun et al., 2002).

Palm tree *Elaeis guineensis* is widely grown in the tropics, while Malaysia, Indonesia, and Nigeria are the largest producers (Mba et al., 2015). Crude Palm oil has the highest content of β-carotene (500–700 ppm), and tocopherols and tocotrienols (600–1200 ppm), (Souganidis, 2013; Mba et al., 2015). Carotenes are antioxidants and tocotrienol prevents



the formation of cholesterol (Sen et al 2007). Crude palm oil is a good source of palmitic acid, linoleic acid, oleic acid and linolenic acid. Crude palm oil had been incorporated in the diets of *C. gariepinus*(Mgbenka and Orji 2008). In a previous experiment, Lim et al., (2001), noted that the growth of African catfish was directly proportional to palm oil additions up to 8%, but not beyond that percentage. Growth and protein utilization efficiency of African catfish fed diets with RBDPO or CPO at the same inclusion level were not significantly different. Conversely, Mgbenka and Orji (2008), stated that African catfish fingerlings accepted feed with fresh palm fruit extract faster than other feeds. Palm oil contains free fatty acids (FFAs), Beta carotenes, vitamin E, and triacylglycerol (TAGs). Crude palm oil contains about 50% saturated fatty acids (SFA) made up mainly of, palmitic acid (C16:0) 44%, 5% stearic acid (C18:0), and traces of myristic acid (C14:0). Palm oil also contains 40% monounsaturated fatty acids (MUFAs) which are made up of 40% oleic acid (C18:1) and 10% polyunsaturated linoleic acid (C18:2) and linolenic acid (C18:3) (Prada et al., 2011). Raw sesame oil has high free fatty acids content and is stable in oxidative conditions. The oxidative stability has been attributed to only sesame oil made from non-decorticated sesame. The high percentage of oil in the seed (60%), shows that sesame is an oil-bearing seed (Enyidi et al., 2014). Sesame seed or its oil had been incorporated into fish feed like Enyidi et al., 2014 for African catfish, *C. gariepinus*. This research is aimed at finding the effects of saturated and unsaturated lipids from sesame seed meal and raw palm oil and their mix, on the fatty acids profile of the feeds, African catfish and digestibility of the feed.

### **MATERIALS AND METHODS**

### *Preparation of crude palm extracts (CPE)*

Fresh palm fruits were obtained from the palm depot ministry of Agriculture at Umuahia Abia State, Nigeria. Fresh palm fruits were sorted and unwanted portions were removed. The palm fruits were washed and 2 kg of the fruits were weighed and placed in a locally produced electric crushing machine set at 200 rounds per minute, for 20 minutes. About 500 ml of water was added during crushing. After 20 minutes and at the end of the crushing palm kernels and the spent mesocarp were removed, squeezed, and discarded. The resultant crude palm slurry was boiled at 100 °C, for 1 hour to remove excess water and concentrate the slurry into a paste. The paste was placed in an airtight plastic container and stored in the freezer at -20°C till used in feed production.

#### *Preparation of crude sesame extract (CSE)*

Fresh fruits of sesame seeds were purchased from grains warehouse Relief grains depot Enugu, Enugu State Nigeria. The grains were sorted and unwanted particles were removed. The sorted sesame seeds were weighed and 2 kg were washed to eliminate dust particles. Washed grains were steeped into 2 liters of clean water and left at room temperature for 24 hours. The soaked grains were decorticated and then rinsed and dried in an electric oven at 40 °C till a 9% dry weight was achieved. The dry sesame seed was milled using an electric crusher set at 200 rounds per minute. About 500 ml of water was added. The mixture of sesame and water was stirred and sieved. The chaff was discarded and the slurry was kept in a stainless steel metal cylinder. The sesame slurry was boiled at 100 °C for 1 hour to remove excess water and the resulting slurry was stored at -20 °C till used for the experiment.in a stainless steel metal cylinder. The sesame slurry was boiled at 100 °C for 1 hour to remove excess water and the resulting slurry was stored at -20 °C till used for the experiment.





# **Production** of feed

The ingredients used in making the experimental feeds are tabulated in Table 1. Specific weights of ingredients were weighed using an electronic weighing machine sensitive to 0.0001 g. The feed was made by varying the composition of crude palm extract (CPE) and crude sesame seed extract (CSE), CPE: CSE as follows: F1, 5:25, F2, 10:20, F3, 15:15, F4, 20:10, and F5, 25:5. There was a control (commercial) diet F6, which was a commercial diet. There is equal inclusion of all protein supplements and basal ingredients for all the experimental feeds (Table1).



**Table 1: Feed composition table of experimental diets varying in inclusion in sesame** seed meal and fresh palm fruit extract used in feeding African catfish Clarias garioninus fingerlings for 90 days

Proximate composition of Vitamin premix per Kg of feed was as follows: Vit.A 4,800000 IU, Vit D: 12000 g; Vit K 0.80 g; Vit B1: 0.40 g; Vit B2:1.20 g; Vit B12: 8.00 mg; Folic acid: 0.80g; Vit C: 100.00 g; Biotin: 0.06; Choline chloride: 80.0 g; Manganese,10.0 g; Cobalt: 0.30 g; Selenium: 0.04 g; Iron: 50.0 g; Copper: 10 g; Iodine: 0.30 g.

The fish meal was Nordic fishmeal (FM) made in Denmark. The bambaranut meal was produced from decorticated bambaranuts, obtained from Enugu in Enugu State Nigeria. The ingredients were ground to powdery form and appropriate weights were mixed using an electric mixer. The mixed ingredients were mixed with 500ml of 100 °C warm water making it 25% moisture in the feed mash. The dough was preconditioned at 100  $\degree$ C using a pressure cooker. The preconditioned dough was then steam pelleted at 100 °C. The pelleted feed was dried in an electric oven at 40  $\degree$ C. The dried feeds were stored in a freezer at -20  $\degree$ C till used.



### *Experimental fish and set up*

African catfish fingerlings of average weight  $3.99 \pm 0.03$  gwere purchased from a reputable fish farm at Umuahia Abia state Nigeria. A total of 126 catfish fingerlings were purchased and distributed into three (3) replicate plastic aquariums per feed type, at 7 fish per aquarium. The catfish were acclimated for 7 days. During the acclimation, period catfish were fed a 35% protein diet. After acclimation, the catfish were fasted for 16 hours to enable gut evacuation of the feed. The dimension of the culture aquarium is length 52 cm, width 52, and height 25cm. The aquaria were supplied a constant flow (c. 0.6 L min-1) of aerated well water (30.0  $\pm$  1.5 °C) and about 40% of the outflow was filtered through a trickle filter, passed through a zeolite chamber, and reused. Average dissolved oxygen was  $-8.2\pm0.2$  mg L<sup>-1</sup>; measured with YSI oxygen meter model 550A (YSI Inc. Yellow Springs, Ohio, USA) and total gas pressure was  $(101.5 \pm 1.0\%)$ ; measured with a P4 Tracker total gas pressure meter (Point Four Systems Inc., Richmond BC, Canada). Ammonia (0.25 ± 0.07 mg  $L<sup>-1</sup>$ ) was measured fortnightly with an ammonia test kit (Tetra Merke, Melle, Germany). Water pH was  $6.9 \pm 0.1$  and alkalinity  $1.13 \pm 0.01$  mmol  $L^1$ .

### *Experimental setup and feeding of fish*

The tanks were subjected to a photoperiod of L12:D12 and the light intensity was c. 8 lux (HD 9221 lux meter, Delta OHM, Padua, Italy). The tanks were cleaned every morning before feeding the fish. The fish were collectively weighed by measuring tank biomass every two weeks. Catfish were weighed in the morning hours between 8 to 9 am before feeding. Fish were hand-fed at 0900 h and 1700 h. The fish were fed to apparent satiation. At the end of the 70-day feeding experiment, five fish were removed from each tank, killed by a sharp blow on the head and their length (to 0.1 cm) and weight (to 0.1 g) were recorded individually. The liver and visceral fat of the sample fish were removed and weighed (to 0.01 g). Sample fish were frozen at -20 $\degree$ C and freeze-dried for proximate analysis. The digestibility of the feed by the fish was measured by acid insoluble ash methods (AIA).

### *Chemical analyses Proximate analysis methods*

The catfish muscle water content was measured from muscle samples taken from two fish per tank frozen at -20°C and freeze-dried to constant weight before being stored at -80°C. Muscle samples were taken from below the dorsal fin and between the pectoral and caudal fins, excluding the skin. The crude protein contents of the feeds and fish were analyzed by the Kjeldahl method from freeze-dried muscle samples. Analyses were made using a Tecator kjeltec model 1002 system using block digestion and steam distillation (Tecator kjeltect Hogänäs, Sweden). The crude protein was calculated as: Crude protein =  $\%$  N x 6.25. The total lipids of fish muscles were measured by reformed chloroform-methanol extraction at a ratio of 2:1. Total lipid was estimated as the difference in weight of nonextracted and extracted muscle samples (Enyidi, 2012). Ash content was calculated by burning a known amount of freeze-dried muscle sample from the catfish in a muffle furnace for 24 hours at 550°C.



### *Determination of digestibility using acid insoluble ash (AIA) method*

To determine the acid insoluble ash, 5g of each treatment feed and the crude palm oil and sesame extract were weighed with an electronic balance and placed in a tarred 50 ml ashing crucible. The feeds and other samples were dried overnight at 100°C. The crucible was allowed to dry within desiccators and reweighed. The samples were ashed for 6 hours at 600°C. The ashes were transferred to a 600ml Berzelius beaker. Approximately 100 ml of 2 N HCl was added to the beaker. The mixture was boiled for 5minutes on a fiber rack. The hot hydrolysate was filtered through Whatman 541 filter paper and then washed with hot distilled water. The filter paper is transferred back to the ashing crucible and ashed for hours at 600°C. The crucible was placed in a 100°C oven to re-dry again. The crucible was cooled in desiccators and then reweighed. The analysis was all done in triplicates.

#### *Analyses of Haemato-biochemical parameters*

Three (3) *C. gariepinus,* per replicate treatment feed, were randomly selected to be used for hemato-biochemical parameters analyses. The parameters analyzed were aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, total protein (TP), total cholesterol (TC), and triglycerides (TG). Fish blood was collected by cutting the tail of the fish from replicates aquaria and drawing with sterilized syringe. The blood was centrifuged at 3000 rpm.

#### *Calculations and statistical analyses For digestibility analysis*

% Acid Insoluble Ash= (Wt. of Crucible + Ash - Wt. of Crucible) x 100 Digestibility (%) = 100 - [(AIA in feed × AIA in feces)/(Nutrient in feces × Nutrients in feed) × 100] MCV = or Fl (Femtoliter)  $MCH = x 10 \mu\mu g$  or Pg  $MCHC = x 100\%$ These parameters were measured by using chemical analyzers (Fuji DRI-CHM 3500i, Fuji Photo Film, Tokyo, Japan).

#### *Hematological analysis*

At the end of the experiment, blood was collected by cutting the tail from replicates of each treatment and used for hematological analysis. Before collecting the blood, the fish were seined out of the pond after reducing the water volume; this is because stress has been identified as a factor affecting the physiological condition and hematology of fish (Ololade and Oginni, 2010). Blood from selected fish was drawn with a sterilized disposable plastic syringe and sterilized needle through the cut tail. The use of a plastic syringe is a necessary precaution with fish blood because contact with glass results in decrease coagulation time. The blood samples (2.0ml) were dispensed into an ethylene diamine tetra acetate (EDTA) bottle for hematological analysis. The hemoglobin (Hb) concentration was measured with Hb test kit using cyanmethemoglobin method, red blood cell (RBC) and white blood cell counts were counted under a light microscope with an improved Neubauer hemocytometer



(Mgbenka and Oluah 2003). The microhaematocrit method of Blaxhall and Daisley (1973) was employed in the determination of blood hematocrit. The hematological indices: mean cell volume (MC V), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated using standard formulae: MCV = or Fl (Femtoliter)  $MCH = x 10 \mu\mu g$  or Pg

 $MCHC = X100%$ 

#### **Determination of fatty acids composition of CPE and CSE**

Total fatty acids, each fatty acid of the feed and fish were analyzed and content was determined by using a total fat determination unit (Model B-815/B-820 Buchi, Flawil, Switzerland). Total lipids were first extracted from the ingredients and feed. Using a sensitive balance, 8.0 g of sesame seeds were weighed. The sesame seed will be milled in a blender to dust. Three replicate samples of 2.5 g each were added into the solvent vessel (glass boiling container). Approximately, 45 mL of n-butanol together with 7 granules of potassium hydroxide (for saponification) were added. Then, 0.26 g of tridecanoic acid C13 was also added to serve as an internal standard. A small quantity of ascorbic acid (Vitamin C) about 2 g was added to act as an antioxidant to prevent oxidation within the extraction vessel. The sesame oil was extracted. Extraction and simultaneous saponification of the samples occurred within the extraction unit (Buchi, Flawil, Switzerland) at boiling temperature for 30 minutes. Then, 40 mL of sodium dihydrogen formic acid mixture was added to convert the potassium salts and fatty acids into free fatty acids, and the mixture was stirred for 3 minutes. The vessels were later removed from the extraction unit and allowed to cool, resulting in a two-phase system with an organic phase containing fatty acids in the upper phase/layer which was separated; 3 mL of the top layer (upper phase) was transferred using a micropipette into a 3-mL vial. The total fatty acid content and composition were then determined by gas chromatography (Model B-820, Buchi, Flawil, Switzerland). Hydrogen carrier gas at a pressure of 225 kPa and mixture gas pressure of 48 kPa with injection temperature of 220 °C and FID detector (flame ionization detector) temperature 260 °C was used in drying. This process followed methods stipulated Zhang et al. (2015). From the initial oven temperature was 130 °C, the temperature was increased at 6.5 °C min<sup>-1</sup> till the final temperature of 260 °C, held for 4 min before terminating the run. The same system of analysis was used for all other samples.

#### *Total lipid determination*

Total lipids from the CPE and CSE and the catfish were determined before the fatty acid determinations using GC-FID. Total lipid extraction followed methods stated in Zhang et al. (2015), using a total fat determination unit (Model B-815/B-820 Buchi, Flawil, Switzerland). Approximately, 8.0 g samples of each of the samples (CPE, CSE, and the catfish) were weighed. The fish was ground to dust using an electric blender. The samples were each divided into three (3) replicate samples, each weighing 2.5 g. The replicate samples were added to the solvent vessel (glass boiling container). Approximately, 45 mL of n-butanol together with 7 granules of KOH was added for saponification. Then, 0.26 g of



tridecanoic acid C13 was included as an internal standard. About 2g of antioxidant ascorbic acid (Vitamin C) about 2 g was added. Extraction and simultaneous saponification of the samples occurred within the extraction unit (Buchi, Flawil, Switzerland) at 100°C for 30 minutes. Then, 40 mL of sodium dihydrogen formic acid mixture was added, converting the potassium salts and fatty acids into free fatty acids, and the mixture was stirred for 3 minutes. The vessels were cooled resulting in a two-phase system. The upper phase was the organic containing fatty acids that were used for the fatty acid analysis. Extraction was done thrice to obtain much quantity of total lipid.

# *Fatty acid determination using Gas Chromatography (GC) flame ionization detector (FID)*

The fatty acids determinations followed methods contained in AOAC Official Method 991.39 (AOAC, 2012). Accordingly, we adjusted the initial samples quantity, temperature, and extracting solvent. About  $100 \pm 0.03$  mg/mL crude palm extract (CPE) or CSE was placed in a screw cap glass tube. Approximately 0.1 mL of palmitic acid solution (10 mg/mL) was added as internal standard. Then 1.5 mL of caustic soda, NaOH in methanol (0.5 N) was added into the tube. A drying agent, nitrogen gas, was blown into the tube from a gas cylinder for 15 seconds. The tube was tightly covered and then vortex using a Vortex Genie2 - 120V USA. The tube was then heated in a VEVOR Water Bath BHS-1 300W, set at 87 °C for 5 minutes, and then cooled. After cooling, 2 mL of  $BF_3$  in methanol (14 %, wt/vol), was added to the mixture. The tube was tightly covered and heated at 87°C for 30 minutes and then cooled. This converts the fatty acids into fatty acids methyl esters (FAME). Approximately, 1 mL of hexane, was added, mixed, and vortex for 5 mins. Then common salt, 3 mL of saturated NaCl solution was added and mixture vortex for 5 min. The mixture was segmented and the upper phase was transferred into the vial. Moisture was absorbed from the vial by including anhydrous  $Na<sub>2</sub>SO<sub>4</sub>$ .

# GC Analysis

The resulting FAME from external standards or sample derivatization was injected separately into the Gas Chromatography instrument (GC). The GC used for the analysis was the 7890A Gas Chromatography System (Agilent Technologies, California, US) equipped with a flame ionization detector set at 280°C and a splitless injector (1 µL) set at 270 °C. The utilized column was a DB-23 (60 m  $\times$  0.25 mm, with a film thickness of 0.25  $\mu$ m). The GC oven protocols followed manufacturers program recommendation, program: 130 °C (hold 2 min), to 170 °C at 6.5 °C/min (hold 5 min), to 215 °C at 2.75 °C/min (hold 12 min), to 230 °C at 30 °C/min (hold 30 min). Carrier gases used were helium and nitrogen of ultrahigh purity grade at flow rates of 11.07 and 31.24 mL/min. The fatty acids identification and Concentration were determined by comparing peak retention time with the respective standards. The concentration of fatty acids was based on the following equation; after (AOAC, 2012).

$$
[{\rm Alx}] = \frac{{\rm Aalxs}}{{\rm ASIs}} \times \frac{[{\rm B}SIs]}{{\rm BS}} \times \frac{{\rm ASle}}{{\rm Aalxe}} \times \frac{[{\rm Balxe}]}{[{\rm BSIe}]} \times 1000
$$

#### **Where:**

 $[ALx] =$  Concentration of fatty acid X on sample  $(mg/g)$ Aalxs = Area of fatty acid X on chromatogram of sample ASIs = Area of palmitic acid (internal standard) on chromatogram of sample [BSIs] = Concentration of palmitic acid (internal standard) on sample BS = Weigh of sample (mg) ASIe = Area of palmitic acid (internal standard) on chromatogram of external standard Aalxe = Area of fatty acid X on chromatogram of external standard [Balxe] = Concentration of fatty acid X on external standard [BSIe] = Concentration of palmitic acid (internal standard) on external standard

# **RESULTS**

#### *Digestibility analysis*

The results of acid-insoluble ash analyses show that the catfish had significantly higher digestibility for crude sesame extract (CSE) (93.34±0.22 %) than for crude palm extract, (CPE)  $78.98 \pm 0.11$ %. Among the treatment feeds, Feed F1 had the highest digestibility of 91.32  $\pm$  0.07%, followed by feed F2, 82.25  $\pm$  0.04%. An equal mixture of the 15% saturated (CPE) and 15% unsaturated lipid (CSE) produced feed that has similar digestibility 78.45  $\pm$ 0.07 % to feed F2 and CPE. The digestibility of feed F4 70.12  $\pm$  0.06% and F5 70.23  $\pm$  0.09 % were not significantly different (P<0.05) (Table 2). The results of the catfish haematobiochemical analysis showed that alanine aminotransferase (ALT), also known as glutamicpyruvic transferase (GPT), and Aspartate aminotransferase (AST) were significantly lower for the fish that received feed F1 followed by feed F2 (P<0.05). Hematological analysis values of the catfish showed that AST and ALT were reducing with the increasing inclusion of unsaturated lipid (Table 2). The catfish fed diets F1 had the lowest AST 82.40±0.07  $\mu/L$ and ALT  $48.60\pm0.05$  µ/L (Table 2). Hepatosomatic index was highest for the fishfed diet F2, 3.35±0.05 and this not significantly different from Hepatosomatic index of fish fed feed F5, 3.22±0.08 (Table 2). The lowest value of hepatosomatic index was recorded for fish fed diet F1, 1.54±0.03 (Table 2).







The main PUFA of CPE is the linoleic acid and the linolenic acid making a total PFA of  $12.8 \pm$ 0.06 %. The main fatty acids of CPE are SFA which constitutes about 49.9±0.08 %of the fatty acids and some MUFA.

### The fatty acid of crude sesame extract and crude palm extracts

The fatty acid profile of CSE and CPE are recorded in table 3. The fatty acids of CPE are dominated by saturated fatty acids (SFAs), which constitute about 49.9±0.08% of the total fatty acids. Among the SAFA, the C16:0 class of saturated fatty acid, hexadecanoic acid, or palmitic acid, comprising 46.0 ±0.02% of the total fatty acid. The next most important fatty acid of CPE was monounsaturated fatty acid, oleic acid, C18:1. Oleic acid accounts for 43.4 ±0.08% of the total fatty acids. Crude palm extract also contains some omega 6 fatty acid, PUFA, C18:2, linoleic acid. Linoleic acid was noted to be present in a small quantity of 12.1±0.04%. The CPE also has small quantities of PUFA linolenic acids 0.7 ±0.03%, and saturated monobasic 18 carbon-chain fatty acid, stearic acid 4.5 ±0.05%. The full profile of fatty acid of CPE is stated in table 3.

<b>Fatty acids (Crude Palm extracts)</b>	% T F A
Lauric acid $(12:0)$	$0.2 \pm 0.06$
Myristic acid $(14:0)$	$1.9 \pm 0.07$
Palmitic acid (16:0)	$46.0 \pm 0.02$
Stearic acid (18:0)	$4.5 \pm 0.05$
Oleic acid $(18.1)$	$43.4 + 0.08$
Linoleic acid (18:2)	$12.1 + 0.04$
Linolenic acid (18:3)	$0.7 + 0.03$
Arachidic acid (20:0)	$0.1 \pm 0.08$
Total SFAs	$49.9 \pm 0.08$
Total MUFAs	$43.4 \pm 0.05$

Table 3: The % Fatty acid composition of crude palm extract used in compounding feed **for** *C. gariepinus*





The fatty acid of CSE is dominated by monounsaturated C18:1 linoleic acid. Oleic acid forms about 45.44±0.01% of the total lipid of sesame crude extract. Crude Sesame extract also has 39.48±0.14 %, C18:2 polyunsaturated fatty acids linoleic acid. There are also 14.75 ±0.00% of hexadecanoic acid, palmitic acid (Table 3). The CSE also contains monounsaturated fatty acid the eicosenoic acid, making up 0.15±0.02% of the total lipids. Generally, CSE has 40.05±0.02% of PUFA, 45.59±0.14% MUFA, and 21.20±0.01% of SAFA (Table 3).

# *Fatty acid composition of feed and catfish*

The fatty acid compositions of the feeds were similar to the fatty acid profile of the CSE and CPE ingredients. Consequently, feed F1, with 25% of CSE had a high content of monounsaturated C18:1, (44.11%) and linoleic acid (18:2n-6) 26.38%. Feed F1 to F5 also have α-linolenic acid (18:3n-3, ALA), as follows; F1, 2.21%, F2, 2.08%, F3,2.0%, F4, 2.1%, and F5, 2.2% of the total fatty acids. Palmitoleic acids  $(16:1\omega)$  were detected in all the feeds, although feed F1 had a higher quantity than all others. The percentage of palmitoleic acids (16:1ω7) per feed is as follows; F1,7.02%, F2, 5.79%, F3, 5.09%, and F4, 5.03%and F5, 5.03% of the total fatty acids respectively. The feed made from CSE (F1, F2, and F3) were found to be higher in their content of eicosapentaenoic acid (EPA; 20:5ω3) as follows; F1, 6.55%, F2, 4.53%, F3, 4.91%, F4, 2.8% and F5, 1.88% of the total fatty acids. Similarly, there was more docosahexaenoic acid (DHA; 22:6ω3), present in feeds containing more CSE as follows; F1, 18.98%, F2,14.01%, F3, 16%, F4, 11.03 and F5, 10.05% (Table 4).









Similarly, feed F5 formulated with 25% CPE was notably higher in the monounsaturated fatty acids like the oleic acids (C18:1), palmitic acid (C16:0) 45.83%. Feeds compounded with high CPE inclusion, feeds F3, F4-F5, had a higher content of homo-Gamma-Linolenic  $C20:3\omega$  6 than the sesame-based diets. The homo-gamma linolenic acids found in the feeds were as follows; F1, 0.19%, F2, 0.17%, F3, 2.24%, F4, 2.42% and F5,2.59% of the total lipids (Table 4). The quantities of linolenic acid ( $18:3\omega3$ ) found in the feeds were virtually similar irrespective of CPE and CSE inclusion percentages. Blending the CSE and CPE was good in terms of growth and weight gain. This is similar to the results of Ng et al. (2013) who noted improved growth when cod liver oil was mixed with palm fruit extract oil. The quantities of linolenic acid (18:3ω3) in the feeds were as follows; F1, 2.21%, F2, 2.08%, F3,2.0%, F4,2.1% and F5, 2.2% of the total lipids (Table 4). Feeds F1 to F4 had some quantity of gadoleic acid C 20:01, and these can be seen in the body composition of the catfish. The sum total of the saturated fatty acids ∑SFA is the sum total of C14:0, C15:0, C16:0, C17:0, C18:0, i15:0, a15:0, i16:0, 16:0FALD, i17:0, i18:0, 18:0FALD, 20:0, and 22:0. The ∑SFA shows that catfish fed with feed F1 had the lowest somatic SFA of 48.58 %. The ∑SFA was the same for catfish fed with either F1 or F2 with a total ∑SFA of 48.58 %. The catfish fed with F3 had ∑an SFA of 52.78%, this was the same as that for F4 fed fish. However, the total saturated fatty acid was 59.07 % for the catfish fed with F5. The catfish fed with F1 had a high percentage of PUFA, 67.94 %, while F2 had 58.19 %, F3 57.9 %, and F4, 47.76%, and F5, 46.43% of the total fatty acids (Table 5).

The total MUFA in the fish was much higher in the catfish fed with F1, 63.65 % than in the reciprocal feed F5, 42.58 %. The total MUFA of catfish fed feed F2 was 59.95 %, and this was higher (P<0.05) than the MUFA of catfish fed F3, 49.35% (Table 5). The percentage of C20-C22 PUFA in the catfish was found to be reduced as the inclusion levels of CSE decreased (Table 5). The catfish fed F1 (25% CSE) had 20-22 PUFA of 42.00 %, while those fed with reciprocal diet F5 had significantly lower value (28.89%) (P<0.05). The catfish somatic fatty acid profile shows that the catfish that were fed Feed 1 to Feed 3, contained more omega 3 fatty acid ( $\omega$ 3) than the rest of the fish. The catfish fed with feed F1(25% CSE) had 71.94% PUFA and also had 42% 20-22 PUFA (Table 5). Conversely, the catfish fed F4 to F5 had more omega 6 fatty acids. We noted that catfishes that were fed F1 (25% CSE) had omega 9 fatty acids of 40.95%. The omega 9 fatty acids of the catfish were progressively reduced with the increasing inclusion of CPE (Table 5). The lowest amount of omega 9 fatty acid was in those catfish fed F5 (25% CPE) 34.14% (P<0.05). The value of Omega 9 in the catfish was lowest for the catfish that received feed F5**.** Arachidonic acid (ARA) was noticed in very little quantities in the experimental fish. The catfish that received feed F1 had about the highest ARA of 0.84% of the total lipid. The least ARA was obtained from the catfish that received feed F5, 0.27%. Eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and omega 3 fatty acid were generally highest for the catfish that received feed F1, 13.76%, 20.21%, and 40.6% respectively. Conversely, the catfish that received F5 had Gammalinolenic acid (GLA, all cis 6, 9, 12- C18:3, ω-6, DGLA 20:3 (ω−6) of, 7.72%; Dihomo-γlinolenic acid (DGLA) of 5.44% and omega 6 fatty acids of 34.96% (Table 5). The value of Omega 3 fatty acids of the catfish was increasing with the increasing inclusion of CSE. However, the values of omega 6 fatty acid in the catfish was increasing with increasing inclusion of (CPE).



Table 5: The fatty acid profile of African catfish fed for 70 days with diets varying in inclusion of crude sesame extracts and crude palm extracts



The ratio of omega 6 to 3 fatty acids (ω-6/ω-3) were for F1, 0.75%, F2, 0.82, F3, 1.10%, F4, 1.3% and F5, 1.61% of the total fatty acids. The analyzed hematobiochemical indices alanine amino transferase (ALS) and alanine amino transferase (AST), revealed low levels for the whole treatment feed.

### **DISCUSSIONS**

The hematobiochemical parameters of the fish were not high indicating that the feed was not deleterious to the fish. The fatty acids content of the catfish resembles the fatty acid content of the feeds. This is in line with previous findings of Qui et al. (2017) who noted that the fatty acid content of yellow croaker (*Larmichthys crocea*), resembled that of their feed. The catfish fatty acid composition of 20:4ω6, 20:5ω3, 20:3ω6 are the replica of the fatty acids of the feeds (Table 4). The experimental feeds contain high quantities of EPA, DHA and ARA. (Fig 1).



Where ARA is arachidionic acid, EPA is eichosapentanoic acid, DHA is docosahexaonic acid and GLS is Gamma-linolenic acid

Figure 1. Relationship of EPA, DHA and crude sesame extract added in catfish diets **varying in the inclusion of crude sesame extract and crude palm oil extracts (CS:CP)** as, feed 1 (25:5), F2(20:10), F3, (15:15), F4,(10:20) and F5(5:25)



The high contents of DHA and EPA was noted to be reducing as the African catfish utilized the experimental diets but the higher digestibility of unsaturated CSE than CPE seems to influence feed utilization. EPA and DHA are essential fatty acids known to enhance cellular synthesis, ionic regulation and cell membranes, pigmentation, and energy reserves (Turchini and Francis 2009). The ratio of EPA:DHA Digestibility of lipids is affected by the lipid source and age of the fish . The catfish fed diet F1 had the highest content of PUFA, MUFA, linoleic acid and 18C chain family fatty acids (Figure 2).



Where SAFA is saturated fatty acids, PUFA is polyunsaturated fatty acids, MUFA, is monounsaturated fatty acids, LIN (18:2ω6 is linoleic acid

# Figure 2. The summary of fatty acids, SAFA, PUFA, MUFA 18: family, 16: family, 20-22 PUFA **and linoleic acid composition of African catfish fed for 70 days with diets**  varying in the inclusion of crude sesame extract and crude palm oil extracts **(CS:CP) as, feed 1 (25:5), F2(20:10), F3, (15:15), F4,(10:20) and F5(5:25)**

The omega 6/omega 3 ratios of the fish were lowest for those fed feed 1 and this suggests the high content of linoleic acid in the feed and fish suggests a precursor to arachidonic acid (AA), which is higher in F1 and reducing from F1 to F5; (Figure 3), note that Linoleic acid is required for the synthesis of prostaglandins and other eicosanoids.

The inclusion of CSE and CPE in the feeds affected the digestibility, nutrient utilization, and fatty acid composition of African catfish. Lipid digestibility in fish is inversely related to saturation of lipids, (Olsen et al., 1998). Saturated lipids and fatty acids are less digested than unsaturated lipids; this could be the reason for the catfish's better digestibility of CSE compared to CPE. Consequently, the better digestibility of feeds F1, F2, and F3, more than F4 and F5, could be because of the higher quantities of MUFA, PUFA, EFA, and C20-22 PUFA in the feeds (Figure 3).



# Figure 3. The relationship between the omega 6 fatty acids, omega 3 fatty acids essential **fatty** acids and the ratios of ω 6 and ω3 fatty acids of African catfish fed diets **varying in inclusion levels of crude sesame extract and crude palm oil extracts as, crude palm oil extracts (CS:CP) as, feed 1 (25:5), F2(20:10), F3, (15:15), F4,(10:20) and F5(5:25)**

The digestion and utilization of the feeds could have been affected by the inclusion of bentonite in the diets formulation. It has been noted that dietary inclusion of bentonite in aquafeed reduces the effects of mycotoxins (Alexander, et al. 2001), while at same time enhance more digestibility and absorption of fatty acids within fish gut. In previous research, Habold, et al. (2009) noted that kaolinite enhanced elevated increase in intestinal triacylglycerol (TAG) hydrolysis and non-esterified fatty acids (NEFA) absorption. Sesame seed contains gadoleic or Eicosenoic (C20:1) of 0.15±0.02, which must be a source of the lipid in the fish. The higher digestibility of feeds F1, F2, and F3 than F4 and F5 could have been due to the lipids and fatty acid composition of the feed. Sesame seed is known to be high in phospholipids and triacylglycerols (Enyidi 2012). Phospholipids are present in low quantities in palm oil 20-80ppm, this enables oxidative stability of the oil but reduces digestibility. Phospholipids are emulsifier that helps in lipids digestion of fish.



The CPE is known to have a high content of palmitic acids (C16:0) 46.0 ±0.02%, Oleic acid (18:1) 43.4 ±0.08 %, and Linoleic acid (18:2), 12.1±0.04% (Figure 4). In this experiment the digestibility of feeds had positive relationship with the dietary content of linoleic acid. The  $R^2$  value of feed digestibility in relationship to linoleic acid was 0.96. The R2 value of the content of linoleic acid in feed was 0.93. The growth of freshwater fish has been linked to the requirement of C18 polyunsaturated fatty acids (PUFA) (Tocher, 2010). The African catfish like other freshwater fish require polyunsaturated omega 6 fatty acids to grow.



### Figure 4. The relationship between Linoleic acid (DARK BAND) of feed and digestibility of feed (LIGHTER BAND), of African catfish feeds varying in inclusion levels of **crude sesame extract and crude palm**

Feed F1, has a good content of (18:2ω6; cis, cis-9,12-octadecadienoic acid) which meets the catfish demands. Meanwhile, feed F5 has a higher content of oleic acids  $18:1\omega$ 9c, 30.59%. Oleic acid is abundant in CPE than in CSE. The Omega 3 fatty acids in the fish are dominated by docosahexaenoic acid (DHA; 22:6ω3). It seems that catfish can produce more DHA than EPA. Our results however show that the feed met the fatty acid requirements of the African catfish. Our results also show that the catfish fed with feed F1 to feed F3 had higher omega 3 fatty acid in its biomass.

# **CONCLUSION**

The catfish digested the CSE more than the CPE. The fatty acid of the fish resembled that of the feed. The fatty acids of catfish fed diets containing CSE were dominated by omega 3 fatty acids especially (DHA; 22:6ω3). DHA. The catfish fed CPE had more omega 6 fatty acids especially dominated by C16:0 lipids. Consequently, omega 6 fatty acid catfish can easily be cultured using our experimental diets.



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