EVALUATION OF DIFFERENT DIETARY OIL SOURCES ON GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF *Clarias gariepinus* JUVENILES

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ABSTRACT
The reported decrease in wild fish stocks has made the replacement of Fish Oil (FO)/codliver oil in aquaculture a priority. The use of vegetable oils (VOs), with lower cost, larger and wider supplies may be a good substitute for FO. The effects of total replacement of cod liver oil (CLO) with VOs, oil palm seed (OPS), Ugwu seed oil (USO)/pumpkin seed oil, soya bean oil (SBO), almond seed oil (ASO)) and a composite of all the VOs referred to as COs on growth performance and nutrient utilization of Clarias gariepinus juveniles was investigated for 12-weeks. Each diet contained 45% CP fed to triplicate groups of 15 juveniles, with initial mean weight 22.82 ± 0.30 g and mean length 15.45 ± 0.04 cm. Fish fed OPS based diet had significantly (P ≤ 0.05) the highest body weight gain (126.34 ± 25.27 g), followed by USO (110.63 ± 15.48). The highest survival rates of (100%) were in USO and Cos but (97.77%) in ASO and CLO (Control) based diets. Significant differences at (P ≤ 0.05) were observed in carcass CP of fish fed OPS based diets (48.09 ± 1.40 g) compared with the CLO's (44.86 ± 0.35 g).This research shows evidence that OPS and USO can effectively replace FO in diets of C. gariepinus.
Keywords: Aquaculture, fish nutrition, animal protein, lipid, catfish.

INTRODUCTION

With global population expansion, the demand for high quality animal protein especially from aquatic sources has risen considerably. Increased aquaculture production is clearly needed to meet this demand in the third millennium, because capture fisheries is showing precipitous decline due to over fishing, habitat destruction and pollution. Further increase in capture fisheries is not anticipated under the current global condition (Dunham *et al*., 2001). Fish farming is being adopted rapidly in Nigeria today. If this development is sustained, aquaculture will create a great impact on the economy and provide proteinous rich food which can be as high as 60% on dry matter basis. However, lack of good quality feed for economic production of fish in Nigeria adversely affects growth rate, survival rate, disease occurrence and total harvest. The success of aquaculture production depends on adequacy of good nutrition. Therefore, the main challenge in aquaculture nutrition in Nigeria is the development of economic and adequate rations for culture of the main farmed fish species. Fish diets must provide adequate energy, protein, vitamins and minerals in proper proportions for fast, efficient growth and the maintenance of health. Choosing the right feed plays an important role in determining the productivity of aquaculture operation.

There has been a trend in commercial fish feed formulation to increase dietary

lipid level inclusion thus increase feed utilization and production respectively. Lipid is a major source of metabolic energy in fish. Being highly digestible, it has a greater sparing action on dietary carbohydrate, thereby playing a definite role in feed utilization. However the demand for fish oil by the aqua feed industry has been predicted to exceed the available resources within the next decade (Barlow and Pike, 1999).

Presently, aquatic feeds have depended heavily on fish meal and fish oil as the source of protein and lipid. However, the feed industry is encountering shortfalls in the availability of these ingredients because of a decline in the number of fish captured in the wild and the increasing human demand for some of the species currently being used for fish meal and oil production. Efforts are being directed in different parts of the world to finding alternative quality ingredients, which ideally are less expensive and readily available for use in practical fish diets. Hence, this study looked at the possibility of incorporating alternative dietary oil sources such as palm oil, almond seed oil, Ugwu/pumpkin seed oil into the diet of *Clarias gariepinus* and the effect these different dietary oil sources have on the growth and nutrient utilization of the juveniles of *C. gariepinus* is relevant at this point.

MATERIALS AND METHODS

Fish, Diets and Experimental Design

The research was conducted in the University of Ibadan, Department of Wildlife and Fisheries Management laboratory. Each of the six treatments was in triplicates using circular plastic tanks. Juveniles of the African catfish, *Clarias gariepinus* were obtained from a local fish hatchery and transported in oxygen bags to the laboratory. The fish were then acclimated to laboratory conditions and fed with a commercial fish feed (35% CP) for 14 days. After acclimatization, groups of fifteen *Clarias gariepinus* juveniles (mean weight 22.82 ± 0.30 g) were randomly stocked into eighteen 45l capacity circular tanks which were filled with 30 litres of water each. Experimental tanks were well aerated using air stones (Lawson, 1995) throughout the period of the experiment.

The diets were fed to the fish at 5% body weight twice daily (between 8.30 am - 9.00 am, and 5.30 pm - 6.00 pm) for 84 days. The weight of each group of fish was taken fortnightly using sensitive top loading balance and the feed adjusted accordingly.

The water quality parameters of dissolved oxygen, temperature and pH were monitored on alternate days early in the morning (7.00 – 8.00 am) on days when the water quality parameters were taken, Digital dissolved oxygen meter (manufactured by American Marine Inc.) was used to take the dissolved oxygen, while the water temperature and pH values of the experimental tanks were measured using Digital/electronic temperature probe (water proof) and a pH meter respectively. The feeding trial was conducted for twelve weeks.

Fish meal, mineral/vitamin premix, soya bean meal, yellow maize, salt and binder used in this study were obtained from an agricultural feedstuff store in Ibadan. The Ugwu (*Telfaira occidentalis*) seeds were bought from Ojoo market, Ibadan and the oil from the seed was extracted using continuous soxhlet extraction technique with hexane. The almond seeds (*Terminalia catappa*) were picked from trees within the campus of the University of Ibadan, Nigeria. The seeds were shelled. The kernels were ground to powder in a hammer mill and the oil from the seeds extracted using the continuous soxhlet extraction technique with hexane. The palm nuts were bought from Ojoo market in Ibadan and the oil from the

seeds extracted locally. The soya bean oil and cod-liver oil was bought from the University Pharmacist.

All the solid ingredients except the liquids, the common salt, vitamin and mineral premix were milled together with hammer milling machine to obtain fine particulates, The crude protein content of the diets were kept essentially at the 45% CP level since this was determined as the protein requirement of juveniles catfish hybrid (Eyo and Falayi, 1999), and *Clarias gariepinus in* Homestead tanks, (Olukunle 2004). Each diet was first mixed, dry and later with just enough warm water to obtain homogenous hard-paste (dough) and pelleted. Using the ingredients, six practical diets containing 45% crude protein, each having different lipid sources was formulated respectively (Table 1). The pellets were sun dried at ambient temperature of 30 °C for three days and stored in air tight plastic at 26 °C.

Sampling Procedures and Analyses

The proximate composition of diets and fish fillets were analyzed according to the methods described in AOAC (2000). On completion of the feeding trial, all fish were starved for 24 hrs (to empty the digestive tract). Liver from two fish per tank were used for histopathological investigations. For histology, fixed (4% buffered formaldehyde) liver specimens were processed manually and embedded in paraffin wax. Sections (5 µ) were cut and mounted on glass slides before staining with Mayers Haematoxilen and Eosine. Stained sections were examined and photographed under light trio ocular (Olympus BX50) microscopy (Takashima and Hibiya, 1995). For the carcass analysis at the end of the study, two fish were pooled together for each replicate tank homogenized in a blender and the proximate composition determined. Moisture was determined by oven-drying at 105 °C over night and until constant weight is obtained. Crude lipid was determined by extraction using petroleum ether for three hours on a soxhlet apparatus; ash was determined from weighed moisture-free samples in a porcelain crucible placed in a muffle furnace at 555 °C for three hours.

Fish, from which blood for haematology was collected, were anaesthetized with 150 mg/l solution of tricaine methane sulphonate (MS-222, Sigma Chemical co. St. Louis, MO, USA) (Wegner *et al*., 1997). Blood samples were taken with 2 ml heparinized syringes and 21 swg needles from the caudal vein of a fish from each treatment and put separately in 2ml heparinized tubes and taken to the laboratory for determination of haematocrit (Hct), haemoglobin (Hb), erythrocyte sedimentation rate (ESR), white blood cell (WBC) and red blood cell (RBC) using the method of Svobodova *et al.* (1991).

The haematological indices of mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were calculated using the total red blood cell count (RBC), haemoglobin concentration (Hb) and hematocrit (Hct) (Dacie and Lewis, 2001). Gross appearance and microscopic appearance of the liver were also examined.

Growth performance, haematological, carcass analysis data collected from the experiment were subjected to one way analysis of variance (ANOVA) to test the significance of variations between the means of growth indices and nutrient utilization parameters determined for fish feed various lipids. Least Significance difference (LSD) was used to determine the level of significance among treatments. Regression analysis was carried out to determine the relationship between the treatments, growth and nutrient utilization parameters using

SPSS statistical package (Version15.0, SPSS Inc. Chicago IL) Windows 2000.

RESULTS

The diets used in the feeding trial, the ingredients composition as well as proximate composition are presented in Table 1. The proximate analysis shows that the value of crude protein was uniform for all the diets. The ether extracts values ranged from 5.64 g in ASO to 6.87 g/100 g CLO based diet which had the highest value. The value of crude fiber ranged from 3.55 g/100 g to 3.92 g/100 g and for ash it ranged from 15.89 to 17.23 g/100g. The cost analyses of experimental diets are highlighted in Table 2. Water quality condition in the experimental tanks showed very little variation throughout the duration of the trial (Table 3). The performance in terms of survival of fish on different diets is shown Table 4.

Table 1: Proximate Composition of diets based with different Lipid sources for *Clarias gariepinus* juveniles

Ingredients	Diet OPS	Diet USO	Diet SBO	Diet ASO	Diet COs	Diet CLO
Fish meal (72%)	36.99	36.99	36.99	36.99	36.99	36.99
Soya bean meal (45%)	36.99	36.99	36.99	36.99	36.99	36.99
Yellow maize (12.5%)	12.40	12.40	12.40	12.40	12.40	12.40
Palm seed oil	5.00	-	-	-	-	-
Ugwu seed oil	-	5.00	-	-	-	-
Soya beans	-	-	5.00	-	-	-
Almond seed oil	-	-	-	5.00	-	-
Mixture of oil	-	-	-	-	5.00	-
Cod liver oil	-	-	-	-	-	5.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix	2.00	2.00	2.00	2.00	2.00	2.00
Proximate Composition (g/100g)						
Crude Protein (%)	46.87	45.68	46.65	45.92	46.66	45.82
Crude Fat (%)	6.79	5.64	6.12	5.98	6.48	6.87
Crude Fibre (%)	3.55	3.66	3.86	3.78	3.92	3.58
Ash (%)	16.94	16.89	17.23	15.89	17.86	17.49
Moisture (%)	6.82	7.62	8.14	8.21	7.56	6.73
NFE (%)	19.03	21.54	17.00	20.22	12.52	20.51

Table 2: **Cost Analysis of Experimental Diets**

Component	OPS	USO	SBO	ASO	COs	CLO
Fish meal (72%)	136.90	136.90	136.90	136.90	136.90	136.90
Soyabean meal (45%)	36.99	36.99	36.99	36.99	36.99	36.99
Yellow maize (12.5%)	7.20	7.20	7.20	7.20	7.20	7.20
Palm seed oil	2. 5	-	-	-	-	-
Ugwu seed oil	-	3.4	-	-	-	-
Soybean oil	-	-	4.5	-	-	-
Almond seed oil	-	-	-	1.00	-	-
Composite oils	-	-	-	-	10.00	-
Cod liver oil	-	-	-	-	-	5.00
Salt	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix	12.90	12.90	12.90	12.90	12.90	12.90
Vitamin premix	26.00	26.00	26.00	26.00	26.00	26.00
Total amount (Cost ₦/kg feed)	222.69	223.64	224.69	220.99	230.19	225.19
FCR	3.18	3.30	3.63	3.47	3.71	3.71
ECR	708.15	734.01	815.62	766.83	854.00	835

Table 3: Mean Bi-Weekly Water Parameters of the Experimental Tanks

Treatments	Parameters	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
1.	Temp (°C)	26.02 ±0.05	25.03 ±0.45	26.03 ±0.00	26.58 ±0.01	25.20 ±0.16	26.00 ±0.01
	DO (mg/L)	6.60 ± 0.0	6.48 ± 0.01	6.70 ± 0.05	5.70 ± 0.00	6.60 ± 0.03	6.60 ± 0.00
	PH	7.20 ± 0.05	6.88 ± 0.01	7.20 ± 0.02	6.93 ± 0.07	6.90 ± 0.06	7.10 ± 0.03
2.	Temp (°C)	25.80± 0.01	25.10±0.08	26.01±0.01	25.10 ±0.04	25.27±0.12	26.00±0.02
	DO (mg/L)	6.50 ± 0.02	6.56 ± 0.05	6.55 ± 0.02	6.70 ± 0.50	6.61 ± 0.01	6.52 ± 0.01
	PH	6.95 ± 0.00	6.84 ± 0.03	7.10 ± 0.21	6.95 ± 0.05	6.90 ± 0.01	7.10 ± 0.02
3.	Temp (°C)	25.70 ±0.01	24.03±0.05	25.90±0.01	25.20±0.01	24.53±0.75	25.70±0.03
	DO (mg/L)	6.46 ± 0.02	6.55 ± 0.02	6.47 ± 0.01	6.70 ± 0.01	6.39 ± 0.04	6.47 ± 0.02
	PH	6.90 ± 0.08	6.84 ± 0.01	7.02 ± 0.01	6.90 ± 0.03	6.78 ± 0.01	6.95 ± 0.05
4.	Temp (°C)	26.00± 0.00	24.27 ±0.38	26.00±0.02	25.80± 0.03	25.17± 0.00	26.05 ±0.01
	DO (mg/L)	6.51 ± 0.02	6.48 ± 0.03	6.51 ± 0.03	6.48 ± 0.02	6.50 ± 0.08	6.55 ± 0.05
	PH	6.95 ± 0.01	6.80 ± 0.02	7.10 ± 0.01	6.95 ± 0.08	6.90 ± 0.02	7.10 ± 0.02
5.	Temp (°C)	25.60 ±0.01	24.20 ±0.16	25.01 ±0.00	25.27 ±0.02	24.20 ±0.05	26.01±0.50
	DO (mg/L)	6.45 ± 0.02	6.12 ± 0.04	6.44 ± 0.01	6.42 ± 0.01	6.04 ± 0.02	6.52 ± 0.04
	PH	6.84 ± 0.01	7.10 ± 0.21	6.90 ± 0.06	6.84 ± 0.01	6.90 ± 0.08	6.88 ± 0.01
6.	Temp (°C)	25.70± 0.01	24.90 ±0.08	25.80±0.02	24.90± 0.00	25.10 ±0.08	25.80 ±0.01
	DO (mg/L)	6.48 ± 0.02	6.82 ± 0.01	6.49 ± 0.02	6.82 ± 0.04	6.59 ± 0.02	6.49 ± 0.01
	PH	7.00 ± 0.08	6.87 ± 0.03	7.00 ± 0.03	6.95 ± 0.05	6.89 ± 0.01	7.01 ± 0.01

Table 4: Growth Performance and Nutrient Utilization of *Clarias gariepinus* juveniles fed diets based with different Lipid sources

Parameters	OPS	USO	SBO	ASO	COs	CLO
Initial Body Weight (g)	22.85 ± 0.43^{a}	22.82 ± 0.30^{a}	22.82 ± 1.22^{a}	22.20 ± 0.63^{a}	22.27 ± 0.67^{a}	22.08 ± 0.76^{a}
Final Body Weight (g)	149.1 ± 24.96^{abc}	133.45 ± 15.48^{d}	116.63 ± 24.67^{c}	127.55 ± 4.59^{e}	113.57 ± 9.56^{b}	105.83 ± 9.68^{b}
Body Weight Gain (g)	126.34 ± 25.27^{abc}	110.63 ± 15.48^{d}	93.81 ± 25.50^{c}	105.35 ± 4.86^{e}	91.30 ± 9.88^{b}	83.75 ± 10.43^{a}
Body Weight Gain (%)	553.10 ± 117.58^{a}	484.88 ± 68.46^{b}	414.40 ± 125.12^{a}	475.20 ± 30.30^{c}	410.7 ± 51.13^{d}	380.71 ± 59.46^{e}
Initial Length (Cm)	15.47 ± 0.06^{a}	15.45 ± 0.08^{a}	15.47 ± 0.08^{a}	15.45 ± 0.04^{a}	15.45 ± 0.04^{a}	15.45 ± 0.04^{a}
Final Length (Cm)	29.97 ± 0.78^{acd}	28.82 ± 1.57^{b}	26.65 ± 1.85^{ab}	28.36 ± 0.66^{e}	27.14 ± 0.88^{d}	27.20 ± 0.83^{c}
Length Increment (cm)	14.50 ± 0.73^{cab}	13.37 ± 1.63^{d}	11.17 ± 1.80^{ad}	12.91 ± 0.69^{e}	11.69 ± 0.87^{b}	11.75 ± 0.80^{c}
Food Conversion Ratio	3.18 ± 0.34^{ab}	3.30 ± 0.20^{c}	3.36 ± 0.36^{d}	3.47 ± 0.19^{e}	3.71 ± 0.15^{a}	3.71 ± 0.25^{b}
Protein EfficiencyRatio	2.70 ± 0.54^{a}	2.42 ± 0.34^{b}	2.01 ± 0.55^{c}	2.29 ± 0.11^{d}	2.08 ± 0.43^{e}	1.83 ± 0.23^{a}
Protein Productivity Value	9.75 ± 2.98^{b}	6.81 ± 5.92^{c}	10.65 ± 5.23^{a}	8.16 ± 6.46^{d}	3.75 ± 0.26^{e}	2.92 ± 0.77^{a}
Protein Intake (g)	2.96 ± 0.59^{b}	2.52 ± 0.35^{c}	2.19 ± 0.60^{b}	2.42 ± 0.11^{d}	2.13 ± 0.23^{a}	1.92 ± 0.24^{e}
Survival Rate (%)	97.77	100	93.3	97.77	100	97.77
Specific Growth Rate	0.97 ± 0.10^{a}	0.91 ± 0.06^{b}	0.84 ± 0.13^{c}	0.90 ± 0.03^{b}	0.64 ± 0.05^{d}	0.81 ± 0.07^{a}
Condition factor:						
Initial	0.62 ± 0.02	0.61 ± 0.00	0.62 ± 0.04	0.60 ± 0.01	0.60 ± 0.02	0.60 ± 0.02
Final	0.55 ± 0.08	0.56 ± 0.04	0.61 ± 0.04	0.56 ± 0.03	0.57 ± 0.04	0.53 ± 0.03
Dfiference	-0.09±0.11	-0.11±0.07	0.01±0.05	-0.06±0.06	0.02±0.06	-0.61±0.06

KEY: Values given in mean ± standard deviation of three replicates.
NOTE: Figures in the same row having same superscript are not significantly different (P > 0.05)

Table 5: Carcass composition of *Clarias gariepinus* (dry matter) fed experimental diets for 84 days

	FISH SAMPLES						
Parameter (% weight)	**INITIAL**	**OPS**	**USO**	**SBO**	**ASO**	**COs**	**CLO**
Crude Protein	46.52	48.09 ± 1.40^{a}	46.60 ± 2.70^{b}	48.56 ± 2.53^{a}	47.27 ± 2.97^{c}	45.27 ± 0.12^{d}	44.86 ± 0.35^{a}
Crude Fat	6.92	6.51 ± 0.56^{c}	7.16 ± 0.27^{a}	6.82 ± 0.83^{d}	6.28 ± 0.78^{e}	5.68 ± 0.89^{ab}	7.06 ± 0.33^{b}
Crude Fibre	1.43	1.37 ± 0.06^{a}	1.42 ± 0.08^{a}	1.39 ± 0.08^{a}	1.30 ± 0.10^{a}	1.29 ± 0.04^{a}	1.37 ± 0.12^{a}
Ash	16.63	16.39 ± 0.45^{b}	16.99 ± 0.09^{a}	16.86 ± 0.99^{c}	16.44 ± 1.00^{d}	15.81 ± 0.91^{e}	15.56 ± 1.28^{a}
Moisture	5.32	5.36 ± 0.57^{a}	5.63 ± 1.48^{a}	5.05 ± 2.05^{a}	6.50 ± 2.44^{a}	7.42 ± 2.21^{a}	4.77 ± 1.02^{a}
NFE	23.18	22.29 ± 1.52^{d}	22.16 ± 3.25^{b}	21.38 ± 2.28^{c}	22.21 ± 2.35^{a}	24.53 ± 1.21^{e}	26.39 ± 2.65^{abc}

KEY: Values given in mean ± standard deviation of three replicates.
NOTE: Figures in the same row having same superscript are not significantly different (P > 0.05)

Survival rate of fish on diets USO and COs showed similar result (100%) while diets based on OPS, ASO and SBO recorded 97.77% survival across board with the least value of 93.33% recorded in CLO based diet. The growth responses in all the treatments are presented in Table 4. There was no statistical difference ($P < 0.05$) in the initial mean weight of the juveniles. However, difference were observed in the final mean weight of the juveniles where diet OPS recorded the highest final mean weight of 149.19 ± 24.96 which was statistically significant ($P < 0.05$).There was a significant difference ($P < 0.05$) in the percentage weight gain and also in the length increment among the diets. Diet OPS (553.10 ± 117.58) and SBO (414.40 ± 125.12) are not significantly different. The highest weight gain was in fishes fed diet OPS compared with USO (110.63 ± 15.48), SBO (93.81 ± 25.50), ASO (105.35 ± 4.86), COs (91.30 ± 9.88) and CLO. (83.75 ± 10.43) The FCR was lowest in diet OPS ($P < 0.05$) which indicate more efficient utilization of the diet by the fingerlings. The SGR was highest in diet 2 and this was significantly different from that of the control diet ($P < 0.05$). The Specific Growth Rate (SGR) with highest value of 0.97 ± 0.10 was recorded in diet OPS and the lowest value of 0.81 ± 0.07 was recorded the fish feed in diet CLO. There were no significant differences between diet OPS, and CLO. Protein efficiency ratio was highest in diet OPS (2.70 ± 0.54).

The crude protein of the experimental fish in all the treatments was significantly different from the value obtained in the initial fish. The Crude fibre and moisture content of the initial fish (prior to the feeding trial) was not significantly different from the values for all the other treatments. The ash content of fish fed diet CLO was significantly lower than those fish fed other diets (Table 5). Results of analysis of the hematological parameter of *Clarias gariepinus* in this study (Table 6) showed that there was no significant difference ($P < 0.05$) in the values of RBC, WBC, ESR, MCV, MCH and Platelet between all the treatments, but the values of Hb, MCHC showed significant differences between the treatments.

Result for the carcass free fatty acid, total cholesterol and triglyceride of the fish fed the experimental diets is presented in Table 7 while the histopathological results on the treatment are shown in Plates 1-6, indicating marked diffuse (fatty change) vacuolation of hepatocytes among fish from the different treatments after the experiment (Plate 1-6).

Table 6: Haematological parameters of African Catfish *Clarias gariepinus* juveniles fed with the experimental diets

Parameters	INITIAL	OPS	USO	SBO	ASO	COs	CLO
PCV (%)	21.00	22.00 ± 2.65[a]	20.33 ± 3.51[a]	18.67 ± 1.15[a]	21.67 ± 4.04[a]	22.0 ±2.650[a]	24.00 ±3.61[a]
Hb (gm %)	7.20	7.07 ± 0.81[b]	3.00 ± 3.51[c]	5.57 ± 0.38[a]	6.90 ± 1.31[d]	7.40 ± 1.06[e]	7.67 ±1.24[a]
RBC ($\times 10^{12}$/L)	1.84	1.79 ± 0.46[a]	1.59 ± 0.12[a]	1.45 ± 0.22[a]	1.95±0.56[a]	1.85 ± 0.52[a]	2.03 ±0.68[a]
WBC ($\times 10^{9}$/L)	16,500	17116.67[a] ±1733.73	19.150[a] ± 975.96	15600[a] ± 4850.78	19966.67[a] ±7823.26	19550.00[a] ±7590.62	19933.33[a] ±1527.53
ESR (mm/hr)	0.20	2.00 ± 1.00[a]	1.33 ± 0.58[a]	1.33 ± 0.58[a]	1.67 ± 0.58[a]	1.67 ± 0.58[a]	1.67 ±1.15[a]
MCV (F1)	114.13	125.94 ± 16.61[a]	129.15 ± 29.01[a]	131.30 ± 24.28[a]	115.15 ± 29.61[a]	122.54 ±19.29[a]	124.86 ±30.14[a]
MCH (Pg)	39.13	40.53 ± 6.03[a]	36.83 ± 9.53[a]	39.19 ± 7.59[a]	36.67 ± 9.52[a]	41.49±9.72[a]	39.79 ±9.23[a]
MCHC (%)	34.29	32.14 ± 0.79[a]	28.41 ± 1.51[abed]	29.81 ± 0.32[c]	31.83 ± 0.41[e]	33.65±3.16[b]	31.91 ±0.37[d]
Platelet	294000.0 0	137333.33[a] ±28095.08	216333.33[a] ±118643.72	131333.33[a] ± 29871.95	159666.67[a] ±70500.59	127333.33[a] ±36501.14	246333.33[a] ±137012.16

KEY: Values given in mean ± standard deviation of three replicates.
NOTE: Figures in the same row having same superscript are not significantly different (P > 0.05)

Table 7: Carcass Free Fatty Acid, Total Cholesterol and Triglyceride in the Experimental Fish

	FISH SAMPLES					
Parameters	**OPS**	**USO**	**SBO**	**ASO**	**COs**	**CLO**
Total Cholesterol (mg/g)	665.83	565.63	2126.32	1050.00	1226.43	1078.00
Triglyceride (mg/g)	6208.57	6108.75	8583.47	4650.00	2777.50	6406.40
Free Fatty Acid (%)	4.75	11.94	5.03	18.13	3.26	10.92

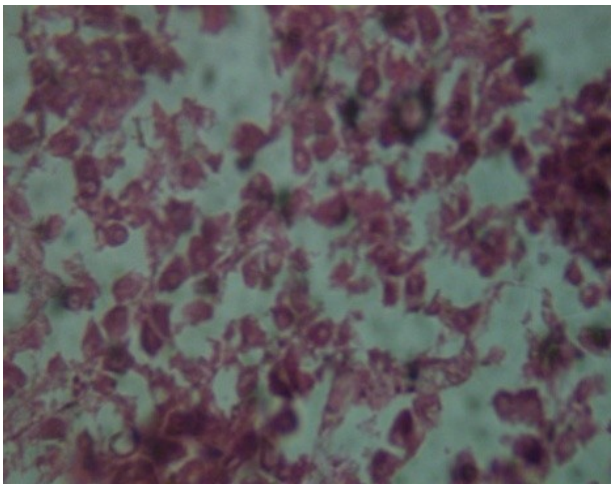

Plate 1: Liver tissue from fish fed COs based diet having severe fatty infiltration

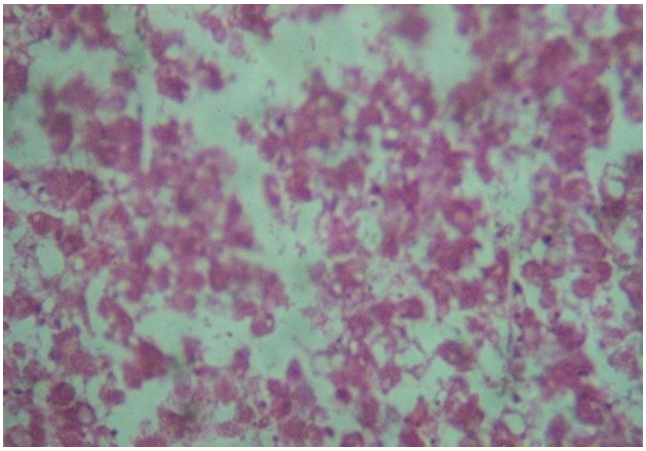

Plate 2: Liver tissue of an experimental fish fed ASO based diet having generalised diffuse hepatic necrosis, and mononuclear cell infiltration

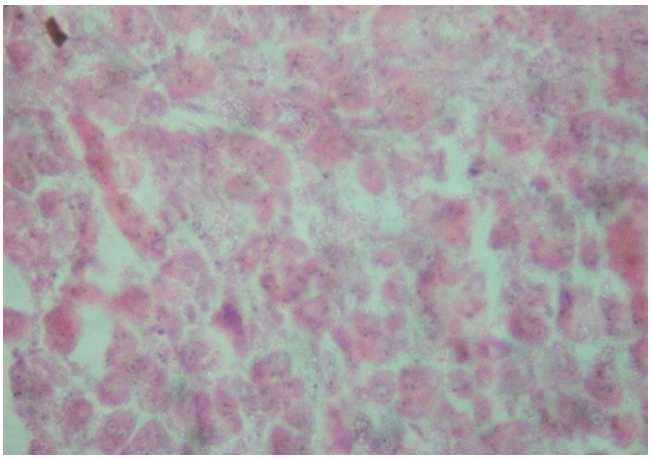

Plate 3: Liver tissue of an experimental fish fed CLO based diet having diffuse fatty degeneration (mild)

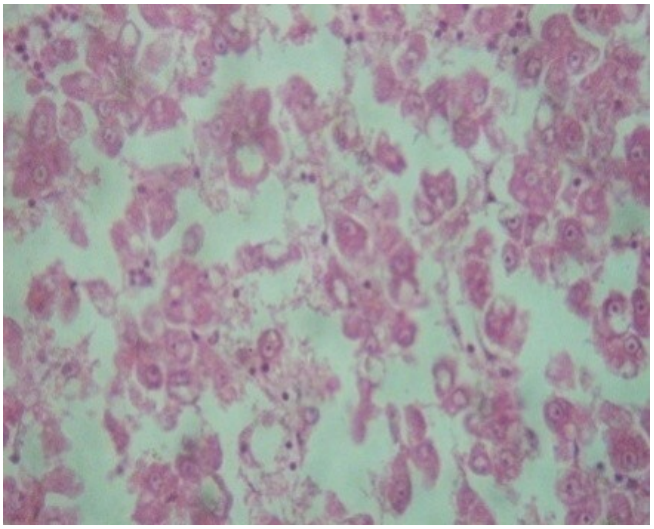

Plate 4: Liver tissue of an experimental fish fed USO based diet having generalised diffuse hepatic necrosis, and mononuclear cell infiltration

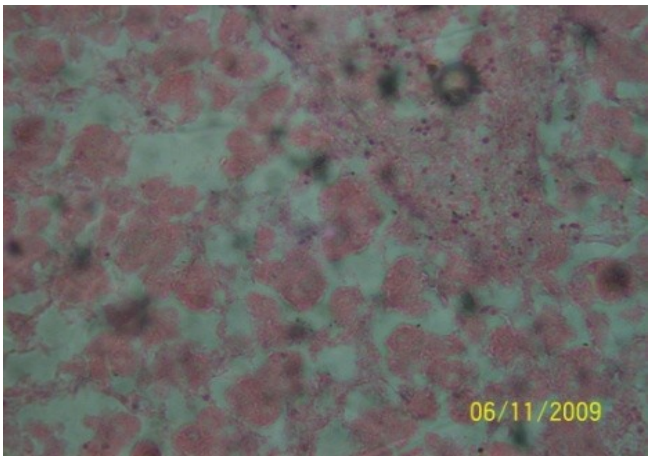

Plate 5: Liver tissue of an experimental fish fed SBO based diet having generalised diffuse hepatic necrosis, and mononuclear cell infiltration

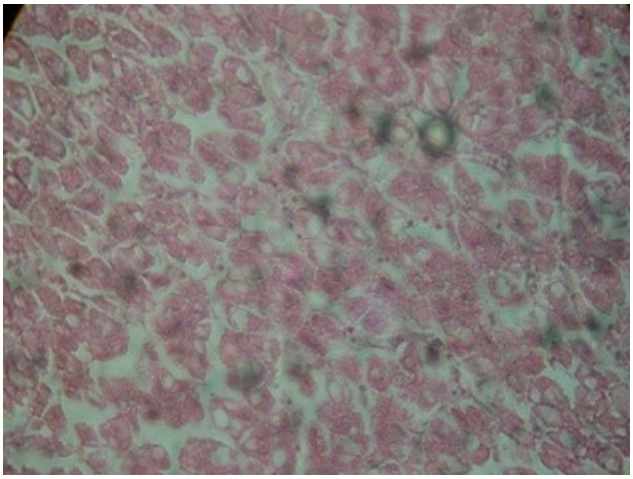

Plate 6: Liver tissue of an experimental fish fed OPS based diet having generalised diffuse hepatic necrosis, and mononuclear cell infiltration

DISCUSSION

Ugwu seed oil (USO) and Oil Palm Seed (OPS) are promising inclusions in replacing FO in feeds of the African catfish, since growth performance were similar among fish fed the different diets, except those fed diet CLO based diet whose growth performance was significantly poorer than all other treatments, Table 4. Fish survival throughout the feeding trial was relatively high for diets USO and COs (100%) but lowest for those fed diet SBO (93.33%), and 97.77% for OPS, ASO, and CLO this could likely be that the n-3 PUFA in the SBO significantly affected the white blood cells and other blood parameters, thereby compromising the immune system. The survival rate of fishes fed COs based diet could be due to the n-3: n-6 PUFA balance obtained from the combination of vegetable oil and animal oil which seems critical in the diet of the African catfish. From the human nutritional point of view, it is considered important that a balance in n-3 and n-6 PUFA be present in the diet to be consumed (Tichelaar, 1993). Fish fed diet OPS performed better in weight gain than fish fed diet CLO. This could be due to the additional n-6 PUFA present in OPS based diet. This results agrees with those of previous studies which have shown that palm oil is a suitable dietary lipid and energy source for some tropical catfish species (Lim *et al*., 2001, Ng *et al*., 2003) and for the red hybrid tilapia, *Oreochromis* sp. (Ng *et al*., 2001). Lim *et al*. (2001) reported that up to 8% of refined, bleached, deodorized, palm olein (RBDPO) or crude palm oil (CPO) can be included in diets of the African catfish with improved performance, protein retention and fillet vitamin E concentration of this fish. Ng *et al*. (2000) also reported that up to 90% of dietary fish oil could be replaced efficiently in the diet of a tropical cat fish, *Mystus nemurus*. The weight gain of fishes fed with (USO) (484.88 ± 68.46) closely followed those fed with OPS based diet 553.10 ± 117.58), ASO (475.20 ± 30.30 g), SBO (414.40 ± 125.12 g), COs (410.73 ± 51.13), and least in CLO (380.71 ± 59.46). The weight gain of fishes fed CLO based diet i.e. the control diet, showed the lowest growth. This might be due to the higher concentration of n-3 PUFA found in cod liver oil. This result agrees with the findings of Ng *et al*. (2001) who found slight depression in growth of hybrid tilapia and also in the African catfish (Ng *et al*., 2003). Fishes fed with a mixture of oils (COs), based diet was observed to have lower weight gain. The report of De Francesco *et al.* (2007) on feed containing the mixture of vegetable oils caused a reduction in the level of plasma cholesterol and circulation of LDL (Low density lipoprotein, transport protein) of rainbow trout.

Specific growth rate with highest value of 0.97 ± 0.10 was observed in diet OPS based diet and the lowest value of 0.81 ± 0.07 in diet CLO. The non significant difference between diet OPS, COs and CLO indicated these fish were able to digest the different diets and convert the diets into body tissues with the same degree of efficiency.

Protein efficiency ratio was highest in OPS based diet (2.70 ± 0.54) which indicated better utilization of protein for growth in juveniles fed this diet.

The result of the experiment showed that diet OPS had the highest value for Protein production value, protein efficiency ratio, food conversion ratio, and protein intake of 9.75 ± 2.98, 2.70 ± 0.54, 3.18 ± 0.34, 2.96 ± 0.59 respectively. Based on these parameters, the diet with the best growth performance is OPS based diet which could be attributed to the high content of carotenoids, tocopherol, vitamin E and n-6 PUFA found in palm oil (Sambanthamurthi *et al*., 2000).

The protein content of the carcass composition did not show significant difference between treatment OPS, USO, ASO and COs ($P > 0.05$) (Table 6), but there was a significant difference between treatments SBO and CLO. The lipid contents of the fish fed with OPS, SBO, ASO and COs were found to be lower than those fed with the control diet (CLO). This could be a result of lipid accumulation in viscera than carcass (Murai *et al.*, 1985) and might be attributed to poor utilization of OPS, SBO, ASO and COs compared to the fish fed fish oil (CLO). The fish fed USO (1.42 ± 0.08) had the highest numerical value of crude fiber while the least value was obtained from COs (1.29 ± 0.04). The values of the NFE showed that there were significant differences between fish fed USO, SBO, ASO and CLO based diets.

Results of analysis of the hematological parameters of *Clarias gariepinus* in this study showed significant difference between the treatment values and the PCV value obtained for the control (CLO) that had the highest value. Hb value of 7.20 g dl for the initial (pre-treatment) *Clarias gariepinus* and the mean values of 7.67 ± 1.23 and 7.07 ± 0.81 obtained for fish raised on diet (CLO and PSO) were similar to the mean Hb values of 7.44% obtained by Etim *et al.* (1999) for *Chrysichthys nigrodigitatus* showing that the oxygen carrying abilities of the blood of these two catfishes are similar. The difference may be due to the different species used by the two authors and the ability to utilize n-6 fatty acids present in vegetable oils which differs from species to species. The value obtained in this study was within the recommended range of 4.1- 10.3 by Blaxhall and Daisley, 1973 for healthy fish.

There was no significant difference in ESR, RBC, WBC ($P \geq 0.05$) among the treatments. The value of the RBC ranged from $2.30 \pm 0.68 \times 10^{12} L^{-1}$ to $1.45 \pm 0.22 \times 10^{12} L^{-1}$ which are similar to those

obtained by Osuigwe *et al*. (2003) for *Clarias gariepinus*. The mean cell haemoglobin concentration (MCHC) differed between treatments and did not follow a clearly defined trend. Fish fed on diet USO showed significantly lower MCHC than fish raised on all the other treatments. Lie *et al.* (1989) reported that an increase in MCHC and MCH values reflect a preserving mechanism in rainbow trout activated at reduced water temperatures. There was no temperature variation in this study, hence no increase relative to the initial MCHC and MCH values were observed.

The result for the fatty acid, total cholesterol and triglyceride of the fish fed on the experimental diets shows that ASO had highest value for free fatty acid, while COs had the least value. Conversely, the muscle protein content was significantly higher in the fish fed OPS (48.09 ± 1.40) and SBO (48.56 ± 2.53) compared with other treatments while both having very low free fatty acid value (4.75% for PSO and 5.03 for SBO). This relationship between muscle protein and lipid content has been observed in previous studies with salmonids (Reinitz and Hitzel, 1980). Shepherd and Bromage (1992), and Rebecca *et al.* (1995) who affirmed that fish utilizes lipids for energy, for cellular structure and for maintenance of the integrity of biomembranes and that neutral fat are far the most important members of the lipid groups in nutrition and cellular physiology in fish. Catacutan (1991) revealed that homoeothermic animals have dietary requirements for essential fatty acids i.e. fatty acids with a double bond (unsaturated) in the ω-6 position, counting from the methyl end of the fatty-acid chain.

Researchers have also found that dietary saturated fatty acids tend to be incorporated into fish fillets within narrowly defined physiological levels (Greene and Selivonchick, 1990; Bell *et al*., 2002) which augments well for lipid sources such as palm oil. Muscle PUFA concentrations are however more labile and have been shown to be positively correlated to dietary PUFA profiles (Greene and Selivonchick, 1990). In this regard, palm oil has the advantage over other vegetable oils such as USO, ASO, and CLO when used in tilapia feeds. The significantly lower PUFA content in the fish fillets of fish fed palm oil-based diets can minimize susceptibility to lipid peroxidation.

Tissue cholesterol concentrations are known to vary depending upon the nutritional status of fish (Kennish *et al*., 1992; Kaushik *et al*., 1995). Hence the observation in this research is in line with these two authors. Fish fed ASO based diet had the highest amount of free fatty acid, while the fish fed with COs had the least. From the standpoint of practical fish culture, dietary lipids are important sources of energy and the only source of essential fatty acids (EFA) in fish and vary from species to species, age to age and size to size (Shepherd and Bromage, 1992).

The result of the economic analysis of experimental diets fed African catfish (C*larias gariepinus*) for 84 days was done based on the price of each raw material, the amounts required to make the different diets and calculated as the cost/kg of each diet. The prices used for the raw material were average prices during the experimental period, due to the fact that there may be significant changes throughout the year. The economic analysis revealed that ASO, OPS, USO, SBO and CLO based diet had better benefit cost ratio over COs based diet, ASO seeds were handpicked freely on the University campus, thus reducing the effective cost. Therefore fish farmers can effectively replace fish oil (CLO) with vegetable oils (ASO and OPS) without adverse effect on growth performance.

The result of this study revealed that there was a marked diffuse (fatty change) vacuolation of hepatocytes among the treatments after the experiment. But this was not due to anti-nutritional factors because this was observed in all the treatments OPS, USO, SBO, ASO, COs and CLO. Environmental stress may cause changes in cellular function that alter the physiology of organ systems in the fish as reported by Vanvuren *et al*. (1994) which is similar to the report of Adeogun (1994) who observed pathological changes like intense vacuolar degeneration of hepatocytes, periportal hepatic necrosis and fibrosis in fingerlings of *Clarias gariepinus* exposed to sub lethal concentration of different part of fruits of *Raphia hookeri* aqueous extracts. Liver receives about 39% of the total cardiac output so it is susceptible to chemical presence in systemic circulation (Bridges, 1990). Also, liver is considered the most important target organ from a toxicological point of view because of its role in detoxification, biotransformation and excretion of xenobiotics (Hassanein *et al*., 1999).

Lipoid accumulations in the experimental fish livers were detected. From the Plates it shows that fish fed diet OPS, SBO, USO and ASO had diffused necrosis and mononuclear cell infiltration. This could be as a result of fat accumulation which results in the rupture of the cytoplasm in the cells, leading to the necrosis of some of the cells. Fish fed diet COs had severe fatty infiltration, this result agrees with Caballero *et al*. (2004) who reported that the reduction of the dietary fatty acids due to the inclusion of vegetable oils in the diets tends to promote fat accumulation in the fish liver. Fish fed diet CLO had diffused fatty degeneration Plate 3 which is milder.

CONCLUSION

In conclusion, the results obtained in this study show that palm oil can replace dietary fish oil (cod-liver oil), in the diet of *Clarias gariepinus*, without significantly compromising growth performance. Ugwu seed oil (USO) was well utilized like OPS. The assessments of utilization of both were based on body weight gain, specific growth rate, protein intake and protein efficiency ratio. The haematology report showed no inhibition to the formation of skeletal tissues, cell formation, blood formation and flow hence its utilization in the aquaculture of *C. gariepinus* should be encouraged as an efficient oil source in catfish feed. In the economic analysis of the cost of diets, COs based diets had the highest cost of ₦ 230.19 for producing 1kg of fish flesh followed by CLO, SBO, USO and OPS with ₦ 225.19, ₦ 224.69, ₦ 223.64 and ₦ 222.69 respectively. Diet ASO had the lowest cost of ₦ 220.99.

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