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Haematological Responses of *Clarias gariepinus* (Burchell, 1822) Broodstock Fed Varying Palm Oil Inclusion Levels

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ABSTRACT

The haematological response of *Clarias gariepinus* fed various palm oil inclusion levels were investigated. Forty-five physically healthy 6 – month old African catfish, *Clarias gariepinus* broodstock of mean weight 380.16 ± 0.03g were fed isonitrogenous (40% crude protein) and isolipidic (6% dietary oil) diets containing graded levels of Palm-Oil (PO) inclusion: 0%, 25%, 50%, 75% and 100%, as Fish Oil (FO) replacements at 4% biomass daily, for 165 days. The dietary effects on haematological characteristics were investigated. Blood analyses result indicated TRT 3 having higher statistical ($P<0.05$) values for Haematocrit (HCT) Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Erythrocyte Sedimentation Rate (ESR): 23.95%, 33.10pg, 35.68gdL^{-1} and 13.60mm/hr, over control (19.03%, 29.02pg, 31.12gdL^{-1}, and 9.08mm/hr) and other treatments: TRT1- (19.72%, 30.47pg, 32.86gdL^{-1} and 11.08mm/hr); TRT2 (20.02%, 30.60pg, 31.80gLl^{-1} and 11.22mm/hr); and TRT4 (16.18%, 26.08pg, 30.98gdL^{-1} and 10.02mm/hr, respectively, while TRT 4 was the least significant in HCT and MCH values. Control and TRT 3 recorded higher significant variations in Mean Corpuscular Volume (MCV): 94.96fl and 95.18fl, respectively, from other treatments: TRT1 (91.19fl); TRT2 (86.68fl); and TRT 4 (85.19fl)). TRT4 and TRT2 were observed to have had the least significant values for the same parameter. The overall result concluded that, 75% PO inclusion level in substitution with FO in *Clarias gariepinus* broodstock diet could enhance their health status.

Keywords: Haematology, Palm oil, *Clarias gariepinus*, Broodstock

INTRODUCTION

In aquaculture, quality feed is very essential for fish health, growth, production, increased yield and profitability. As the demand for good quality formulated aqua feeds continues to be higher, Fish Oil (FO), an essential component in aqua feed has become so scarce due to stagnation in the marine and pelagic fish production, resulting in its long-term supply problem, high cost of aqua feed production, unnecessary stress and lean purse of fish farmers.

FO in aqua feed serves as a source of energy and Essential Fatty Acid (EFA) called omega -3 fatty acid. Eicosapentanoic Acid, (EPA) (20:5ɲ -3) Docosa Hexanoic Acid (DHA) [22:6ɲ -3] and Arachidonic Acid (ARA) [20: 4ɲ -6] belong to this EFA. It also contains vitamins A, E, and K, but low proportion of essential linoleic acid and a high percentage of oleic acid. Polyunsaturated Fatty Acid (PUFA) contents in fish oil improve diet digestibility and utilization, enhance immune system, and serve as stress resistance, due to the presence of high level of unsaturated fats such as linoleic and linolenic acids, vitamins A and E, FO, when used in broodstock fish diet, enhances fish larvae weight and their resistance to osmotic shock (Aby – ayad *et al*, 1997). However, FO scarcity with its attendant lipid quality problem, as marine fish oil is very susceptible to oxidation, has undoubtedly resulted in fish farmers and aqua feed producers seeking alternative dietary lipids. Omega-3s.

in FOs are highly unstable molecules that tend to decompose and, in the process, unleash dangerous free radicals. Research has shown that, omega -3s are found in a more stable form in vegetables, fruits and beans (Hunter, 1990). Vegetable Oils (VOs) are a source of energy and essential fatty acids that are needed for normal and structural development. They aid absorption of fat – soluble vitamins A, D, E and K, and improve digestibility in fish due to high level of unsaturated fats in them. Essential fatty

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acids in VOs are linoleic and linolenic acids. They are used to build specialized fats called omega-3 and omega -6 fatty acids, which deficiencies cause abnormalities in the liver and kidney, changes in blood, decreased growth rate and immune function (Mantzioris *et al*., 1994).

African catfish utilize efficiently, fatty acid contents of plant oils for better immune system and sound health status attainment. Many successful research works in respect of partial substitution of FO with VOs in fish diet has been carried out (Ng, 2004, Ademola and Olubodun, 2009) but, little studies relating to VOs effects on fish immune system are being recorded.

Oil palm (*Elaeis guineensis*), from which Palm-Oil (PO) is extracted is a native of West Africa. Among major oil seeds, oil palm accounts for the smallest percentage (5.5 percent) of all the cultivated land globally, but produces the largest percentage (3.2 percent) of total output. Palm oil production is expected to grow by more than 25 percent by 2020 to a global level of more than 68 million tons (The Palm Oil History, 2012).

It is considered to be the best alternative to FO, due to its natural, richness in carotenoid, pro-vitamins and lycopene content along with tocopherol and tocotrienol while, most VOs contain almost exclusively tocopherols. It is much less saturated than other plant oils, and reduces stress in African catfish due to bioaccumulation of tocopherols and tocotrienols, thereby reducing pathological conditions (Koh, 2006). It is against this background that; this study is designed to evaluate the effect on haematological parameters of *Clarias gariepinus* fed diet with graded levels of palm oil.

MATERIALS AND METHODS

Experimental Diet

The feed ingredients used in this experiment were procured from within Ibadan metropolis. The experimental diets were formulated to contain 0, 25, 50, 75, and 100% of palm-oil in replacement of fish oil in triplicates, giving a total of forty-five diets. All the diets were isonitrogenous (40% crude protein) and isolipidic (6% oil). The diets were prepared be mixing the ingredients together in a Hobert-200 mixer. Each diet was mixed with each graded level of PO. The diets were moistened with water until a desirable paste – like consistency was formed, and later pelletized using starch as a binder with the aid of a local pelletizer of different particle sizes: 4.5mm and 6mm dies, according to the fish size. All the diets were dried at ambient temperature for three days, after which they were labelled in dry air-tight plastic container, and stored in a cool, dry and safe place. The experimental diets were analyzed in triplicates for their proximate composition (AOAC, 2000). The percentage composition of the diets is shown in Table 1.

Fish source and experimental site

A total of physically healthy forty-five 6 – month old *Clarias gariepinus* broodstock consisting of thirty females (383.63 ± 0.03g) and ten males (385.00 ± 0.05g were procured from the fish farm of Department of Animal Science and Fisheries Management, Bowen University, Iwo, Nigeria. The experiment was carried out on the fish farm of the Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria.

Experimental fishes and management

A total of physical healthy forty-five 6 months old *Clarias gariepinus* broodstock averagely sized 380 ± 0.03g were randomly allotted to 5 treatments in a Completely Randomized Design, in concrete tanks of size 1m x 1m x 1m, after acclimatization for a period of two weeks. Their body weights were recorded prior to the feeding trial (week O). The fish were served pelleted experimental diet twice daily at 4% live body weight from 9.00 – 10.00 hour, and 16.00 – 17.00 hour, and feed intakes were taken each time. Body weights were taken twice daily on weekly basis throughout the feeding trial period. Water quality parameters were maintained at optimum levels. The feeding trial took a period of 168 days.

Table 1: Gross Composition (g/100g) dry matter of palm-oil-based diets fed to *C. gariepinus* broodstock

Ingredients	**Palm oil inclusion levels (%)**				
	0	**25**	**50**	**75**	**100**
Yellow Maize	25.20	25.20	25.20	25.20	25.20
Groundnut	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
Palm 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	1.00	1.00	1.00	1.00	1.00
Binder (Starch)	1.00	1.00	1.00	1.00	1.00
Proximate Composition (%)					
Moisture	7.18	5.75	5.98	5.45	5.39
Ash	16.53	16.02	15.12	16.25	14.98
Crude Protein	41.02	40.37	40.32	40.56	40.28
Crude Lipid	13.94	12.82	12.74	12.94	12.58
Crude Fiber	2.32	1.02	0.98	1.14	0.88
Nitrogen Extract	27.92	26.72	26.66	26.82	26.02
Gross Energy (Kcal/kg)	4570.32	4518.44	4522	4557.48	4497.17

Water quality

Temperature was taken daily with the aid of mercury- in- glass thermometer. pH and Dissolved Oxygen (DO) were measured weekly using a calibrated pH metre and through Winkler's method (Boyd,1990), respectively. Also determined weekly, was ammonia content, using A.O.A.C (1995). All the water quality parameters (Table 3) were maintained at optimum levels: Temperature (26.75^{0}C), pH (6.54), oxygen (5.47mgL^{-1}) and ammonia level (2.33mgL^{-1}).

Haematological studies

At the start of the feeding trial (week 0), and at the expiration of the 168 days experimental period, blood samples were collected from randomly selected fish specimens in triplicates, with a view to assessing palm oil dietary effect on the haematological parameters of the broodstock fish. Fish samples were anaesthetized with 150mg/liter of tricaine (MS – 222). According to Osuigwe *et al*. (2005), while, blood was drawn from their posterior caudal vein according to Ayuba *et al*; (2012), and 2mls was decanted in heparinized bottles containing lithium heparin anticoagulant for determination of White Blood Cell (WBC), Red Blood Cell (RBC), Haemoglobin concentration (HB), Haematocrit (HCT) and Erythrocyte Sedimentation Rate (ESR).

White blood cells and RBCs were counted using Neubauer's improved haematocytometer using Turks and Hyem's solution respectively, as a diluting fluid, while HB was calculated using cyano-methemoglobin method by Dacie and Lewis (2001). HCT (packed cell volume), which is the percent volume of packed red cells following centrifugation, was estimated by centrifuging the blood sample for 5 minutes at 10,000 (cpm), as described by Lagler *et al*, (1977). ESR was determined following the procedure of Svobodova *et al*., 1991. MCH, MCHC, and MCV were determined according to Dacie and Lewis (2001). The following blood indices were determined. The MCHC, MCH, and MCV were determined using the total RBC, HB and HCT values, following Dacie and Lewis (2001) formula:

i. WBC (Leucocyte) = No of cells counted X 0.25 X 200 (10^4mm^3),
ii. RBC (Erythrocyte) = No of cells counted X 10 X 200 (10^6mm^3),
iii. MCV (fl) - Size of red blood cells or, volume of a single red cell in fish blood.
iv. $HB = \frac{\text{Value obtained}}{100} \times 17.2mg/100mL$

v. Haematocrit (HCT) **-** The percentage volume of packed red cells following centrifugation.

$$= \frac{HCT \times 100}{RBC\ (10^6 \mu l^{-1})}$$

vi. MCHC $(gdL^{-1}) = \{Hb\ (gdL^{-1}) \times 10\}$ Hct x 100

vii. MCH (pg) = $\frac{\{Hb\ (gdL^{-1}) \times 10\}}{RBC\ (10^6\ \mu l^{-1})}$

ESR (mm/hr) values were determined following the procedures of Svobodova *et al*. (1991). The volume of ESR within the given time interval = the difference between 100% and the percentage part presented by corpuscular volume.

Statistical analysis

Data obtained from the experiment were subjected to One-way Analysis of Variance (ANOVA). Duncan's multiple range test Duncan (1955) were used to differentiate between treatment means at 95% confidence level (P<0.05).

RESULT

The ingredients and percentage nutrient composition of the diets fed to the fish for a period of 168 days are represented in Table 1. Moisture, ash, and protein contents ranged between 5.39 and 7.18%, 14.98 and 16.53%, and 40.28 and 41.02%, respectively. Crude lipid content varied from 12.58 to 13.94%, while fiber content ranged between 0.88 and 2.32%. Gross energy values of the diets fell between 4497.17 and 4570.32kcal/kg. Diet containing 100% PO recorded the lowest values while, control (0% PO) had the highest values in all. The findings reflected the good quality of the diets and their acceptability, which must have improved growth, and enhanced the health performance of the fish. *Clarias gariepinus* fed graded levels of PO in substitution for FO in the trial diet, exhibited statistically varied ($P < 0.05$) values in the haematological parameters except WBC, RB and HB when compared with the initial and control (Table 2).

Table 2: Haematological parameters of *C. gariepinus* broodstock fed varying levels of palm oil-based diets

Blood parameters	Initial	Palm oil inclusion levels (%)				
		0	25	50	75	100
WBC ($X10^3/\mu l$)	4.07±0.02^a	4.28±0.04^a	4.32±0.01^a	4.48±0.01^a	5.28±0.03^a	4.11±0.02^a
RBC($X10^6/\mu l$)	1.98±0.00^a	2.22±0.02^a	2.28±0.01^a	2.55±0.02^a	2.99±0.01^a	2.07±0.03^a
Hb (gdL^{-1})	4.10±0.01^a	5.22±0.01^a	5.86±0.03	6.12±0.02^a	6.66±0.02	4.38±0.01^a
Ht (%)	14.85±0.01^c	19.03±0.03^b	19.72±0.01^b	20.02±0.02^b	23.95±0.03^a	16.18±0.01^c
MCH (pg)	24.97±0.03^c	29.02±0.01^b	30.47±0.02^b	30.60±0.03^b	33.10±0.00^a	26.08±0.00^c
MCHC (gdL-1)	30.32±0.01^b	31.12±0.01^b	32.86±0.02^b	31.80±0.03^b	35.68±0.00^a	30.98±0.01^b
MCV (fl)	89.00±0.02^c	94.96±0.03^a	91.19±0.01^b	86.68±0.02^d	95.18±0.01^a	85.19±0.02^d
ESR (mm/hr)	10.48±0.02^b	9.08±0.01^b	11.08±0.01^b	11.22±0.01^b	13.60±0.01^a	10.02±0.01^b

a, b , means along the same row with any identical superscript are not significantly different(p> 0.05). **Keys:** WBC = White Blood Cell, RBC = Red Blood Cell, Hb = Haemoglobin, Ht = Haemotocrit, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean corpuscular, Haemoglobin Concentration, MCV =Mean Corpuscular Volume, ESR = Erythrocyte Sedimentation Rate.

The Ht, MCH, MCHC, MCV and ERS; 23.95%, 34.19pg, 35.68gdl^{-1}, 95.18fl and 13.60mm/hr respectively, in TRT 3, were statistically significant ($P < 0.05$) than other treatment levels, followed by TRT 2 (20.02%, 30.60pg), TRT 1 (19.72%; 30.57 pg), and control (19.03%; 29.02pg) for only HCT and MCH respectively, while TRT 4 (16.18% and 26.08pg) and initial (14.85% and 24.97pg) respectively, were the least significant. MCV values of TRT 3 (95.18fl) and control (94.96fl) were significantly higher than other treatment levels and initial: TRT 1 (91.19fl), TRT 2 (86.68fl), TRT 4 (85.19fl) and initial (89.00fl), while TRT 4 had the least significant values. TRT 3 was significantly higher in ESR value (13.60mm/hr), followed by TRT 2 (11.22mm/hr), TRT 1 (11.08mm/hr) and the initial (10.48mm/hr), while TRT 4 (10.02mm/hr) and control (9.08mm/hr) were the least significant.

The recorded values of WBC, RBC and HB did not differ statistically ($P > 0.05$) among treatment levels, control and the initial. It is worth observing that, values for all the parameters with the exception of MCHC and MCV increased gradually with increase in PO substitution in the diet except in T4.

Water quality parameters (Table 3) such as temperature, pH dissolved Oxygen and ammonia which ranged from 26.61 – 26.88^{o}c, 6.40 – 6.54, 5.39 – 5.54gdl^{-1} and 2.28 – 2.34gdl^{-1} respectively were similar.

DISCUSSION

The values of the chemical composition of the diets which fell within the ranges obtained in the past findings of Ochang *et al*. (2007) were lower than what were recorded in the previous study of Marimuthu and Muralikrishma, (2010). Protein contents were similar to what were contained in Dada and Olugbemi, 2013, while the lipid and ash content remained higher than what were recorded by the same authors. Age of fish is one of the factors determining the nature, type and chemical composition of feed to be served to a particular specie.

The effects of FO substitution with PO in *Clarias gariepinus* diet was manifested by changes in the haematological process taking place within the body system of the experimental fish, in comparism with the initial and control. The importance of haematology according to Joshi *et al*; 2002, lies in determining the health status of fish. The recorded values of WBC, HB, HCT, MCH, MCHC, MCV and ESR affirm the past findings of Etim *et al*. (1999), Subhadra *et al*. (2006), Ochang *et al*. (2007), and Oluyemi *et al*. (2008). A gradual increase in WBC values across board corroborated the findings of Joshi *et al*. (2000b) that, survival of fish can be correlated with increase in antibody production. RBC, HCT, PCV, and ESR determine anaemic condition in fish were within normal ranges Falaye *et al*. (1999) and Osuigwe *et al*. (2004), as none of them suffered haemorrhage resulting from trauma or, other symptoms which might have been due to lack of cobalt or copper in the trial diet, which aids assimilation of iron, as well as vitamins such as folic acid, vitamins B6 and B12, while HB values, as affirmed by previous findings of Etim *et al*. (1999) indicated that the oxygen-carrying capacity of the catfishes were similar.

Erythrocyte counts together with packed cell volume are highly valuable blood parameters that could be used as a tool in aquaculture and fishery management for checking anaemic conditions in fish, and a decrease in the erythrocyte count or, in the percentage of haematocrit indicates the worsening state of an organism (Adeyemo, 2007). The higher the level of FO substitution with PO, the higher the HCT values between treatments. This was contrary to the obtained values of Ekanem (2001). Low haematocrit values are of a consequence of deficiency of copper in the diet, which hindered synthesis in the liver of *Salvelinus alpinus* (Yurkowski, 1980). The values of Hb which confirmed the previous findings of Etim *et al*. (1999) and Ochang *et al*. (2007) indicated that, the oxygen- carrying capacities of the blood of the two catfishes were similar. The Hb values were lower than what was obtained by Subhadra *et al*. (2006) for the largemouth bass.

The gradual increase in some of the haematological parameters including MCH and MCHC, with increase in PO levels, in substitution with FO in the trial diet, resulted in sound immune system. Stronger and better immune system of the experimental fish in T3 might have been due to proper and efficient dilution of FO and PO. Poly Unsaturated Acid (PUFA) in FO as well as vitamin E molecule, alpha-tocopherol and 70 – 80% alpha – tocotrienol in PO function in fish health and disease (Lim *et al*., 2001; Ng, 2004). PUFA contents in FOs are responsible for sound immune system, stress resistance, and other health parameters in fish (Koven *et al*., 2001).

Inclusion of omega -3 fatty acid in control without dilution with PO, resulted in decreased haematological parameters' values, which gradually increased with increase in PO levels across board, corroborated the past findings of Nwabueze *et al*. (2011), that a reduction in the levels of haematological parameters with the continued inclusion of fish oil in *Heterobranchus bidorsalis* juvenile fish diet depicted a reduction in immunity.

Lower values of WBC, RBC, HCT, MCH and MCHC in TRT 4 proved that, a balance between µ-3: µ-6 seems critical in *Clarias gariepinus* broodstock. Good quality diet, conducive environmental condition, as well as proper management of the fish might have played a good role in the health process of the experimental fish. Seasonal variations, nutrition, size, genetic properties, population density, lack of food supply, and environmental stress affect the haematological data of fish (Blaxhall, 1973).

Water quality parameter

Water quality parameter values were within the optimum recommended range for culture of *Clarias gariepinus* (Viveen *et al*., 1986; Ajani *et al*., 2011).

Conclusion

This shows that, PO inclusion level in substitution of FO in the diet of *Clarias gariepinus* is practicable and nourishing, as it contains good nutrient composition that enhances the health and welfare of fish. Therefore, it is recommended that, 25% FO incorporation with 75% PO in this study, is a reliable source of essential fatty acids and vitamins required by apparently healthy *Clarias gariepinus* broodstock for better immune response.

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