HAEMATOLOGICAL AND SERUM BIOCHEMICAL PROFILES OF *Clarias gariepinus*-FED DIFFERENT INCLUSIONS OF DIETARY OIL

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ABSTRACT
The haematological and biochemical profiles of Clarias gariepinus fed different dietary oil inclusions were compared. One hundred and twenty juvenile C. gariepinus were acclimated for one week and fed twice daily at 3 % body weight. The experiment was done in a duplicate static weekly renewal bioassay. Fifteen fish samples were each randomly distributed into eight tanks with A1 & A2 as controls and B1 & B2 (10 % palm oil), C1 & C2 (10 % coconut oil) and D1 & D2 (10 % fish oil) as dietary treatments. A significant (P<0.05) increase in weight of C. gariepinus-fed dietary inclusions was observed. Fish-fed palm oil had increased levels of neutrophils and platelets. Fish-fed coconut oil in diet had increased lymphocytes and eosinophils. Total cholesterol, HDL, LDL, VLDL, ALB, and Na^{+} levels were highest in dietary coconut oil. Fish-fed fish oil diet had the highest level of urea and a lower weight gain. This study showed that 10 % palm oil and coconut oil inclusions in C. gariepinus diet may serve as alternatives to fish oil without any harmful effects on fish haematology and serum biochemistry. Palm oil, a cheaper source of energy may be a better dietary replacement.

Keywords: Dietary oils, *C. gariepinus*, haematology, serum biochemistry

INTRODUCTION

The global growth in aquaculture production increases the demand for aqua feeds which provide nutrients for faster fish growth, survival and sustainability of healthy life (Hodar *et al*., 2020). A high percentage of the total cost of fish production comes from procurement of aquaculture feeds. One of the major component of feed for farmed fish is fish oil, mainly because of its high content of n-3 HUFA (highly unsaturated fatty acids), which are considered essential fatty acids for fish (Sargent and Tacon, 1999). Fish oil can be utilized to partially spare dietary protein from use as energy in aquaculture feeds and limit cost of production (Ajani *et al.*, 2011).

Fatty acids are naturally occurring in fish which they acquire from plankton and algae in their habitats (Tasbozan and Gokce, 2017) with varying composition of fatty acids, depending on a variety of factors such as species, diet, season, age, geographical location and environmental conditions. In aquaculture, dietary lipids play important role as source of energy for growth and development of fish (Kyoung-Duck *et al*., 2012). Lipids supply about double the energy as proteins and carbohydrates (Sotolu, 2010).

With an increase in the global demand for fish oil coupled with its high cost, a growing interest in evaluating alternative oils to replace fish oil in fish diet has resulted (Ochang, 2011; Babalola *et al.*, 2011; Babalola and Apata, 2012). According to Babalola *et al.*, (2016), vegetable oils are rich in C18 polyunsaturated fatty acids (PUFAs), being precursors of highly unsaturated fatty acids (HUFAs). Changes in the sources of dietary lipids have been found to affect fish health and ability to resist disease. Lipids are known modulators of immune responses against pathogens (Binder *et al*., 2016). Jalili *et al.* (2019) reported that the quality of dietary phospholipids and fatty acids may have effect on the modulation of immune response of fish.

Dietary lipids, mainly vegetable oils and animal fats are readily digestible energy sources in fish diet (Qiu *et al*., 2017). Several alternative oils to fish oil have been evaluated (Ajani *et al*., 2011). Differences in haematological parameters, immune response and serum biochemical variables as a result of dietary vegetable lipid sources have been reported for fish (Babalola *et al*., 2009; Demir *et al*., 2014, Tijani *et al*., 2015). However, it has been demonstrated that fish species may respond differently to different dietary lipids in fish feeds (Li *et al*., 2019). The comparative effects of palm oil, coconut oil and fish oil on the haematological and serum biochemical profiles of *Clarias gariepinus* was investigated.

MATERIALS AND METHODS

The study was conducted in the Department of Fisheries, Delta State University, Asaba Campus, Asaba, Nigeria. The study lasted 3 months in 2018. One hundred and twenty, 6 weeks old juvenile *Clarias gariepinus* (mean weight 3.5±0.2 g, mean total length of 4.8±0.06 cm and mean standard length of 3.8±0.03 cm) purchased from a fish farm in Asaba were used. Fish were acclimated in stock tank for one week during which fish were fed twice daily by 7.00 am and 7.00 pm. Average water quality variable were temperature, 27.5^{0}C- 29.5 ^{0}C; pH, 6.5-8.0 and dissolved oxygen, 4.5 mg/L - 4.8 mg/L.

The experiment was carried out in duplicate static weekly renewal bioassay of fifteen fish distributed into eight tanks with A1& A2 as control, B1 & B2 (10 % Palm oil), C1 & C2 (10 % Coconut oil) and D1 & D2 (10 % Fish oil) inclusion in diet as treatments. Fish were fed experimental diets (Table 1) twice daily at 3 % body weight. Table 2 shows the proximate composition of experimental diet analyzed according to AOAC (1984).

Table 1. Percentage composition of experimental diets

Ingredients	A (control)	B (10 % Palm oil)	C (10 % Coconut oil)	D (10 % Fish oil)
Fish meal	27.82	27.82	27.82	27.82
Groundnut cake	13.91	13.91	13.91	13.91
Soybean meal	27.82	27.82	27.82	27.82
Maize	17.44	17.44	17.44	17.44
Lysine	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5
Dichromium Phosphate	0.5	0.5	0.5	0.5
Vitamin Premix	1.0	1.0	1.0	1.0
Vitamin C	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Palm oil	-	10.0	-	-
Coconut oil	-	-	10.0	-
Fish oil	-	-	-	10.0

Growth parameters determined were weight gain, total length gain, Specific growth rate (SGP), Feed conversion ratio (FCR) and Protein efficiency ratio (PER) (Nalawade and Bhilave, 2011; Panase and Mengumphan, 2015).

SGR = $100\ [\ (Logn\ final\ weight\ -\ Logn\ initial\ weight)]/Time\ (days)$

FCR = $Total\ feed\ (g)/Weight\ gain\ (g)$

PER = $Weight\ gain\ of\ fish\ (g)/crude\ Protein\ intake\ \ (g)$

Table 2. Proximate composition of experimental diets

Percentage Composition (%)	A- control	B	C	D
Moisture	7.5	4.5	8.6	5.2
Ash	10.7	12.0	13.3	8.6
Crude fibre	4.9	4.2	3.1	6.1
Crude protein	33.4	39.1	37.5	37.0
Fat extract	17.8	21.0	23.0	15.1
NPE	25.7	19.2	16.5	28.0

Haematological parameters such as packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) and white blood cells (WBC) were determined. Fish blood measuring 2.5 ml was withdrawn through the fish caudal vein with 0.5 ml put a bottle containing EDTA for haematology. For haematological indices, mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular Haemoglobin (MCH) and Mean corpuscular Volume (MCV) were calculated (Dacie and Lewis, 2001). Two ml fish blood were collected and put into sterile test tubes, allowed for 30 minutes to clot and centrifuged at 2000 revolutions for 10 minutes and the serum harvested (Tomlinson *et al*., 2013). Serum biochemical parameter levels determined such as total cholesterol, low density lipoprotein (LDP), high density lipoprotein (HDL), triglycerides, very low density lipoprotein (VLDP), sodium ions, chloride ions, urea and albumin were done using Randox Laboratory Test kit ((Randox Laboratory, UK)).

Data obtained were subjected to one way analysis of variance using SPSS version 21 to determine the effect of experimental diet on growth, haematological and serum biochemical profiles with significant means separated at P<0.05.

RESULTS

Table 3 shows growth performance of *C. gariepinus*-fed different oil rich diets. There was a significant (P<0.05) increase in the final weight of *C. gariepinus*-fed treatment dietary inclusion as compared with fish in control diet. FCR in control was similar with treatments 1 and 3 except treatment 2 with slightly reduced levels.

Table 3. Growth performance of *C. gariepinus* fed different oil rich diets.

Growth Parameters	A- Control	B- Palm oil	C- Coconut oil	D- Fish oil
Final weight (g)	57.96 ± 1.80 ^{a}	74.19 ± 1.92 ^{b}	74.16 ± 1.65^{b}	68.60 ± 1.41^{a}
Initial weight (g)	9.49 ± 0.46 ^{a}	9.11 ± 0.48 ^{a}	9.09 ± 0.48^{a}	9.41 ± 0.22 ^{a}
Weight gain (g)	47.47 ± 0.46 ^{a}	65.08 ± 1.44^{b}	65.07 ± 1.17^{b}	59.19 ± 0.19^{b}
Final Total length (cm)	32.91 ± 0.88 ^{b}	32.76 ± 0.78^{b}	28.28 ± 1.10^{a}	32.69 ± 0.75^{b}
Initial Total length (cm)	4.81 ± 0.02 a	4.78 ± 0.01 a	4.80 ± 0.02 a	4.81 ± 0.01 a
Total length gain (cm)	28.10 ± 0.86 a	27.98 ± 0.77 a	23.48 ± 1.08 a	27.88 ± 0.74 a
SGR (%)	2.99 ± 0.04 ^{a}	2.64± 0.04 ^{a}	2.97 ± 0.09^{a}	2.97 ± 0.09^{a}
FCR (%)	1.04 ± 0.02 ^{ab}	1.27 ± 0.02^{ab}	0.92 ± 0.04 ^{a}	1.11 ± 0.03^{ab}
PER (%)	2.52^{a}	2.42^{a}	2.56^{a}	2.37^{a}

Means with different superscripts on the same row are significantly different at P<0.05

The levels of haematological parameters (Table 4) shows that PCV and Hb were significantly higher (P<0.05) in fish-fed fish oil. WBC level was significantly (P<0.05) reduced in fish in control compared with palm oil, coconut oil and fish oil. Fish-fed palm oil had increased levels of neutrophils and platelets. Fish-fed coconut oil in diet had increased lymphocytes and eosinophils while control fish had significantly (P<0.05) lower monocytes and eosinophils. MCHC was similar for all diets but fish-fed coconut oil in diet has slightly higher levels.

Table 4. Haematological profile of *C. gariepinus* fed different oil rich diets.

Blood Parameters	A- Control	B- Palm oil	C- Coconut oil	D- Fish oil
PCV (mg/dL)	34.75 ± 0.94^{b}	36.50 ± 0.86^{b}	30.75 ± 0.75^{a}	39.75 ± 0.25^{c}
Hb (g/dL)	11.60 ± 0.30^{b}	12.17 ± 0.28^{b}	10.27 ± 0.25^{a}	13.22 ± 0.07^{c}
WBC Total (mm^3)	5675 ± 278^{a}	7100 ± 433^{b}	6500 ± 310^{ab}	9225 ± 317^{c}
Neutrophils (g/L)	60.75 ± 0.94^{b}	64.75 ± 1.4^{c}	51.75 ± 1.30^{a}	59.50 ± 1.04^{b}
Lymphocytes (g/L)	31.50 ± 0.64^{b}	25.25 ± 1.79^{a}	38.00 ± 0.81^{c}	30.75 ± 0.47^{b}
Monocytes (g/L)	7.5 ± 0.64^{a}	9.00 ± 0.40^{b}	8.25 ± 0.25^{ab}	9.00 ± 0.40^{b}
Eosinophils (g/L)	0.0^{a}	0.25 ± 0.25^{a}	1.50 ± 0.28^{b}	0.50 ± 0.28^{a}
Platelets (mL)	27000 ± 12247^{b}	380000 ± 14142^{d}	196250 ± 1547^{a}	307500 ± 12500^{c}
MCH (FL)	111.49 ± 0.62^{a}	122.02 ± 0.33^{a}	97.60 ± 0.70^{a}	95.24 ± 0.75^{a}
MCV (Pg)	3.55 ± 0.80^{a}	3.68 ± 0.60^{a}	3.05 ± 0.74^{a}	2.76 ± 0.50^{a}
MCHC (g/L)	32.00 ± 0.00^{b}	31.09 ± 0.01^{ab}	32.40 ± 0.02^{b}	31.00 ± 0.01^{ab}

Means with different superscripts on the same row are significantly different at P<0.05.

Serum biochemical profile of *C. gariepinus*-fed different oil rich diets is presented in Figure 1. Total cholesterol, HDL, LDL, VLDL, ALB and Na^+ were highest in fish-fed dietary inclusion of coconut oil. Results show that total cholesterol exhibited a significant (P<0.05) increase in dietary coconut oil inclusion. Fish-fed dietary fish oil inclusion had highest level of urea and Cl^-. Fish in control experiment recorded higher level of triglyceride. HDL and LDL levels were significant (P<0.05) different.

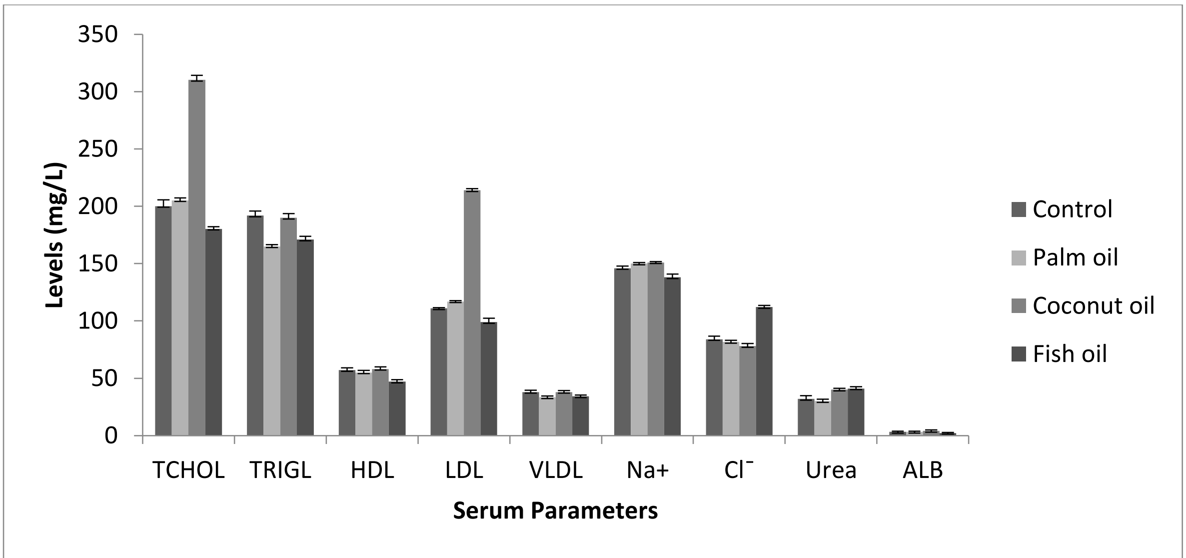

Figure 1. Serum biochemical profile of *C. gariepinus* fed different oil rich diets (TCHOL- Total cholesterol, TRIGL- Triglyceride, HDL- High density lipoprotein, LDL- Low density lipoprotein, VLDL- Very low density lipoprotein, ALB- Albumin, Na^+ - Sodium, Cl^- - Chloride).

DISCUSSION

Weight gain and final weight were higher in *C. gariepinus*-fed dietary inclusions of palm oil, coconut and fish oil than in fish in control. This is similar to the reports of Ng (2004) and Sotolu (2010) who observed that substantial quantities of vegetable oil including coconut oil and palm oil did not have any negative effects on growth performance and can be used as energy substitutes in fish diets. PCV and Hb levels were similar to levels of 37.25 and 10.10 for culture and 31.17 and 8.40 for wild *C. gariepinus* respectively obtained by Adedeji *et al.* (2009). While levels of WBC and MCV were lower than levels obtained. The reduced level of PCV of fish with coconut dietary inclusion could suggests the presence of a toxic factor in the blood. Oyawoye and Ogunkunle (2014) reported that a toxic factor such as haemagglutin may have adverse effect on blood formation. The PCV and blood protein levels for both the control and test diets were within normal range as reported by Omotoyin (2006). The insignificant increase in MCH and MCV in fish-fed palm oil rich diet may be an important factor in fish health. MCV, MCH and MCHC are particularly important in the diagnosis of anaemia in most animals (Aderolu and Akinremi, 2009).

Total cholesterol levels were elevated in fish-fed coconut oil dietary inclusion than in other diets. Levels were however lower than that reported by Aderolu and Akinremi (2009). Levels obtained for triglycerides were not significantly different in treatment diets and control fish. Levels of total cholesterol, triglyceride and LDL obtained in this study for fish-fed coconut oil dietary inclusion were higher than the reported limits by Okoye *et al*. (2016). Jafer *et al.,* (2019) reported that coconut oil has better LDL lowering effect than palm oil, even though levels were not significant. Chinwong *et al*., (2017) observed that coconut oil increases HDL cholesterol levels in humans. Sun *et al.,* (2015) reported that palm oil consumption increases LDL cholesterol and was due to the fact that palm oil contains a high amount of saturated fats with inconsistent effects on blood lipids. *C. gariepinus*-fed fish oil dietary inclusion had lower levels of total cholesterol, triglyceride, HDL and LDL. Fish oil reduced serum total cholesterol and triglycerides. Qin *et al*. (2015) noted that fish oil has been used for treatment of cardiovascular diseases via triglyceride reduction and inflammation modulation.

Fish fed fish oil diet had highest level of urea and a lowest weight gain was recorded in control, while fish fed palm oil diet had the highest weight gain. Ajeniyi and Solomon (2014) observed that higher weight of fish resulted in reduced level of urea and that this may defer from fish species due to influence of diet, season and environmental stressors on weight of fish. Na^+ was higher than Cl^- levels which was different from the trend observed by Yelwa and Solomon (2016) who reported a higher Cl^- level than Na^+ and noted that NaCl helped the body to maintain normal fluid balance. Martemyanov (2001) observed that a common range in levels of Na^+ is maintained in species with a decrease in levels during reproduction due to spawning stress. Serum albumin levels were not significantly different in treatments and control and also similar to levels earlier reported for *C. gariepinus* (Okoye *et al.,* 2016). Andreeva (2010) and Januar *et al.* (2015) reported that fish serum albumin levels are species and family dependent and also may be affected by differences in environmental conditions. Though Haroun *et al.,* (2006) reported that coconut is cheap, readily available in Nigeria compared to other oils, has antiviral, anti-protozoal, anti-bacterial properties, most stable at warmer temperatures, resistant to oxidative rancidity and it is not directly consumed by man so there is less competition on its usage, palm oil is relatively more available than coconut oil.

CONCLUSION

Dietary inclusions of palm oil, coconut oil and fish oil improved growth performance of *C. gariepinus* more than fish fed control diet. Fish fed fish oil in diet had highest level of urea and a lower weight gain, while fish fed palm oil in diet had the highest weight gain. This study has shown that 10 % dietary inclusions of palm oil and coconut oil may be included in diets of *C. gariepinus* as alternatives to fish oil without any deleterious effects on fish haematology and serum biochemistry. However, since palm oil is a cheaper and more readily available source of energy than coconut oil, palm oil can serve as a dietary replacement for fish oil.

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