

Original Research Article

Determination of Biochemical Responses of Genetically-Male Tilapia (*Oreochromis niloticus*) after Replacing fish oil with Plant-based oils in their Diets

ABSTRACT

This study evaluated biochemical responses of genetically male tilapia after replacing fish oil with plant-based oils in their diets. The sources of oils were coconut, olive, crude palm, sunflower and sesame seed. These oils were incorporated at 7% level **and were used to formulate** five isonitrogenous (42.5% CP) diets. **In the control experiment (sixth diet) fish oil was used. Fish were reared in 1m x 1m x 1m floating net-hapa. Feeding trial lasted for 12 weeks. At the end of the experiment, blood samples were collected and tested for serum protein, electrolytes and tissue lipid peroxidation. Results of the study revealed that the ratio of albumin to globulin was statistically similar ($p > 0.05$) in all fish group examined. The results of serum electrolyte showed that olive oil treated fish recorded the highest ($p < 0.05$) level.** The amount of malondialdehyde (MDA) formed in fish tissues increased ($p < 0.05$) in sunflower oil group by 69%, 43%, 20% and 32% in the liver, kidney, gills and heart respectively. This indicated that reactive oxygen species may be associated with the metabolism of sunflower oil leading to peroxidation of membrane lipids of the respective organs. However, this level of MDA deposition had not been associated with any negative effects on fish health. Inferences from this study conclusively support the direct use of these plant oils as total replacement for fish oil in diets of *Oreochromis niloticus*.

Keywords: Genetically male tilapia, fish oil, plant oils, substitution, fish diet and health

INTRODUCTION

Aquaculture plays a major role in the overall agricultural sector of the Nigerian economy. The country with hundreds of rivers and ponds is notable for being a fish-loving nation where fish plays an important role in the diets, constituting the main and often irreplaceable animal protein source in both urban and rural households (Otubusin, 2011). The major fish species cultured in Nigeria include catfishes and tilapia. Tilapia is one of the most widely cultured fish in the world. Currently, farmed tilapia represents more than 75% of world tilapia production (FAO, 2013), and this contribution has been **growing** exponentially. **The growth recorded in the global tilapia production are traced to several factors.** Among these are: **possibilities for** genetic improvement, ease of culture and high adaptability to a wide range of environmental conditions (Ponzoni *et al.*, 2008). **However, in tilapia production, the males usually grow faster than the females. This had been so since the females mature earlier than the males and this early sexual maturation diverts energy from growth to reproduction. This usually results into prolific breeding, overcrowding and competition.** The most effective solution to this problem is to produce and grow only male fish. **Fish scientists had provided solution to this problem through the application of basic genetics. This led to the introduction of the genetically male tilapia (GMT).** The GMT so far developed has proved to be excellent production fish in both extensive and intensive systems using ponds, raceways, cages and tanks (Eknath *et al.*, 2007). **Genetically improved tilapias** are now in use in more than 20 countries around the world (Gupta and Acosta, 2004) including Nigeria (Effiong, 2015).

Aquaculture feed accounts for more than 50% cost in intensive aquaculture operations (NRC, 1993). The major concern in the industry is the fact that major ingredients (fish meal and fish oil) are scarce and expensive. The issues around the use of fish meal and fish oil in compound aquaculture diets are wide ranging and complex (FIN, 2006). **Aquaculture production has increased spontaneously in the past years while catches from the wild are dwindling. The increased aquaculture production was traceable to the use of fish meal and fish oil obtained from small pelagic fisheries (clupeids). However, during the El Niño events of 1998 – 2004 (FAO, 2013) there had been a major drop in the production of the clupeid fisheries. This affected aquaculture production greatly. Fears had been raised that if this trend continues aquaculture may not bridge the gap between the high demand and limited supply.** In view of this, establishing a sustainable fish culture requires identification of alternative protein and oil sources. There have been considerable research efforts to find suitable, cost-effective, non-conventional and terrestrial alternative ingredients which can totally or partially replace fish meal in aqua-feeds production (Ufodike *et al.*, 2011a and b; 2012). Presently, the most urgent problem to be solved in the industry relates to fish oil replacement. Thus this study was conducted to evaluate the effectiveness of replacing dietary fish oil (cod liver) with vegetable oils (coconut, palm, olive, sunflower and sesame) on serum proteins, lipid profile, electrolytes and tissue lipid peroxidation of super male tilapia.

Materials and Methods

Experimental Design

The experimental setup composed of an outdoor concrete tank (8m x 5m x 1.65m) situated at the Vika Farms Limited, Mbak Etoi, Uyo, Nigeria. The farm is located at geographical coordinates of Latitude: 5° 3' 0" North and Longitude: 7° 56' 0" East. 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 8.5m x 6.0m bamboo raft with eighteen 1.5m x 1.5m apartments fit able with eighteen 1m x 1m x 1m hapas constructed and placed to fit on the concrete tank as described by Otubusin (2000).

Experimental Diet Preparation

Six isonitrogenous diets (42.5% protein) were prepared using fishmeal, soybean meal, groundnut cake and corn flour as main ingredients (Table 1). **The Pearson Square method was used to balance the proportion of each ingredient used. The control diet had fish oil as the main lipid source while in the other five diets coconut, olive, crude palm, sunflower and sesame oils were used to replace fish oil. The various oils were incorporated at 7% of the diet. The feed ingredients were procured from reputable agro-vet store. Each proportion was carefully weighed out according to diet and mixed thoroughly to obtain a homogenous paste. Thereafter, these were made into pellets using 2mm meat mincer, air-dried, labelled accordingly and stored in the freezer for subsequent use.**

Fish Rearing and Management

The experimental set-up comprising of 18 floating net-hapa were randomly stocked with catfish fingerling (mean weight 4.5±0.10g) at a density of 20 fish/unit. Fish were fed at the rate of 5% of their body weight three times daily at 08:00, 13:00 and 18:00hrs. Feeding trial lasted for twelve weeks.

Table 1: Composition (g/kg) of experimental diets containing different lipid sources

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
FM	186.00	186.00	186.00	186.00	186.00	186.00
SBM	186.00	186.00	186.00	186.00	186.00	186.00
CFL	182.00	182.00	182.00	182.00	182.00	182.00
GNC	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
Premix*	0.5000	0.5000	0.5000	0.5000	0.5000	0.5000
Fish oil	70.00	-	-	-	-	-
Coconut oil	-	70.00	-	-	-	-
Olive oil	-	-	70.00	-	-	-
Palm oil	-	-	-	70.00	-	-
Sunflower oil	-	-	-	-	70.00	-
Sesame oil	-	-	-	-	-	70.00

* 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.

Blood Collection and Preparation

At the end of the feeding trial, 5ml blood per fish was collected from vertebral blood vessel using 2ml disposable syringes and needle and transferred to plain sample bottles. The separation of serum from whole blood was performed by centrifugation using a bench top centrifuge at 3,000rpm for 15 minutes at 25°C. **The serum was separated and stored at 4°C for biochemical analyses (Rudneva and Kovyreshina, 2011).**

Biochemical Measurement

The concentrations of total cholesterol (TC), Triglyceride (TG), HDL- cholesterol and LDL cholesterol were determined by enzymatic colorimetric test described by Fossati and Prencipe (1982). Total protein level in serum was determined by biuret method (**Cheesbrough (1987)**) using standard Fortress diagnostic kit. Serum ions: bicarbonate (HCO₃⁺), sodium (Na⁺), chloride (Cl⁻) and potassium (K⁺) were analyzed colorimetrically using standard Parksha Neochem analysis kit according to **Cheesbrough (1987)**. Lipid peroxidation was determined by measuring malondialdehyde formation in fish tissues (liver, kidney, heart and gills) as described by **Farombi et al. (2008)**. **The biochemical measurements were conducted at the laboratory of the Department of Biochemistry, University of Uyo, Nigeria.**

Statistical Analysis: Data analyses were carried out using Statistical Package for Social Sciences (SPSS 19.0, 2010 version) and level of significance was set at $p \leq 0.05$.

RESULTS

The results of lipid profile are presented in Table 2. From the results fish fed sesame oil diet had the lowest lipid profile among all tested diets. Total cholesterol level was highest (3.67 mmol/l) in group fed coconut oil diet with no significant effects ($p > 0.05$) from those treated with palm oil in their feed. Triglyceride followed similar trend with no significant effects ($p > 0.05$) between control and sunflower oil diets. The group fed olive oil diet had significantly

higher ($p < 0.05$) high density lipoprotein level than all other groups while the control diet recorded the highest level of low density lipoprotein. Results of serum protein, albumin, globulin, and albumin/globulin ratio are presented in Table 3. Fish fed palm oil based diet had significantly higher ($p < 0.05$) total protein (8.31%). Albumin and globulin levels followed similar trend. No significant differences ($p > 0.05$) were observed between fish fed the control diet and those fed sesame oil-based diets. The albumin/globulin ratios were similar in all tested groups. Results of serum electrolytes revealed that there was a significantly higher ($p < 0.05$) level of sodium concentration in fish fed palm oil diet. Fish fed olive and palm oil diets had similar levels of potassium and bicarbonate levels. In general, sesame oil treated group recorded the least electrolyte levels (Table 4).

Table 2: Lipid profile (mmol/l) of natural male tilapia fed diets containing different lipid sources

Variables	Control	CCO	OLO	PMO	SFO	SSO
TC	3.77±0.02 ^{ab}	3.98±0.05 ^d	3.67±0.01 ^a	3.90±0.03 ^{cd}	3.84±0.02 ^{bc}	3.74±0.02 ^{ab}
TG	1.37±0.00 ^{bc}	1.42±0.01 ^d	1.33±0.01 ^a	1.39±0.01 ^c	1.37±0.01 ^{bc}	1.35±0.00 ^b
HDL	1.04±0.02 ^a	1.08±0.01 ^a	1.13±0.01 ^b	1.03±0.02 ^a	1.04±0.01 ^a	1.01±0.02 ^a
LDL	2.11±0.02 ^c	2.26±0.06 ^b	1.96±0.05 ^a	2.14±0.03 ^b	2.18±0.03 ^{bc}	2.13±0.02 ^b
VLDL	0.62±0.00 ^{bc}	0.64±0.00 ^e	0.61±0.00 ^d	0.63±0.00 ^d	0.62±0.00 ^{cd}	0.61±0.00 ^b

Data are mean ± standard error: means in the same row with the same superscript were not significantly different ($p > 0.05$). Where: TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein. CCO, coconut oil; OLO, olive oil; PMO, palm oil; SFO, sunflower oil; SSO, sesame oil

Table 3: Serum protein (mmol/l) of natural male tilapia fed different lipid diets

Indices	Control	CCO	OLO	PMO	SFO	SSO
Protein	7.26±0.04 ^a	8.12±0.03 ^b	7.80±0.03 ^b	8.31±0.12 ^c	8.07±0.01 ^b	7.28±0.02 ^a
Albumin	3.99±0.02 ^a	4.47±0.02 ^b	4.29±0.02 ^b	4.58±0.02 ^c	4.44±0.01 ^b	4.01±0.01 ^a
Globulin	2.76±0.02	3.09±0.01	2.94±0.02	3.16±0.05	3.07±0.01	2.77±0.01
A/G ratio	1.45±0.00	1.45±0.00	1.45±0.00	1.45±0.00	1.45±0.00	1.45±0.00

Data are mean ± standard error: means in the same row with the same superscript were not significantly different ($p > 0.05$). Where: CCO, coconut oil; OLO, olive oil; PMO, palm oil; SFO, sunflower oil; SSO, sesame oil

Table 4: Serum electrolyte (mg/1000g) of natural male tilapia fed different lipid diets

Indices	Control	CCO	OLO	PMO	SFO	SSO
Na ⁺	132.11±1.1 ^{ab}	133.42±0.5 ^{bc}	134.31±0.8 ^{bc}	134.95±0.7 ^c	130.9±0.5 ^a	130.7±0.3 ^a
K ⁺	2.84±0.02 ^d	2.71±0.01 ^b	2.75±0.00 ^c	2.77±0.01 ^c	2.69±0.01 ^{ab}	2.66±0.01 ^a
Cl ⁻	61.26±3.6 ^a	108.11±3.1 ^c	133.33±6.5 ^d	88.29±4.7 ^b	68.47±4.8 ^a	63.06±1.8 ^a
HCO ₃ ⁺	20.5±0.5 ^d	15.0±0.6 ^b	18.5±0.5 ^c	19.83±0.2 ^c	15.0±0.6 ^b	12.5±0.6 ^a

Data are mean values ± standard error: means in the same row with the same superscript were not significantly different ($p > 0.05$). Where: Na⁺, sodium; K⁺, potassium; Cl⁻, chloride; HCO₃⁻, bicarbonate; CCO, coconut oil; OLO, olive oil; PMO, palm oil; SFO, sunflower oil; SSO, sesame oil

A significant increase ($p < 0.05$) in lipid peroxidation (as malondialdehyde formation) was observed in fish tissues (liver, kidney, gills and heart) following exposure to different oil-based diets (Figure 1). **The highest percent MDA formed was observed in fish fed the palm**

oil-based diet. The levels were 41% in the liver, 14% in the kidney, 39% in the gill and 14% in the heart. These results indicated that reactive oxygen species may be associated with the metabolism of crude palm oil leading to peroxidation of membrane lipids of the respective organs.

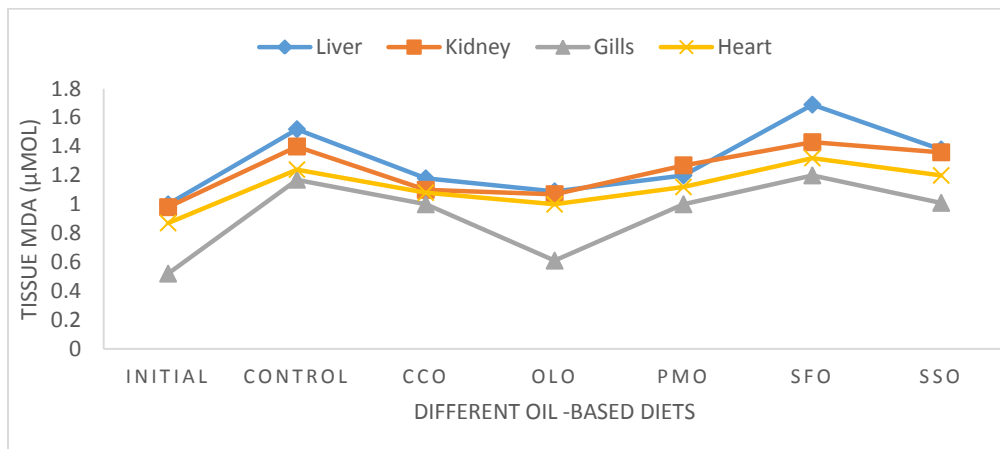


Figure 1: Tissue lipid peroxidation (µmol MDA formed/g net tissue) of natural male tilapia fed different lipid diets

DISCUSSION

Substantial use of vegetable oils as energy source in fish diets have yielded positive growth responses in fish (Babalola and Adebayo, 2007). Results obtained in this study showed that plant oils could be used as excellent nutrient base in tilapia feed manufacture. All the experimental diets were adequately consumed by fish with no sign of stress. This may imply that there was no palatability problem and feed were adequately utilized. This report is similar to the observation of Aderolu and Akinyemi (2009) in the utilization of coconut and peanut oils, and Sotolu (2010) in the utilization of sesame seed and palm oils by *Clarias gariepinus*. **Ochang *et al.* (2007) reported that vegetable oil could replace fish oil in catfish diet at up to 12.5% level without any negative influence on feed intake. Thus, the 7% replacement level adopted in this study appeared to be within acceptable limits that ensures balance in lipid components for normal fish growth (NRC, 1993).**

The highest HDL and lowest LDL levels recorded in fish fed olive oil diet could be linked to high oleic and linoleic acids present in this oil, **since** both MUFA and PUFA had been reported to play significant roles in reducing blood cholesterol levels (Mensink and Katan, 1992). Furthermore, a decrease in serum LDL cholesterol has been reported in Atlantic salmon (Jordal *et al.*, 2007) and rainbow trout (Richard *et al.*, 2006) fed plant oil-based diets, possibly because of the decreased content of dietary cholesterol in the diets. A reduction in cholesterol might also result from the content of phytosterol in the dietary plant oils used; since (Genser *et al.*, 2012) documented that phytosterol has cholesterol lowering effect. In this study, high level of triglyceride was observed in fish fed coconut oil diet. This indicated efficiency in hepatic metabolites compound, normal physiological function of the metabolism and high immune level of fish fed the diets. This result agreed with Marina *et al.* (2009) who reported that coconut is composed mainly of medium-chain triglycerides which may not carry the same risks as other saturated fats thus may create a more favourable blood cholesterol profile **for human**

consumption. This study is significant as it has revealed that none of the lipoprotein level of tested fish was significantly altered by the experimental diets; suggesting that no critical injury was caused by fish oil substitution with the different vegetable oils. However, coconut and olive oils outperformed other tested oils in their cholesterol lowering and health – promoting properties. Serum proteins play a key role in maintaining osmotic pressure and viscosity of fish blood. Of particular interest is the correlation of the albumin/globulin ratio **as such** low levels have been linked to nephrosis in fishes (Sandnes *et al.*, 1988). **Moreover, serum albumin plays a role in osmotic pressure of protein and different fish have differ distribution of the various fractions (Baker, 2002).** The Elasmobranchii, for instance, are known to use urea which is present in their blood in very large amounts to maintain the osmotic pressure of their blood approximately equal to that of their environment. In teleosts (such as tilapia), it has become a matter of interest to determine whether lipid type has any influence upon the concentration and distribution of their serum proteins. The data presented in this study revealed that the concentrations of serum proteins were in normal range **of between 6.0 to 8.0g/dl** which, indicated that the animals were apparently healthy throughout the experimental period.

The basic function of electrolytes in the body lies in controlling fluid distribution, intra and extracellular acid-basic equilibrium, maintaining osmotic pressure of body fluids and normal neuro-muscular irritability. The levels of these ions observed in fish serum in present study suggested that no critical injury was caused by fish oil substitution with the different vegetable oils. The levels of Na^+ and K^+ obtained in this study were similar to other study (Wei *et al.*, 2011). The increase in the concentration of Na^+ found in the blood serum of tilapia fed all the oil diets showed no practical effect on the ionic functions mentioned above. On the other hand, the K^+ concentration in the serum was significantly decreased in fish which in combination with the increase in Na^+ indicated normal function of the heart and a non-toxic damage to the central nervous system of the fish since high level of K^+ had been linked to inhibition of heart function and neurotoxic damages (Adedeji, 2010). Also, the HCO_3^+ and Cl^- functionally participate in maintaining normal irritability of the heart, muscles and nerves, as well as the selective permeability of cell membranes. Therefore, the significant increase ($p < 0.05$) in the concentrations of the above ions in fish fed olive and palm oil diets showed a non-toxic effect of these oils on fish.

Clinical diagnosis of disease and damage to the structural integrity of fish has been assessed by monitoring the level of malondialdehyde (MDA) formed in fish tissues such as liver, kidney, gills and heart (Gutteridge and Wilkins, 1982). MDA is a product of lipid peroxidation (Devaki *et al.*, 2004). High levels lead to disorganization of membrane by peroxidation of unsaturated fatty acids. Under such condition, the ratio of polyunsaturated to other fatty acids is altered (Ochang *et al.*, 2007). The obvious consequence being a decrease in membrane fluidity and death of cell (Shen and Liu, 2006). **The results of the present study revealed that these parameters were not altered by the experimental diets. This may mean that partial substitution of fish oil with vegetable oils did not cause any deleterious effects on the liver, kidney, gills and heart of tilapia.**

CONCLUSION

The present study indicated that all the experimental oils (olive, coconut, crude palm, sunflower and sesame) had no detrimental influence on fish health and could be used to **partially** replace fish oil in fish feed manufacture. However, the use of PUFA-rich oils such as sunflower should be carefully regulated while palm and olive oils are highly recommended.

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UNDER PEER REVIEW