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Utilization of dietary oils on growth and fatty acid composition of genetically-male tilapia, *Oreochromis Nilotsicus*

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Abstract
The study investigated utilization of dietary lipids of plant origin (coconut, olive, palm, sunflower and sesame) on growth and fatty acid compositions of genetically-male tilapia, *Oreochromis niloticus.* The control diet contained fish oil. These oils were incorporated at 7% level of the diet and used to produce a 42.5% crude protein balanced diets. Fish (weight 2.50±0.02g and standard length 4.3±0.00cm) were randomly stocked at 20fish/floating net-hapa (1m^3) and fed at 5% body weight for 12 weeks. All experimental procedures followed standard methods. Results revealed the highest final weight (56.42g), average daily growth (0.64g), specific growth rate (3.69%/day) and final standard length (18.43cm) were obtained in fish fed palm oil-based diet. Lauric (12:0), myristic (14:0) and palmitic (16:0) acids were the main saturated fatty acids while oleic (18:1) was the main monounsaturated fatty acids. Long chain PUFAs such as EPA, DHA and ARA were observed in fish fed olive, sunflower and sesame oil diets. This showed that these oils would be beneficial to human health. The study concludes that replacing fish oil with these plant oils consistently resulted in major changes in growth and fatty acid profile of the male tilapia.

Keywords: plant oils, nutrient utilization, fatty acid profile, male tilapia

Introduction

The Nile tilapia, *O. niloticus* is an important commercial freshwater fish species that commands high consumer demand in Nigeria. The country is reported to be a fish-loving nation where fish plays an important role in the diets of the citizen constituting the main and often irreplaceable animal protein source [1]. Production of tilapia has steadily increased over the last decade with farmed tilapia representing more than 75% of world tilapia production [2]. The reports of [3] opined that production of this fish had peaked supply list among freshwater fish culture in Nigeria with intensive farming rapidly expanding at an annual rate of about 12%. Factors such as genetic improvement and ease of culture had contributed to the rapid global growth of tilapia production [4]. However, the Nigerian aquaculture sector is currently faced with the problem of inadequate supply and prohibitive cost of quality fish feeds. Thus, fish feed manufacturers are currently faced with the need to reduce feed costs to match the fluctuating prices of fish products, since increase in the cost of imported feed ingredients could greatly cuts into the profit margin. Therefore, knowledge of the optimal level of non-protein nutrients such as lipids can be used effectively in reducing feed costs in aquaculture.

Lipids in aquaculture feed play important roles as concentrated sources of energy and essential fatty acids for growth, development and proper functioning of physiological processes [5]. They form essential component of steroids and phospholipids which are used as precursors in the synthesis of vitamins and hormones [6]. Lipids are also known to be vehicles for absorption of fat–soluble vitamins and exhibit many hormonal activities. Aquaculture has traditionally used products from industrial fisheries, namely fish meal and oil, for the production of both scientifically and commercially high valued products. However, the reports of [2] estimated the use of fish oil in aqua-feeds since the year 1999 and projections of usage in 2015 and 2030 and the overall results revealed that aqua-feed industry used a greater proportion of fish oil supplies than other sectors. World demand for fish has also been on a steady rise as a result of ever increasing world population, the often reported health benefits attributed to fish and the omega-3 and omega-6 contents. Demand for fish is predicted to keep increasing. Thus, consumer demands can only be met through aquaculture.

Consumers would usually desire to know the nutritional constituents of fish species obtained from different sources. This could be answered through data on fatty acid composition of body lipid. The essential fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are important in human diets. A daily intake of 500mg EPA and DHA had been recommended for prevention of coronary heart disease in human [7]. The human body can make some of the fats it needs from foods consumed but these two essential fatty acids – linoleic and linolenic acids cannot be synthesized. Thus, emphasis had been placed on the increased consumption of fish and fish products, which are rich in PUFA of the n-3 and n-6 series [8]. In Nigeria, increasing attempts to develop practical diets for farmed fish using unconventional materials have been successful [9, 10, 11]. However, the application of replacements should ensure that the end product remains a good source of omega-3 and omega-6 [12]. The overall picture is therefore a gradual substitution of fish oil with suitable plant alternatives for increase growth and productivity. In view of this, the present study was carried out with the aim of investigating the growth and fatty acid compositions of genetically male tilapia.

Materials and Methods

Experimental Site and Design

An outdoor concrete tank (L x B x H: 8m x 5m x 1.65m) located at Vika Farms Limited, Mbak Etoi, Uyo, Akwa Ibom

State, Nieria (geographical coordinates of Latitude: 5o 3' 0" North and Longitude: 7o 56' 0" East) was used for the production stage of the study. This tank was equipped with both inlet and outlet facilities and a 5,000 litre capacity overhead tank served as water reservoir. The experimental design was made up of a module consisting of 8.5 x 6.5m bamboo raft with eighteen 1.5m x 1.5m apartments fittable with 1m x 1m x 1m net-hapas constructed and placed on the concrete tank. The net-hapas had top covers that prevent fish from jumping out as well as protecting fish from aerial predators. The tank was washed and filled with water to a depth of 1.2m. Eighteen net-hapas were fitted to the compartments representing six treatments with three replicates each. Each hapa was rigged and suspended at a depth of 0.75m in water. The float lines were tied to the four corners of each compartment using kuralon rope (No 15) as described by [13].

Diets Preparation and Fish Rearing

The experimental diets containing 42.5% protein were prepared using fishmeal, soybean meal, groundnut cake and corn flour as main ingredients (Table 1). The plant oils from coconut, olive, palm, sunflower and sesame were used to replace fish oil (control) at 7% level of the diets. Pearson Square method was used to balance the proportion of each ingredient used for diet preparation. Each portion was carefully weighed out according to diet and mixed thoroughly to obtain a homogenous paste. Thereafter, they were made into pellets, air-dried, separately labelled according to diets and stored for subsequent use. Tilapia fingerling (mean weight 2.50±0.02g and standard length 4.3±0.00cm were stocked at 20 fish/net-hapa. Fish were fed at 5% body weight three times daily and feeding trial lasted for 86 days.

Table 1: Composition (g/kg) of experimental diets containing different oil resources

Ingredients	Control	Coconut	Olive	Palm	Sunflower	Sesame
Fish meal	186.00	186.00	186.00	186.00	186.00	186.00
Soybean meal	186.00	186.00	186.00	186.00	186.00	186.00
Corn flour meal	182.00	182.00	182.00	182.00	182.00	182.00
Groundnut cake	375.00	375.00	375.00	375.00	375.00	375.00
Lysine	0.300	0.300	0.300	0.300	0.300	0.300
Methionine	0.3000	0.3000	0.3000	0.3000	0.3000	0.3000
Fish premix*	0.5000	0.5000	0.5000	0.5000	0.5000	0.5000
Fish oil Coconut oil	70.00 -	- 70.00	- -	- -	- -	-
Olive oil	-		70.00	-	-	-
Palm oil	-	-	-	70.00		-
Sunflower oil	-	-	-	-	70.00	
Sesame oil	-	-	-	-	-	70.00
Estimated protein (%)	42.50	42.50	42.50	42.50	42.50	42.50

* Fish Premix (per kg of diet): Vitamin A: 10,000,000 I.U.D; D3: 2,000,000 I.U.D; E: 23,000mg; K3: 2,000mg; B1: 3000mg; B2: 6,000mg; niacin: 50,000mg; calcium Pathonate: 10,000mg; B6: 5000mg; B12: 25.0mg; folic acid: 1,000mg; biotin: 50.0mg; choline chloride: 400,000mg; manganese: 120,000mg; iron: 100,000mg; copper: 8,500mg; iodine: 1,500mg; cobalt: 300mg; selenium: 120mg; antioxidant: 120,000mg.

Determination of Growth and Economic Performances

Growth performances were evaluated using standard formulae by [14] thus:

Mean weight gain (MWG) (g) = Final weight (g) –Initial weight (g)

Average daily growth (ADG) = MWG (g)/length of feeding trial (t) (days)

Specific growth rate (SGR) (%/day) = $100(\ln W_2 - \ln W_1)/T_2 - T_1$

Where: W_2 = Weight at time T_2; W_1 = Weight at time T_1

Feed conversion ratio (FCR) = Total dry feed fed (g)/MWG (g)

Protein efficiency ratio (PER) =MWG (g)/total protein fed (g)

Condition factor (CF) = CF = $100W/L^3$ [15]

Where: W = Weight (g) and L = Length (cm).

Survival rate (SR) (%) = 100(Number at end of feeding trial/Number at start of feeding trial)

Lipid Extraction and Fatty Acid Analysis

The method of [16] was employed in the extraction of total lipids and preparation of fatty acid methyl esters. Fatty acid analysis was conducted on a gas chromatograph (Hewlett Parkard: 6890). This was fitted with an automatic sampler (Model: AS 2000B) and flame ionization detector. The conditions used were: Omega wax fused silica capillary column BPX-70 (60m x 0.32mm i.d., 0.25µm film thickness) (SGE; Melbourne, Australia). The starting temperature was 108ºC, which was raised to 115ºC at a rate of 8ºC/min and held for 10 minutes. The temperature was finally raised to 240ºC at a rate of 8ºC/min and held for another 10 minutes. The sample size was 1µl and flashed through helium as the carrier gas at a rate of 1.6 ml/min with inlet pressure of 12 psi. Fatty acids methyl esters were identified in comparison to an external standard (SupelcoTM 37 component FAME Mix).

Statistical Analysis

Data collected were analyzed using one - way Analysis of Variance (ANOVA) to determine variations that exist among treatments. The New Duncan Multiple Range Test was used to separate means. All analyses were performed at 95% confidence interval. Statistical Analyses were done using Statistical Package for Social Sciences (SPSS) software (Version 22.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results

Results of growth performances (Table 2) revealed that the highest mean final weight (56.42g), mean weight gain (53.87g), average daily growth (0.64g), specific growth rate (3.69%/day) and mean final standard length (18.43cm) were obtained in fish fed palm oil-based diet. This was followed by those fed the control diet, and the poorest growth response of fish was obtained in group fed coconut oil-based diet. When these results were compared with those obtained in control group, there were significant variations ($p < 0.05$) in all the growth indices tested among various diets. It was observed that fish fed palm oil diet had the best food and protein efficiency ratios. Fish condition factor was significantly different ($p < 0.05$) among tested diets with the best value (1.22) recorded in sunflower oil-based diet. Survival rates of fish fed different oil sources were statistically similar ($p > 0.05$), ranging between 98.33% and 100%. Increment in size of tilapia fed different oil-based diets for 12 weeks showed that the best performance was observed in group fed palm oil-based diet. When mean weight gain of fish was plotted against specific times, at which the weights were measured, the graph appeared as sigmoid curves (Figure 1). The growth curves rise up slowly with an increasing slope, representing an increasing rate of growth up to the end of the feeding trial.

Table 2: Growth Performance of Catfish Fed Diets Containing Different Lipid Sources

Indices	Control	Coconut	Olive	Palm	Sunflower	Sesame
MIW(g)	2.54±0.02	2.53±0.02	2.55±0.01	2.55±0.01	2.54±0.01	2.54±0.01
MISL(cm)	4.3±0.00	4.3±0.00	4.3±0.00	4.3±0.00	4.3±0.00	4.3±0.00
MFW(g)	45.6±1.56^{c}	39.33±0.3^{a}	42.57±0.53^{b}	56.42±0.55^{d}	41.13±0.63^{ab}	39.75±0.14^{a}
MFSL(cm)	16.83±0.2^{d}	14.4±0.1^{b}	15.33±0.17^{c}	18.43±0.07^{e}	14±0.00^{a}	14.07±.07^{ab}
MWG(g)	43.05±1.6^{c}	36.8±0.32^{a}	40.02±0.53^{b}	53.87±0.56^{d}	38.59±0.62^{ab}	37.21±0.14^{a}
ADG(g/day)	1.51±0.02^{c}	1.44±0.00^{a}	1.47±0.01^{b}	1.64±0.01^{d}	1.46±0.01^{ab}	1.44±0.00^{ab}
SGR(%/day)	3.44±0.02^{c}	3.26±0.01^{a}	3.35±0.01^{b}	3.69±0.03^{d}	3.31±0.01^{ab}	3.27±0.04^{a}
FCR	1.63±0.07	1.62±0.03	1.67±0.02	1.56±0.02	1.59±0.02	1.61±0.01
PER	31.06±1.3	31.57±0.59	30.24±0.38	32.33±0.34	31.80±0.40	31.63±0.21
SR (%)	100±0.00	100±0.00	98.33±1.67	100±0.00	98.33±1.67	100±0.00
CF	0.7±0.04^{a}	1.15±0.03^{c}	0.88±0.00^{b}	0.72±0.01^{a}	1.22±0.02^{cd}	1.18±0.01^{d}

Data are mean ± standard error: means with different superscript within a row are significantly different ($p < 0.05$).
Where: MIW=mean initial weight; MISL=mean initial standard length; MFW=mean final weight; MFSL=mean final standard length; ADG=average daily growth; SGR=specific growth rate; FCR=food conversion ratio; PER=protein efficiency ratio; SR=survival rate; CF=condition factor

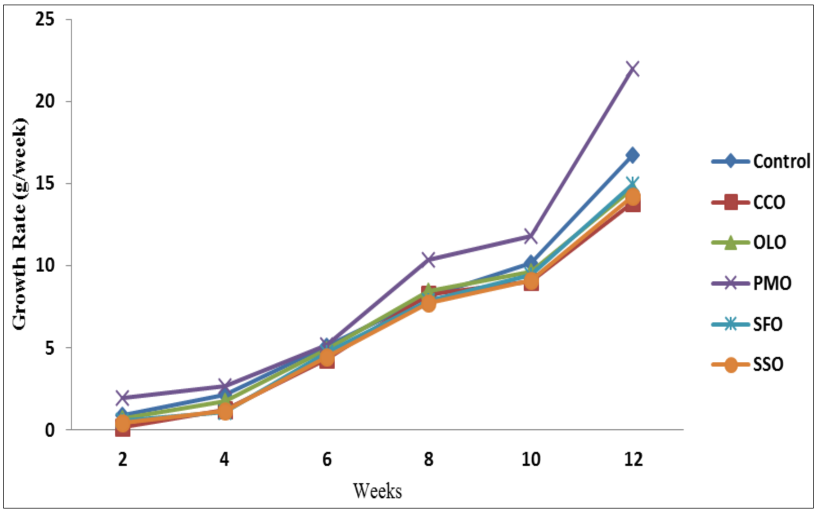

Fig 1: Biweekly growth curve of natural male tilapia fed diets containing different dietary lipids for 12 weeks

The profile and percentage composition of the different fatty acids in muscle lipid of genetically male tilapia are contained in Table 3. From the results, Lauric (12:0), myristic (14:0), and palmitic (16:0) acids were the main saturated fatty acids while oleic acid (18:1) was the main monounsaturated fatty acids. This study showed that replacing fish oil with vegetable oils consistently resulted in major changes in fatty acid profile of fish.

Table 3: Muscle fatty acid (% of total fatty acid) of genetically male tilapia fed diets containing different lipid sources

Indices	Control	Coconut	Olive	Palm	Sunflower	Sesame
10:0	0.16±0.03	2.33±0.04	-	-	-	-
12:0	1.49±0.7^{b}	47.5±0.16^{c}	0.03±0.00^{a}	0.79±0.04^{ab}	0.03±0.00^{a}	0.42±0.14^{a}
14:0	5.61±0.3^{c}	9.24±0.12^{d}	0.58±0.2^{a}	2.37±0.28^{b}	0.41±0.02^{a}	0.36±0.02^{a}
14:1	0.45±0.05^{a}	8.48±0.17^{d}	1.9±0.12^{b}	2.81±0.1^{c}	0.43±0.16^{a}	0.69±0.24^{a}
15:0	0.25±0.1^{a}	-	1.31±0.04^{c}	-	0.73±0.21^{b}	0.32±0.05^{a}
16:0	5.39±0.04^{c}	6.23±0.12^{c}	2.38±0.14^{a}	23.47±0.18^{e}	2.23±0.12^{a}	3.37±0.12^{b}
16:1	6.32±0.32^{e}	4.18±0.1^{d}	0.56±0.12^{a}	9.63±0.15^{f}	2.38±0.18^{b}	3.78±0.07^{c}
17:0	0.98±0.14	-	-	-	0.35±0.05	-
17:1	0.26±0.04	-	-	-	0.4±0.04	0.68±0.10
18:0	7.13±0.12^{d}	6.5±0.25^{c}	4.26±0.08^{a}	5.57±0.12^{b}	5.33±0.10^{b}	5.74±0.06^{b}
18:1	16.24±0.15^{b}	8.62±0.17^{a}	72.34±0.24^{e}	42.23±0.12^{d}	23.3±0.09^{c}	42.35±0.04^{d}
18:2n-6	27.33±0.01^{d}	4.32±0.21^{a}	7.6±0.19^{b}	12.42±0.58^{c}	38.31±0.22^{e}	23.44±0.22^{d}

18:3n-3	23.43±0.25^{f}	1.52±0.15^{b}	6.3±0.19^{c}	0.6±0.23^{a}	23.46±0.18^{e}	16.59±0.18^{d}
18:3n-6	0.54±0.15	-	-	-	-	-
20:0	0.22±0.02^{b}	0.07±0.02^{a}	0.47±0.05^{c}	-	0.14±0.18^{ab}	-
20:1	0.59±0.01^{c}	-	0.04±0.00^{a}	-	0.34±0.04^{b}	-
20:2n-6	0.03±0.00^{a}	-	0.29±0.01^{b}	-	0.39±0.04^{c}	0.42±0.03^{d}
20:3n-3	0.06±0.01	-	-	-	-	-
20:3n-6	0.51±0.1^{c}	0.05±0.01^{a}	0.86±0.07^{d}	-	0.19±0.1^{b}	0.1±0.00^{a}
20:4n-3	0.30±0.00^{b}	0.04±0.00^{a}	0.71±0.64^{c}	0.03±0.00^{a}	0.05±0.00^{a}	0.77±0.02^{c}
20:4n-6	0.60±0.00^{b}	0.23±0.00^{a}	0.16±0.03^{a}	0.06±0.00^{a}	2.15±0.03^{c}	0.07±0.1^{a}
20:5n-3	0.17±0.09^{a}	-	0.43±0.05^{b}	-	0.78±0.14^{c}	-
22:1	0.04±0.00	-	-	-	-	-
22:6n-3	0.54±0.03	-	-	-	0.78±0.02	0.69±0.16
23:0	0.78±0.06	-	-	-	-	0.33±0.03
24:0	0.77±0.05	-	-	-	-	-
∑SAT	28.2±0.95^{c}	83.87±0.44^{e}	11.36±0.31^{a}	44.64±0.21^{d}	12.29±0.08^{b}	15.35±0.19^{b}
∑MUFA	16.24±0.15^{b}	8.62±0.17^{a}	72.34±0.24^{e}	42.43±0.25^{d}	23.3±0.09^{c}	42.35±0.04^{d}
∑PUFA	50.77±0.25^{d}	5.84±0.35^{a}	13.9±0.18^{b}	13.09±0.35^{b}	61.78±0.29^{e}	40.03±0.27^{c}
∑n-3	30.5±0.26^{f}	4.56±0.16^{b}	8.44±0.72^{c}	0.7±0.02^{a}	25.08±0.26^{e}	19.06±0.24^{d}
∑n-6	26.99±0.23^{d}	2.0±0.21^{a}	7.62±0.14^{b}	12.48±0.58^{c}	39.66±0.3^{e}	26.61±0.07^{d}
∑n-3/n-6	1:1.13±0.02^{b}	1:2.32±0.2^{c}	1:1.11±0.08^{b}	1:0.06±0.02^{a}	1:0.63±0.01^{b}	1:0.72±0.00^{b}

Data are mean ± standard error: means with different superscript within a row are significantly different ($p < 0.05$)

Discussion

The present study has showed that replacing fish oil with vegetable oils consistently resulted in major changes in growth performances and fatty acid profile of genetically male tilapia. In a previous study by [17] no detrimental effects were observed in growth performances when fish oil was totally replaced with vegetable oils in the diets of Nile tilapia. Rather, a slight but significant reduction in feed intake and weight gain were noted. Earlier reports by [18] stated the feasibility of total replacement of fish oil by vegetable oils in turbot fish meal-based diets. In the present study it was observed that the use of well-balanced plant protein diets with a low inclusion level of fish meal (200 g/kg), just to cover essential fatty acids needs, vegetable oils could replace up to 100% of fish oil without adverse effects on growth performance of tilapia. This was in accordance with previous results on tilapia fed low-fish meal content diets which the dietary fish oil was replaced by vegetable oil alternatives [19]. The FCR and total feed intake were not significantly ($p >$ 0.05) different among treatments. The result indicated that fish were able to digest the diets and converted into body tissues with the same degree of efficiency irrespective of the lipid source in the diets. Studies by [8] revealed that vegetable oils are cheaper and less subjected to oxidation than fish oil. They enable growth and feed conversion without significantly affecting the flesh, growth and organoleptic qualities as obtained in fish oil [20]. Thus, the results of the present study agreed with other findings for Nile tilapia [19] and other fish species [21]. Results on nutrient efficiency showed that PER values were not significantly different ($p <$ 0.05) between control and alternative oil diets and the best value was recorded in palm oil diet. Similar and comparable results were reported in tilapia by [22, 23]. Amongst the plants oils used in this study, palm oil diet produced the best growth performance and net protein utilization. This result was in agreement with that obtained in other warm water finfish species by [24].

The results of replacing fish oil with vegetable oils consistently led to major changes in fatty acid profile of tilapia. In the present study, the dietary fatty acid profile of tilapia reflected the fatty acid composition of oil in diets. The presence of linoleic and linolenic acids in diet resulted to increase in the levels of 20:4n-3, ARA, EPA, and DHA in fish muscle lipids. This could be explained by the bioconversion of linolenic acid to corresponding longer-chain fatty acids by the fish. The highest levels of ARA and 20:3n-6, were detected in fish fed the sunflower oil diet, which was rich in linoleic acid. Fish fed the palm oil diet, which had very low amount of linolenic acid, seemed to maintain the DHA level at a certain point independent of the diet. This demonstrated the paramount role of DHA in cell membrane structure in the selective deposition of these fatty acids. The presence of omega-3 long chain PUFAs such as EPA, DHA and ARA observed in fish fed olive, sunflower and sesame oil diets proved that these oils are extremely beneficial to human health [25]. Thus, the results of this study suggested that in formulating feed for tilapia palm oil is recommended as sole dietary lipid source to achieve the highest percentage growth while all the experimental oils had omega-3 long chain PUFAs which are beneficial to human health.

Conclusion

The present study showed that different vegetable oils in diets resulted in positive growth responses, whole body composition and fatty acid profile of tilapia. The study also showed that all the experimental oils had omega-3 long chain PUFAs which are beneficial to human health. However, palm oil is recommended as sole dietary lipid for maximum growth while olive, sunflower and sesame oils are excellent sources of essential fatty acids required in human diets.

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