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EFFECTS OF REPLACING DIETARY FISH OIL WITH VEGETABLE OILS ON HEAMATOLOGICAL PROPERTIES OF AFRICAN CATFISH (Clarias gariepinus)

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ABSTRACT

Dietary Fish oil replaced with vegetable oils was evaluated as an ingredient in practical diet for *Clarias gariepinus* reared in net fish hapas (1m x 1m x 1m) suspended by bamboo poles in an earthen pond. 25 fingerlings of *Clarias gariepinus* were distributed inside each hapas of 20 – twenty net fish hapas which amounted to 500 fingerlings reared for complete 56 days (8-eight weeks). After 8 weeks blood were extracted by puncturing the dorsal aorta into EDTA bottles which was taken to the laboratory for haematological analysis. The packed cell volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC), white blood cell (WBC), of the treatment (T_3) increased significantly relative to the control. while WBC decreased significantly in treatment two (soyabean oil) compared with the control. Others heamatological analyses (MCV, MCH and MCHC) showed that there is no significantly difference ($P>0.05$) between the values of these haematological parameters. Therefore, the significant difference were observed in the levels of RBC ($P<O.05$) which may be as a result of the stress the fish were exposed to during the study.

Keywords: *Clarias gariepinus*, haematological properties, fish oil and vegetable oil

INTRODUCTION

Fish meal has been a popular protein supplement for poultry and animal feeds for many decades. The composition of fish meals vary according to the species of fish, method of processing, and whether fillets have been removed for separate markets. Anchovies and menhaden are processed whole, with resulting meals containing 64 and 61% protein, respectively (National Research Council, 1994). By contrast, Zaviezo and Dale found tuna meal (from which fillets had been removed) to contain an average of only 53% protein, but higher levels of calcium and phosphorous than the whole fishmeal. Catfish has become an increasing popular human foodstuff in the United States, with significant production centered in the state of Mississippi. By the mid-1980s, the volume of processing waste had reached a level that justified its rendering as a distinct ingredient. As catfish meal is relatively new, its composition has not been reported in the scientific literature. The purpose of the current study was to evaluate a number of catfish meal samples to provide nutritionists with a baseline of data for use in commercial feed formulation (Zaviezo and Dale, 1994). Lipids are defined as fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds. They are a broad group of naturally occurring molecules including fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules (Fahy, 2005).

Lipids (fats) in aquaculture feeds are also utilized by fish as a source of energy which partially spares or substitutes for protein. Lipids being sources of highly digestible energy are also the only source of essential fatty acids needed by fish for normal growth and development. Lipids act as transport media for fat soluble vitamins, sterols etc. some forms of lipids like the phospholipids, are major constituents of cellular membranes and are important for the maintenance of membrane flexibility and permeability. Lipids are added to feeds to enhance the flavor of feeds and texture (Fahy, 2005).

Fish oil is oil derived from the tissues of oily fish. It is recommended for a healthy diet because it contains the omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), precursors to eicosanoids that reduce inflammation throughout the body (Moghadasian, 2008). Fish do not actually produce omega-3 fatty acids, but instead accumulate them from either consuming microalgae that produce these fatty acids, as is the case with fish like herring and sardines, or, as is the case with fatty predatory fish, by eating prey fish that have accumulated omega-3 fatty acids from microalgae. Such fatty predatory fish like mackerel, lake trout, flounder, albacore tuna and salmon may be high in omega-3 fatty acids, but due to their position at the top of the food chain, these species can accumulate toxic substances. For this reason, the FDA recommends limiting consumption of certain predatory fish species (e.g. albacore tuna, shark, and swordfish) due to high levels of toxic contaminants such as mercury, dioxin, PCBs and chlordane. More than 50 percent of the world fish oil production is fed to farmed salmon. (FAO, 2008). Over the years, fish oil has also sparked a lot of interest in the study treating clinical depression and especially, bipolar disorder. While there have been limited studies done on the subject, there have been notable trials that showed significant evidence to suggest that the omega-3 fatty acids in fish oil acted as a mood stabilizer, or at least, enhanced the benefit of SSRI medications. It is also interesting to note that the countries that indicated the highest intake of fish in their diets also correlated with the lowest rates of depression among citizens. (WebMD, 2008).

The level of availability of vegetable oils compared to fish oil has tended to be stable and increasing due to the increase of production of agricultural products and the rather low demand for vegetable oils. This makes vegetable oil a good alternative to fish oil in terms of its availability, sustainability, and it's price. Furthermore, recent research work has shown that vegetable oils have nutritional properties which satisfy the energy and nutritional requirements of fish. (NRC, 1993). This is a pale yellow fixed drying oil produced by solvent extraction from soybean seed (*Glycine max*). It is soluble in alcohol, chloroform and ether. It is also known as Chinese bean oil. Soybean oil has a neutral flavor and well balanced fatty acid profile, which makes it a desirable ingredient. Liquid soybean oil is low in saturated fat, contains no trans fat and is high in mono and poly saturated fats. It is also a principal source of omega-3 fatty acid and a primary source of vitamin E. Soybean oil is low in saturated fat, rich in the essential fatty acid and is an excellent source of vitamin E. Like all plant fats, soybean oil has no cholesterol. It is a good source of both linoleic and linolenic acids which are essential to human. Linoleic is about 50% while linolenic is about 7% of the fat content. It is also found to be rich in alpha-linolenic acid, representing about 7% of the fats in soybean, an essential unsaturated fatty acid which helps to lower the risk of stroke. Soybean is a good source of vitamin E because one serving spoon provides more than 10% of the minimum daily

requirement. Soybean oil also contains lecithin, which lowers blood level of cholesterol. The poly unsaturated fatty acid reduces blood level of Low Density Lipoproteins (LDLs) and High Density Lipoproteins (HDLs) too (Fahy, 2005). To produce soybean oil, the soybeans are cracked, adjusted for moisture content, heated to between 140°F and 190°F, rolled into flakes, and solvent-extracted with hexane. The oil is then refined, blended for different applications, and sometimes hydrogenated. Soybean oils, both liquid and partially hydrogenated, are exported abroad, sold as "vegetable oil," or end up in a wide variety of processed foods. Most of the remaining residue (soybean meal) is used as animal feed.

Groundnut oil is a slightly thick, dark-yellow oil, also known as peanut oil or arachis oil. Its major component fatty acids are oleic acid (56.6%) and Linolenic acid (26.7%) butit also contains some palmitic, arachidic, arachidonic, behonic, lignoceric and other fatty acids in lesser amounts. Groundnut oil also known to have a high content of palmitic, oleic and linolenic acids. Like whole groundnuts, groundnut oil is dangerous to anyone with peanut allergy (Bulrit, 2010) Groundnut oil is of great use due to its medicinal purposes and is used in the treatment of arthritis and acne blackheads. Natural red palm oil is produced from the fruit of the palm, *Elaeis guineensis*. It is liquid at room temperature and as the name implies, it has rich bright red color which is due to the high content of natural carotenoids. The carotene content is about 500mg/kcal. Palm oil is usually red in color as a result of its composition of carotenes (Beta carotene and alpha caroteneoten). It has 15 times more provitamin A carotene than carrots and 300 times more than tomatoes. In addition to beta carotene, alpha carotene and lycophene , it contains at least 10 other carotenes along with tocopherols and toctrienols. It is widely used as cooking oil. Palm oil contains about 50% saturated fatty acids monosaturated oleic acid is also a constituent of palm oil which is also a natural source of toctrienol, a part of vitamin E. family (Fahy, 2005).

Palm oil which has a low n-6 PUFA is currently the second most abundant vegetable oil world wide after soybean oil. Global production of crude palm oil (CPO) exceeds 28 million tonnes and is the most traded and abundantly available vegetable oil in the world. Shea butter is a slightly yellowish or ivory colored natural fat extracted from the seed of the African shea tree (*Vitellaria paradoxa*) by crushing, boiling and steaming. It is widely used in cosmetics as a moisturizer, salve or lotion. Shea butter is edible and may be used in food preparation, it is also used in the chocolate industry as a substitute for cocoa butter though the taste is quite different. Shea butter oil is a complex fat that contains many non-saponifiable components (i.e. substances that cannot be fully converted into soap by treatment with alkali). Haematology can be a useful tool for monitoring health status, detecting illness, and following the progress of disease and response to therapy. Despite advances in fish medicine in recent years, interpretation of fish haematology often is hampered by a lack of meaningful reference values and the bewildering diversity of fish species O'Neal and Weirich, 2001.). A multitude of intrinsic and extrinsic factors cause normal and abnormal variation in hematologic data. This article provides an overview of some of the hematologic abnormalities in fish induced by infectious agents and environmental, husbandry, and nutritional issues (Vosylienë, 1999).

The application of haematology studies to the investigation of animal and human disease process is well accepted and considered to be a routine procedure in diagnostic studies (Ezeri, 1989). There have been attempts to apply haematology parameters like Haematocrit, Haemoglobin, Erythrocyte count, Leucocytes, Mean corpuscular volume, Mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration to the study of abnormal physiological processes in fish. The Haematocrit will vary, depending on the health and physiological conditions of the individual fish. Haemoglobin in the blood is a rapid method of detecting disease conditions in fish including anaemia. Obtaining a count of erythrocytes in fish blood is a useful Tool because of the association of abnormal red blood cell (RBC) counts with disease conditions. The main function of red blood cell is to transport oxygen from the lungs to the body cells. Most white blood cell exhibit amoeboid movement and can squeeze between the cells in the walls of capillaries and enter the intercellular spaces. White blood cells help to depend the body against diseases. Blood also protects the body against disease- causing micro-organism. The aim and main objectives of this project are to study effects of haematology responses of *Clarias gariepinus* fed with different oil ingredients like palm oil, shea butter, fish oil, groundnut oil, soyabean in their feed and to evaluate the relationships between different haematological parameters of *Clarias gariepinus* fed with different oil ingredients in their feed.

MATERIAL AND METHODS

The feeding trial was conducted in net fish hapas (1m x 1m x 1m) suspended by bamboo poles in an earthen pond located at Alogi Obantoko, Abeokuta, Nigeria. 20 hapas were suspended at ¾ of their volume and tied to carefully arranged bamboo poles. *Clarias gariepinus* fingerling was used for the experiment. The fish were acclimatized for two weeks in plastic tanks. 25 fingerlings of fish were thereafter stocked in each hapa.

Collection of blood was carried out via puncturing the dorsal aorta into EDTA bottles which was taken to the laboratory for Haematological analysis.. The experimental set-up consisted of 5 treatments which replicated thrice, fish fingerlings were randomly distributed at the rate of 25 fish per hapa. After stocking, fish were fed with prepared diet according to their treatment at 5% body-weight daily. At the end of 8-eigth weeks, the blood sample was extracted from the fish and the analysis was done in other to determine the effect of each oil used (groundnut oil, soyabean oil, palm oil, shea butter and fish oil) on the blood parameters like Haematocrit, Haemoglobin determination, Erythrocyte count, leucocyte count, Mean corpuscular volume, Mean corpuscular haemoglobin, and Mean corpuscular haemoglobin concentration.

Determination of haematological properties

The following parameters were used to assess the effects of dietary treatments on the heamatological profile of *Clarias gariepinus* at the end of the feeding trial.

Haematocrit

The haematocrit (HCT) which is known as the packed cell volume (PVC) of each individual was determined y contrifigation of blood in blood placed capillary tube (with one end sealed) using haematocrit centrifuge.

Erythrocyte Count

The calculation of the number of red blood cells per cu.cm must take to consideration.

a. The number of cells counted

b. The dilution of the blood (1:200)
c. The volume of the diluted blood counted, which is equals the depth of which the cells are counted (0.2sq.mm) or 0.02cu.mm. therefore the number of red blood cells per cu.mm is equal to

$$\#\text{ cells counted} \times \frac{1\text{ cu.mm}}{0.02\text{cu.mm}} \times 200 = \#\text{ cells counted} \times 10{,}000$$

Haemoglobin

Haemoglobin level was determined colorimetrically by the cyamethemoglobin method. Blood was mixed with drabkin's reagent, which contained ferricyanide and cyanide. The ferricyanide oxidized the iron in the heamatolgical to methemoglobin. The methemoglobin. The methemoglobin counted with the cyanide to form cyamethemoglobin. The cyamethemoglobin produced a colour which was measured in the colorimeter at a wavelength of 540nm.the fish haemoglobin concentration (Cpt) was calculated by comparing the optical density (O.D) with that of standard.

$$Cpt = \frac{ODpt \times Cstd}{ODstd} = g/dl$$

ODpt — optical density concentration
ODstd - optical density standard
Cstd - concentration standard
Leucocyte Count (white blood cell)

The dilution of the blood (1:20)
The volume of diluted blood counted which is equal to the depth of the chamber (0.1nm)x the area in which the cells are counted (45qmm) or 0.4 cu.mm. therefore, the number of leukocytes per cu.mm = number of cells counted x1 cu.mm x 20 or number of cells counted x50. A small amount of blood is accurately diluted with 2% acetic acid which destroys the non- nucleated erythrocyte and makes clearly visible the nuclei of the leukocytes. Nucleated erythrocytes remain intact.
Mean corpuscular volume (MCV)
The mean volume of blood cell was estimated using the relationship

$$MCV = \frac{\text{Pack Cell Volume}}{\text{Erythrocyte Count}} \times 100$$

Mean corpuscular haemoglobin (MCH)
The haemoglobin content of a single RBC was calculated as
MCH = Haemaglobin/ Erythrocyte count (pg)*
Mean corpuscular haemoglobin concentration (MCHC)
MCHC = Haemoglobin concentration(g/100ml) x 100/ pack cell volume(%) (g/100ml)

Statistical Analysis

All data obtained were subject to analysis of one way analysis of variance (Anova) and the differences among means were tested for significance $P< 0.05$ using LSD.

Table 1: Composition of Experimental Feed

	% composition Treatments				
Ingredients	**1**	**2**	**3**	**4**	**5**
Fishmeal	27.82	27.82	27.82	27.82	27.82
Groundnut Cake	13.91	13.91	13.91	13.91	13.91
Soybean meal	27.82	27.82	27.82	27.82	27.82
Maize	17.44	17.44	17.44	17.44	17.44
Lysine	0.50	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50	0.50
Dichromium phosphate	0.50	0.50	0.50	0.50	0.50
Vitamin premix	1.00	1.00	1.00	1.00	1.00
Vitamin C	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Fish oil	10.00	-	-	-	-
Groundnut oil	-	10.00	-	-	-
Palm oil	-	-	10.00	-	-
Shear butter oil	-	-	-	10.00	-
Soybean oil	-	-	-	-	10.00
Total	100.00	100.00	100.00	100.00	100.00

Treatment 1 – fish oil
Treatment 2 – groundnut oil
Treatment 3 – soybean oil
Treatment 4 – shear butter oil
Treatment 5 – palm oil

Table 2: Proximate Analysis on Fish and Feed Prior to Project Commencement

	Moisture (%)	Ash (%)	Crude fibre (%)	Crude protein (%)	Fat extract (%)
Treatment1 (fish oil)	5.38	9.70	5.70	8.40	14.45
Treatment2 (groundnut oil)	5.62	9.90	5.40	12.35	19.00
Treatment 3 (soybean oil)	6.14	10.30	4.50	10.60	18.10
Treatment 4(shear butter oil)	8.48	10.50	3.50	13.85	20.61
Treatment 5 (palm oil)	3.54	10.10	4.80	11.65	20.71
Fish sample	78.13	5.50	0.02	16.60	20.6

RESULT

Results of effects of replacing dietary fish oil with vegetable oils (soyabean oil, groundnut oil, sheabutter oil and palm oil) on the haematology of Claries gariepinus are presented on Table 3. The blood valves of Cat fish, namely the Haemoglobin (Hb), packed cell volume, (PCV) White Blood Cell (WBC), Mean Corpuscular Volume, Mean Corpuscular Haemoglobin (MHC) and Mean Corpuscular Haemoglobin Concentration (MCHC) levels, increased,

whereas Hb level content varied between 10.7gldl (Diet 2) and 12.8gldl and was significantly different (P< O.05). PVC (38%) was recorded and the lowest PVC (32%) for diet 2. Reduction in packed volume cell and Haemoglobin concentration of fish may be as a result of essential fatty acid, mineral and vitamin differences or imbalance and heat treatment, have been known to cause such deficiencies in legumes.
In the light of the present study the mean value of PVC was 35 in the control group (T,) which was decrease significantly from other treatments (32, 38, 34, 33) in groups 2, 3, 4 and 5, respectively with groups 2, 4 and 5 having significantly (P = 0.83, 0.84, 0.80 respectively) lower PCV compare to the control.

Table 3: Haematological profile of experimental fish

	PCV (%)	Hb (g/dl)	RBC (mil/mm3)	WBC(104/mm3)	MCV (um3)	MCH (ug)	MCHC (%)
T_1 (control)	35 ± 0.81 [b]	11.8 ± 0.09 [b]	4.0 ± 0.08 [b]	5,200 ± 84.62 [a]	88 ± 0.70[ab]	30 ± 0.81[a]	34 ± 0.90[a]
T_2	32 ± 0.83 [c]	10.8 ± 0.06 [e]	3.6 ± 0.06 [d]	5,000 ± 84.63 [b]	89 ± 0.70 [a]	30 ± 0.82 [a]	34 ± 0.90 [a]
T_3	38±0.84 [a]	12.8 ± 0.06 [a]	4.4 ± o.07 [a]	5400 ± 84.60 [a]	86 ± 0.71 [b]	29 ± 0.83[a]	34 ± 0.93 [a]
T_4	34 ±0.80 [bc]	11.4 ± 0.08 [c]	3.8 ± 0.06 [c]	5,200 ± 84.62 [a]	89 ±0.62 [a]	30 ± 0.82[a]	35 ± 0.91 [a]
T_5	33 ±0.82 [c]	11.1 ± 0.07 [d]	3.8 ± 0.07 [c]	5,200 ± 84.61 [a]	87 ± 0.67 [b]	29 ± 0.80 [a]	33 ± 0.92 [a]

PCV = Packed Cell Volume (%)
Hb = Haemoglobin (g/dl)
RBC = Red Blood Cell (mil/mm3)
WBC = White Blood Well (104/mm3)
MCV = Mean Corpuscular Volume (cu.microns)
MCH = Mean Corpuscular Haemoglobin (u/mg)
MCHC = Mean Corpuscular Haemoglobin Concentration (%)

DISCUSSION

Studies have shown that the nutritive values of food have profound influence on the haematological properties. Effects of replacing dietary fish oil with vegetable oils on the haematology of *Claries gariepinus* were seen in the result from the blood analysis of each treatment. Haemoglobin concentrations reflect the supply of an organism with oxygen and other organism itself tries to maintain them as much stable as possible (Ezeri, 1998).
The study shows that mean haemoglobin in the control was 11.8, 10.7 in treatment 2, 12.8 in treatment 3, 11.4 in treatment 4 and 11.1 in treatment five. A decrease in the concentration of haemoglobin reflects the supply of an organism state and developing anaemia. Since haemoglobin in the blood is a rapid method of detecting disease conditions in fish. A decrease in the concentration of haemoglobin in blood is usually caused by the effect of toxic metals in gills as well as decrease in oxygen; which also suggests anaemia in Catfish. Haematological indices (RBC count, concentration of hemoglobin and haematoint) have been reported to indicate secondary responses of an organism to irritants (O'Neal and Weirich, 2001). The MCV, MCH and MCHC increased

considerably in all treatments compared to the control. However, the increase in MCV was significant (P<O.05) only in treatment 3 and 5 (P = 0.7 and 0.67, respectively), while the increase in MCH and MCHC recorded by the treatments was significant (P< O.05) only in treatment T_3 and T_5. Mean Corpuscular Volume (MCV) was estimated to calculate the size of red blood cell while the mean corpuscular haemoglobin (MCH) and mean corpuscular Haemoglobin concentration are used to calculate the concentration of Haemoglobin in fish blood. Therefore, MCV and MCH values were calculated using the PCV, Hb and RBC. If MCV, MCH and MCHC decrease it means that the condition of the blood of the fish is normal; the value decreased sparingly in treatment 3. Between MCV and MCH there is no significant different (P>0.05) within these parameters for different diets. It can be concluded that fish oil replaced with soybean oil out of other vegetable oils used is acceptable to *Clarias garipinus* and it could be used instead of fish oil at 10% inclusion level for optimal growth performance and normal haematological parameters of African catfish.

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