# *Original Research Article*

**Evaluation 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 to produce five isonitrogenous (42.5% CP) diets. The sixth diet contained fish oil and served as control experiment. Feeding trial was conducted in floating net-hapa ($1m^3$) system for 12 weeks. At harvest, blood samples were taken and examined for serum protein, electrolytes and tissue lipid peroxidation. Results showed that kidney function parameter (albumin/globulin ratio) was similar ($p > 0.05$) in all tested groups while statistically higher ($p < 0.05$) serum ions were observed in olive oil treated fish. 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 is an integral component of the overall agricultural production system in Nigeria. 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 exponentially growing in recent years. Several factors have contributed to the rapid global growth of tilapia. Among these are: genetic improvement, ease of culture and high adaptability to a wide range of environmental conditions (Ponzoni *et al*., 2008). However, a major problem in tilapia culture is that females grow slower than males. Early sexual maturation diverts energy from growth to reproduction and unwanted breeding results in overcrowding and competition. The most effective solution to this problem is to produce and grow only male fish. Researches have addressed this problem in an innovative way through the application of basic genetics, to develop a unique product in genetically male tilapia (GMT) (Mair *et al.*, 1997). The GMT so developed has proved to be excellent production fish in both extensive and intensive systems using ponds, raceways, cages and tanks (Eknath *et* *al.*, 2007). They 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). One of the main areas of contention is the presumption that although aquaculture production is expected to continue its rapid rise in the foreseeable future, catches from small pelagic fisheries, which are the sources of fish meal and oil are expected to remain static, or even decrease due to major drop in supply during the El Niño events of 1998 – 2004 (FAO, 2013). Fears have been raised that this trend may have disastrous consequences for the ecosystem, fueling concerns that aquaculture may not be a net contributor to world fish supplies, but instead, adding more pressure on wild fisheries (Allan, 2004). 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 x1.65m) situated at the Vika Farms Limited, Mbak Etoi, Uyo, Nigeria. The farm is located at geographical coordinates of Latitude: $5^{o}$ 3' 0'' North and Longitude: $7^{o}$ 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 (41.00% protein) were prepared using fishmeal, soybean meal, groundnut cake and corn flour as main ingredients (Table 1). In diet 1 (control), fish oil served as the lipid source. In diets 2 to 6, coconut, olive, crude palm, sunflower and sesame seed oils were used as total replacement for fish oil respectively. The various oils were incorporated at 7% of the diet. All ingredients were procured at the same time to avoid variations associated with batch differences. They were carefully weighed out, mixed, made into pellets using 2mm meat mincer, air-dried and labeled separately according to diets.

### Fish Rearing and Management

Each floating hapa was randomly stocked with *Clarias gariepinus* (4.5±0.10g) at 20 fish per rearing system. Experimental diets were fed to triplicate groups of fish at 5% of their body weight. This amount was divided into three equal portions and fed at 08:00, 13:00 and 18:00hrs respectively over a period of 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 $25^{o}$C. The serum (supernatant) was transferred into well-labelled sample bottles using a Pasteur pipette and stored in a refrigerator at a temperature of $4^{o}$C for biochemical analyses.

**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 (Henry *et al*., 1974) 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 Henry *et al.* (1974). Lipid peroxidation was determined by measuring malondialdehyde formation in fish tissues (liver, kidney, heart and gills) as described by Bull and Marnett (1985). All biochemical analyses were carried out at Biochemistry laboratory, University of Uyo.

**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). 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.

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±0.02b[c] | 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.

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 ($p > 0.05$).

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; $HCO_3$, bicarbonate.

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 (Table 5). Fish fed palm oil diet, exhibited the highest percent MDA formation of 41%, 14%, 39% and 14% compared to the initial levels in the liver, kidneys, gills and heart respectively. 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.

Table 5: Tissue lipid peroxidation (µmol MDA formed/g net tissue) of natural male tilapia fed different lipid diets

| Source of Tissue | Initial | Control | CCO | OLO | PMO | SFO | SSO |
|---|---|---|---|---|---|---|---|
| Liver | 1.00[a] | 1.52±0.03[c] | 1.18±0.04[b] | 1.09±0.08[a] | 1.20±0.02[b] | 1.69±0.01[cd] | 1.38±0.02[bc] |
| Kidney | 0.98[a] | 1.40±0.01[bc] | 1.10±0.02[a] | 1.07±0.01[a] | 1.27±0.01[b] | 1.43±0.01[c] | 1.36±0.02[bc] |
| Gills | 0.52[a] | 1.17±0.02[c] | 1.00±0.05[b] | 0.61±0.01[a] | 1.00±0.11[b] | 1.20±0.01[c] | 1.01±0.01[b] |
| Heart | 0.87[a] | 1.24±0.01[bc] | 1.08±0.01[b] | 1.00±0.02[a] | 1.12±0.02[a] | 1.32±0.03[c] | 1.20±0.05[b] |

Data are mean ± standard error: means in the same row with the same superscript were not significantly different ($p > 0.05$).

## 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) earlier showed that vegetable oils can replace fish oil up to 12.5% inclusion level in catfish diets without feed intake associated problems. The 7% inclusion levels of the various oils used in the present study appeared to be within acceptable limits that ensured balances 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. This is because 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. 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 which low levels have been linked to nephrosis in fishes (Sandnes *et al.*, 1988). Moreover, it has been established that serum albumin plays the predominating role in exerting osmotic pressure of the proteins. Different kinds of fish vary sharply in the total serum proteins and in the distribution of the various fractions. 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 which, indicated that the animals were apparently healthy throughout the experimental period.

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 is commonly assessed by monitoring the level of malondialdehyde (MDA) formation in fish tissues such as liver, kidney, gills and heart. 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). In this study these parameters were insignificantly ($p > 0.05$) altered by the experimental diets, suggesting that no critical injury was caused by fish oil substitution with vegetable oils and the oils did not exert 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 totally 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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