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Effects of Fish Oil Replacing Groundnut Oil on Growth and Digestibility Coefficient of African Catfish, *Clarias gariepinus* (BURCHELL, 1822), Broodstock

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ABSTRACT
A study on the effect of Fish Oil (FO) replacing Groundnut Oil (GO) on *Clarias gariepinus* growth was conducted. Forty-five (45) 6-month old *Clarias gariepinus* broodstock (thirty females and fifteen males) weighing averagely 380.42g were randomly allocated to fifteen treatments (n=3), in concrete tanks sized, 1m x 1m x 1m, in the ratio of 2 females to 1 male. Five isonitrogenous and isolipidic diets (40% crude protein and 6% dietary lipids) were formulated. Fish oil was replaced with groundnut oil at 0, 25, 50, 75 and 100%, denoting treatments T1 Control), T2, T3, T4 and T5, respectively. The experimental fish were fed at 4% biomass, twice daily for 168 days. Results revealed P<0.05 for final weight (614.72g), weight gain (234.21g), specific growth rate (1.65), feed conversion ratio (2.20), and protein efficiency ratio (0.90), for the control group. T3 ranked next: 606.35g, 225.12g, 0.86, 2.90, and 0.76, respectively while, T5 recorded the least: 573.72g, 193.60g, 0.69, 4.18 and 0.60, respectively for the above parameters. The control recorded the highest statistical apparent digestibility coefficient for protein and apparent digestibility coefficient for values (82.00% and 83.00%), followed by T3 (80.00% and 82.00%), T2 (25% GO) (76.00% and 77.00%) and T4 (75% GO) (75.00% and 76.00%), respectively, while T5 (100% GO) recorded the least values (72.00% and 74.00%) for the two parameters. Control recorded highest total body carcass protein (66.13%), moisture (6.78%), and lipid (5.98%), while T3 had highest total body carcass ash (4.95%) and fiber (5.61%), thus, connoting that GO can serve as a veritable substitute for FO at 50% inclusion level, in fish diet.

Keywords: Fish oil, Groundnuts oil, growth, digestibility coefficient, *Clarias gariepinus* broodstock

INTRODUCTION

Dietary lipids, mainly vegetable oils and animal fats are readily digestible energy source in fish diet. They can be utilized to partially spare dietary proteins from use as energy in aquaculture feeds and limit ammonia production (Ajani *et al*., 2011). They also aid digestibility of certain protein-rich feeds, improve palatability, feed conversion ratio, reproductive efficiency, alleviate stress, and easily metabolized in their body system.

Fish Oil (FO), which is derived from oily fish tissues of herring and salmon, is very important in fish nutrition, as they are sources of energy, essential fatty acids, vitamins, and antioxidants needed for adequate growth, sound health, successful reproduction, and general maintenance. It's being highly nutritious and dense, makes it an important component of fish feed, for enhancing fish growth and reducing water pollution (Ochang, *et al*., 2007).

There would be an increase in cost of fish oil which would culminate into increase in cost of aquaculture feed, a situation that is now arising because of the problem of overfishing in our water bodies, resulting in dwindling catch and unfavorable environmental changes thus, making FO unavailable or, inadequate for use in aqua feeds (New and Wijkstrom, 2002). This has resulted in the use of Vegetable Oils (VOs) which are relatively cheaper and readily available, as viable alternatives to FO (wholly, or partially) in fish feeds.

Different types of vegetable oil have been used for fish rearing with good results on their survival and growth rates (Villata *et al*., 2008). There are however, little studies on the effects of oil-based diets on broodstock fish performance, in respect of fish growth vis-à-vis the essence of apparent digestibility

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coefficient in their growth process. Quality aqua feeds are not justified by nutritional composition but, by the degree to which a fish utilizes such feeds for sustenance. Digestibility evaluates the richness of a diet, which is determined by the fish ability to digest, absorb and assimilate the nutrients in that diet. When carbohydrate component of a diet is utilized, the possibility of reducing the nitrogenous waste in a water body to the barest minimum is very high. Uneaten feeds, in a water medium, result in environmental pollution, which is dangerous to the existence of other aquatic animals. High levels of carbohydrates and proteins in faeces decrease faecal integrity and, as such increase the dissolution of the faecal matter, when expelled into the water column and thereby deteriorating water quality where fish are reared (Glencross *et al*., 2004) hence, the greater need for nutrients digestibility.

Groundnut Oil (GO), derived from groundnut (*Arachis hypogaea L.*), an herbaceous legume, which grows throughout the tropical and warm temperate regions of the world, is recognized as second only to palm oil in Africa in terms of availability, and sustainability. It is a high energy source The oil is edible and naturally has deep yellow color with pleasant nutty aroma and sweet taste, due to lack of linoleic acid, known for causing deterioration of oil flavor while, the refined type has light yellow and neutral taste (Diop *et al*., 2004).

It is one of the stable cooking oils with longer shelf life, due to its higher proportion of Unsaturated Fatty Acids (UFA), low proportion in saturated fats, lack of cholesterol content and presence of essential fatty acid (linoleic acid- omega-6) (Aderolu and Akinremi, 2009). It has very good lipid profile and has Saturated, Mono Unsaturated and Polyunsaturated Fatty Acids (SFA: MUFA: PUFA= 18: 49: 33), in healthy proportions. The essence of this study therefore, is to assess the growth performance and nutrient digestibility of *C. gariepinus* fed GO replacing FO at different levels

MATERIALS AND METHODS

Experimental stock and management

Forty-five (45) 6-month old *C. gariepinus*, of mean weight (380.42g±0.02) and length (36.47cm±0.01), procured from Bowen University, Iwo, Oyo State, Nigeria, were randomly divided into fifteen concrete tanks of 1m x 1m x 1m, and stocked at the rate of 3 fishes (2 females: 1 male) per tank after 2 weeks acclimatization period, in an outdoor cylindrical tank, covered with fine mesh net. Water was changed every other day and refilled with already fertilized water from adjacent pond, with the aid of a 2.5Hp pumping machine. Water temperature, dissolved oxygen, ammonia and pH levels were maintained at 27.08 ± 0.01^oC, 5.51 ± 0.02 mgL^{-1}, 2.22 ± 0.01 mgL^{-1} saturation level, and 6.62 ± 0.02, respectively.

Experimental diet

Five isonitrogenous diets containing 40% crude protein and 6% lipid were formulated such that, FO was replaced with 0, 25, 50, 75 and 100% of GO (Table 1). Each of the prepared diets for the 6-month old fishes, was passed through a mincer of 4.5mm in diameter, blended and pelleted for a period of 14 weeks of the feeding trial. The pellet size was later increased to 6mm in diameter at their attainment of an average weight of 498.22g and, maintained till the expiration of the experiment. All the pelleted diets were dried at ambient temperature of 30^0C for 3 days. The dry pellets were packed individually in labeled dry air-tight plastic containers. The feeds were sub-divided into two equal parts and served by hand, twice daily, in triplicates, at 4% Live Body Weight (LBW). The fishes were starved for twenty-four hours before the commencement of the feeding trial, to allow for uniformity in the fish stomach condition and to induce their appetite. Fish survivability was monitored daily. Proximate composition of experimental diets were performed in triplicates according the method described by AOAC (2000).

Growth performance and nutrient utilization measurements

The growth parameters estimated while the feeding trial lasted were Weight Gain (WG), Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), Protein Efficiency Ratio (PER) and Nitrogen Metabolism (NM), as well as Apparent Digestibility Coefficient for energy (ADC_{energy}) and Apparent Digestibility Coefficient for protein ($ADC_{protein}$), which determined the palatability of the diet to the fishes. The experimental fishes in each treatment tank were weighed fortnightly, using DRONCO$^{(R)}$

kitchen scale. Mean body weight was taken in triplicates for each treatment, from which average weight was determined (Table 2). Quantities of feed fed to the fishes were measured at intervals, while uneaten diets and faecal deposition were flushed away. The survivability (Table 2) of the fishes was adequately monitored twice daily.

Table 1: Ingredients and Nutrients Composition of the experimental diets fed to *C. gariepinus* broodstock for 168 days

Ingredients	**Levels of Fish Oil Inclusion (%)**				
	0	**25**	**50**	**75**	**100**
Yellow Maize	25.20	25.20	25.20	25.20	25.20
GroundnutCake	48.20	48.20	48.20	48.20	48.20
Fish Meal	16.10	16.10	16.10	16.10	16.10
Cod liver Oil	6.00	4.50	3.00	1.50	0.00
Groundnut Oil	0.00	1.50	3.00	4.50	6.00
Oyster Shell	1.50	1.50	1.50	1.50	1.50
Bone Meal	1.00	1.00	1.00	1.00	1.00
Fish Premix[a]	1.00	1.00	1.00	1.00	1.00
Binder (Starch)	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Proximate Composition (%)					
Moisture	7.20	7.10	7.28	7.12	7.02
Ash	16.52	15.42	15.60	15.18	14.01
Crude Protein	41.08	40.72	40.94	40.68	40.38
Crude Lipid	12.92	11.84	12.27	11.69	11.48
Crude Fibre	2.35	1.78	1.92	1.00	0.98
Nitrogen Free Extract	27.81	27.01	27.12	26.98	26.72
Gross Energy (Kcal/kg	4584.10	4334.00	4345.22	4312.70	4328.44

Three male and three female fish were weighed for determination of proximate composition (initial carcass value). Two male and two female fish were sampled for the final carcass composition at the end of the experiment. *C. gariepinus* were minced for moisture and ash contents. Moisture was determined by drying pre-weighed samples at 105^0C for 25-hrs. Ash content was determined by incinerating the dried samples in a muffle furnace at 500^0C for 15-hrs, while Soxhlet extraction with petroleum ethers was used for fat determination. Crude protein was analyzed by Kjeldal method. Fish samples were sacrificed for visceral, liver and gonads to be removed. They were subsequently dried on a clean towel and weighed individually, using Analytical Scale. The dried visceral, livers and gonads were stored at - 4^0C for further analysis.

Digestibility study

Digestibility of the experimental diets was carried out using In-vitro method according (AOAC, 2000). Each of the weighed sample (5g) groundnut oil-based diets was put into test-tube and was made to incubate under anaerobic condition. The incubation was done with pepsin, for protein digestibility. Rumen liquor was used for energy digestibility. Incubation process was at the same temperature (35^0c) and at a neutral pH (7.0) under anaerobic condition, in a water bath. The differences between protein and, gross energy of the incubation feeding samples before incubation and the residual after incubation were used as indices for protein and energy digestibility determination, respectively.

Growth performance and data computation

At the end of the trial, growth and nutrient utilization performance parameters such as weight gain, final weight, specific growth rate, fish conversion ratio, protein efficient ratio and survival rate were estimated using the following formulae:

$$\text{Mean Initial Weight } (MW_1) = \frac{\text{Total weight of fish at the start of the feeding trial}}{\text{Total number of fish at the start of the trial feeding}}$$

$$\text{Mean Final Weight } (MW_f) = \frac{\text{Total weight of fish at the end of the feeding trial}}{\text{Total number of fish at the end of the feeding trial}}$$

Mean Weight Gain (MWG in g) = MWf – MWi

$$\text{Specific growth rate (SGR in \%)} = \frac{[\ln(Wf) - \ln(Wi)] \times 100}{\text{Period of experiment}}$$

Where LnWf = Log of Final Weight; LnWi = Log of Initial Weight.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total dry feed fed (g)}}{\text{Total weight gain (g)}}$$

Protein efficiency ratio (PER) = Weight gained by fish (g)/Protein fed or consumed (g)

Protein consumed = [(% of protein in diet X total quantity of feed consumed (g))/100]

Survival rate (%) = [(Nf – Ni/Ni) x 100]; Ni = Initial number of fish at the beginning of experiment, Nf = Final number of fish at the end of the experiment

$$\text{Protein Digestibility} = \frac{\text{Final crude protein (g)} - \text{Initial crude protein}}{\text{Initial crude protein (g)}}$$

$$\text{Energy Digestibility} = \frac{\text{Final gross energy} - \text{Initial gross energy}}{\text{Initial gross energy}}$$

The calculations of MWG, FCR, SGR, PER, NM, %SURV, ADC_{energy}, and $ADC_{protein}$, are as described by Eyo and Mgbenka (1992), AOAC (2000), Nwanna (2003), Fafioye *et al*., (2005), Khan and Abidi (2007), Du *et al*; (2008), Sogbesan and Ugumba (2008), Sotolu and Faturoti (2008).

Statistical analysis

Data obtained from the experiment were subjected one-way Analysis of variance (ANOVA) descriptive statistics. Differences between the means were determined using Duncan (1955) Multiple Range test and with the aid of Statistica Version 5.1.

RESULT

Ingredients and nutrient composition of the diets are presented in Table 1. Crude protein and moisture contents ranged from 40.22 to 41.08% and 7.12 to 7.20%, in T5 and control (T1) diets, respectively. The lowest and highest ash values (14.01, 16.52) were found with T5 and (T1), respectively, while gross energy values varied between 4328.44 in T5 and 4584.10 in (T1). Crude fiber and lipid values, which were highest in T1 and lowest in T5, ranged from 0.98 – 2.34 and 11.48 – 12.92%, respectively.

Growth performance and nutrient utilization values of the fishes are shown in Table 2. The initial weights of the fishes were uniform, while significant differences (P< 0.05) were observed in the WG and SGR within the treatments. Highest WG and SGR values (234.21g and 1.65), respectively were recorded by fishes on T1, followed by fish on T3 (225.12g and 0.86), respectively. The least values for the parameters (193.60g and 0.69), respectively, were obtained from fishes on T5. Percentage survivability of fishes, which was 100% in all the treatments, was not statistically different (p> 0.05). FCR varied significantly (p<0.05), with control fishes recording the lowest value (2.20). PER also varied significantly, and ranged between 0.60 (T5) and 0.90 (T1). There was no significant differences (p> 0.05) in NM among the treatments.

Higher (p<0.05) ADCp and ADCe values were obtained in control (82.00%; 83.00%) followed by T3 (80.00%; 78.00%), compared to T2 (76.00 %; 77.00 %,) and T4 (75.00%; 76.00%), while T5 (72.00%; 73.00%) recorded the least value for the parameters.

Body carcass composition (Table 3) revealed significant differences (p< 0.05) between the initial (fishes at the start of the study), T1 and other treatments for crude protein content. Body crude protein content of the initial and T5 had lowest values (59.18 and 60.95%), respectively, compared to T1 (66.13%) and other treatment groups; T2, (64.20%), T3 (64.39%), and T4 (63.58%). However, no significant differences (p > 0.05) were recorded for crude fiber, ether extract, ash and moisture, though T3 recorded higher numerical values for crude fiber and ash (4.77%; 4.95%) than control fish (4.02%; 4.75%) and other treatments; T1 (4.33%; 4.25%), T3 (4.69%; 4.50%), and T4 (4.01; 3.96)across board. Body lipid content was numerically higher in T1 (6.35), followed by T3 (6.28). T5 recorded the least (5.22). Fishes at the start of the study recorded the highest value (7.88%) for moisture, while T1 had the least (5.87%). The minimum and maximum values for water quality parameters (Table 4) were not statistically different ($p > 0.05$) in all treatments, with temperature (27.02±0.01ºC; 27.12±0.01 ºC), pH (6.33± 0.01; 6.94± 0.02), dissolved oxygen (5.44± 0.01; 5.57± 0.01 mgL^{-1}), and ammonia (2.15± 0.01; 2.29± 0.02 mdL^{-1}).

Table 2: Growth performance and apparent digestibility coefficient of *C. gariepinus* broodstock fed varying groundnut oil inclusion levels diet

Parameters	Groundnut oil Inclusion levels (%)				
	0	25	50	75	100
W_i (g)	380.51±0.03	380.95±0.05	380.64±0.03	380.00±0.02	380.02±0.03
W_f (g)	614.72±0.03^a	594.25±0.05^c	602.25±0.04^b	585.45±0.04^d	573.72±0.05^e
WG (g)	234.21±0.01^a	213.30±0.05^c	225.12±0.03^b	205.45±0.03^d	193.60±0.03^e
% Surv	100.00±0.01^a	100.00±0.01^a	100.00±0.00^a	100.00±0.02^a	100.00±0.00^a
SGR	1.65±0.03^a	0.81±0.04^c	0.86±0.02^b	0.80±0.03^c	0.69±0.02^{cd}
FCR	2.20±0.02^a	3.51±0.02^c	2th.90±0.03^b	3.92±0.00^c	4.18±0.01^d
PER	1.64±0.01^a	0.73±0.03^b	0.76±0.02^b	0.68±0.02^c	0.60±0.01^{cd}
NM	50.46±0.04^a	48.92±0.01^a	49.90±0.00^a	48.06±0.03^a	47.02±0.02^a
$ADC_{protein}$ (%)	82.00±0.03^a	76.00±0.04^b	80.00±0.03^a	75.00±0.04^b	72.00±0.03^c
ADC_{energy} (%)	83.00±0.02^a	77.00±0.03^b	82.00±0.04^a	76.00±0.04^b	74.00±0.02^b

Means with the same superscript within the same row were not significantly different ($p > 0.05$).
W_i (g) = Initial Weight (g), W_f (g) = Final Weight (g), % Surv = Percentage Survivability, SGR = Specific Growth Rate (%/g), FCR = Feed Conversion Ratio, PER = Protein Efficiency Ratio, NM =Nitrogen Metabolism, $ADC_{protein}$ = Apparent Digestibility Coefficient for protein, ADC_{energy} = Apparent Digestibility Coefficient for energy

Table 3: Carcass composition (%) of *C. gariepinus* (dry matter) before and after the feeding trial

Parameters	Initial	Groundnut oil Inclusion levels (%)				
		0	25	50	75	100
CP (%)	59.18±0.03^b	66.53±0.01^a	64.20±0.03^a	64.39±0.02^a	63.58±0.01^a	60.95±0.05^b
CF (%)	3.75±0.01^a	4.02±0.02^a	4.31±0.02^a	4.77±0.04^a	4.69±0.03^a	4.01±0.02^a
CL (%)	5.01±0.01^a	6.34±0.03^a	5.48±0.01^a	6.12±0.02^a	5.36±0.01^a	5.22±0.01^a
Ash	3.98±0.02^a	4.75±0.04^a	4.25±0.01^a	4.95±0.02^a	4.50±0.02^a	3.96±0.01^a
Moisture	7.88±0.02^a	5.87±0.02^a	6.02±0.02^a	5.95±0.02^a	6.60±0.04^a	6.98±0.01^a

Note: Means with the same superscript within the same row were not significantly different ($p > 0.05$).
Key: CP = Crude Protein, CF = Crude Fibre, CL = Crude Lipid.

Table 4: Water quality parameters of the experimental fish fed Groundnut oil-based diet for 168 days

Parameters	Groundnut oil inclusion levels				
	0	25	50	75	100
T (^{0}C)	26.18±0.00^a	27.07±0.01^a	27.12±0.01^a	27.09±0.02^a	27·02±0.01^a
pH	6.46±0.01^a	6.94±0.01^a	6.64±0.02^a	6.33±0.03^a	6.74±0.01^a
DO (mg/l)	5.40±0.01^a	5.51±0.01^a	5.55±0.02^a	5.44±0.02^a	5.57±0.01^a
Ammonia	2.22±0.00^a	2.29±0.01^a	2.20±0.02	2.15±0.01^a	2.26±0.01^a

Note: Means with the same superscript within the same row were not significantly different ($p > 0.05$).
T= Temperature, DO = Dissolved Oxygen,

DISCUSSION

The results above indicated that, the quality and adequacy of the diets supported fish growth and contributed to the improvement of their health condition thus, indicating that, broodstock catfish require adequate combination of μ-3 poly unsaturated essential fatty acid and carotenoid contained in FO and μ-6 Essential Fatty Acid (EFA), as well as the presence of antioxidants (vitamins C and E) in groundnut oil which have protein-sparing effect (protein retention in the fishes' body) for proper growth, and general survival. However, the good performance records of fishes on T3 might be due to appropriate essential fatty acids combination of the two oils, which meets the requirements needed for proper fish growth (Steffens, 1997; Watanabe, 1982).

The good FCR values were an indication of a better efficient feed utilization, which was reflected in their growth performance. Also, PER values also indicated that, the lipid content in the diets had a sparing effect on ingested protein thus, allowing more protein to be deposited in the fishes' tissues. Similar observations were made in past studies by Regost *et al*. (2001), and, Page and Andrews (1973). Inverse relationship of lipid to moisture contents was confirmed by Olukunle (2011) and Shafaeipour *et al*. (2008). High crude protein values (> 40%) in the groundnut oil diets suggested that, the lipid had protein-sparing effect and is a mark of improvement in feed efficiency (Babalola and Apata, 2006; Winfree and Stickney, 1981).

ADCp values are comparable to the findings of Ghulam *et al*. (2015) and Booth *et al*. (2008), while ADCe values are lower than those recorded by the same authors. Variations in ADCp and ADCe values might be due to differences in fish type, age and size, feed ingredients and the particle size of each of the ingredients, quantity of each of the ingredients added, feed processing method, their level of interactions, and general fish management. Variations in digestibility parameters could be caused by methodological factors (Allan *et al*. 1999), and fish size (Henken *et al*., 1985). The higher ADCp values indicated that, *Clarias gariepinus* broodstock capable of efficiently digesting protein components of the diet, due to the presence of proteolytic enzyme in the fish body system which hydrolyses the protein in the diet. When there is an adequate supply of lipids in feeds, lower protein content may limit the capacity of fish to digest lipids maximally, as compared to the higher protein treatments (Johnston, 2002).

The quantity of feed served throughout the experimental period was optimum enough for efficient digestion and assimilation processes to have taken place, thus, resulting in higher ADCe and ADCp, improved PER, better FCR and improved weight gain (WG). Over-feeding of fish may cause overloading of stomach and intestine, and decrease the efficiency of ingestion, digestion and assimilation (Jobling, 1986). The optimum ration level and feeding frequency are important for maximum growth, greater food intake, higher feed utilization, higher nutrient retention efficiency, for stable body conformation and composition in fish (Booth *et al*; 2008). The least growth rate observed in fishes on T5 revealed that, 100% groundnut oil without FO inclusion was not adequate for protein-sparing hence, its inadequacy in the growth of *Clarias gariepinus* broodstock.

The water quality parameters fell within recommended ranges (Ajani *et al*; 2011; Olukunle, 2000). They were reported to be conducive enough, for the fishes to have adequately fed well, with no stress and exhibit optimum growth performance, as higher temperature is noted to adversely affect feed intake and feed digestion (Ayodele and Ajani, 2011; Hogendoorn *et al*. 1983).

Conclusion

Groundnut oil in the diet of *Clarias gariepinus* broodstock enhanced higher utilisation of diet, as revealed by their performance. The study therefore concludes that, GO can be used to replace FO, in the diet of *Clarias gariepinus* broodstock at 50% inclusion level, without compromising their growth, apparent digestibility and survival.

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