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Haematological Changes in the Blood of *Clarias gariepinus* Fed *Chrysophyllum albidum* Seedmeal Replacing Maize

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ABSTRACT

This study was conducted to investigate haematological changes in the blood of *Clarias gariepinus* fed diets containing *Chrysophyllum albidum* seedmeal replacing maize. Five isonitrogenous diets containing maize, which was replaced by *Chrysophyllum albidum* at a rate of 0, 25, 50, 75 and 100% were made. The diet without *Chrysophyllum albidum* seedmeal served as the control. Experimental diets were assigned randomly to the tanks and each group of fish was fed 5% body weight in equal proportion per day. The fish fed diet 1 had the highest PCV while the fish fed diet 3 had the lowest PCV. There was significant difference ($p < 0.05$) in the PCV of the fish fed various dietary treatments. However, there was no significant difference ($p > 0.05$) in the PCV of the fish fed diet 2, 3, 4 and 5. A similar trend, as observed for PCV, was also observed for Hb, RBC, MCV, MCH and MCHC. There was no significant difference ($p > 0.05$) in the WBC of the blood of the fish fed various dietary treatments so there were also neutrophils and lymphocytes.

Key words: *Clarias gariepinus*, *Chrysophyllum albidum*, maize, dietary treatments

INTRODUCTION

A review of the animal and aquafeed industries in Nigeria made by Fagbenro and Adebayo (2005) revealed that most catfish feeds are farm made, using locally available ingredients such as maize, soybean, fish meal, blood meal, rice bran and fish oil, etc. Olurin *et al.* (2006) reported that maize is the major source of metabolisable energy feed ingredient in most compounded diet for catfish spp. This is because, it is readily available and digestible. However, the increasing prohibitive cost of this commodity has necessitated the need to search for alternative ingredients that will serve as a replacement. More so, FAO reported insufficient quantities of maize that are produced in Nigeria were predominantly used for human consumption. Osuigwe *et al.* (2005) reported that high cost and scarcity of maize in formulated diet has led to the use of under utilised energy sources; such as cassava root meal, wheat bran and sorghum meal.

Chrysophyllum albidum, from the Sapoteacea family is commonly found in the Central Eastern and Western Africa (Amusa *et al.*, 2003). They are distributed in Nigeria, Uganda, Niger, Cameroun and Cote d'Ivoire. It is often called the white star apple are distributed throughout the southern part of Nigeria (Iwalewa *et al.*, 2006). Across Nigeria, it is known by several local names. In South-western Nigeria, the fruit is called "agbalumo" and known as "udara" in South eastern

Nigeria and is generally regarded as a plant with diverse ethno-medicinal uses (Amusa *et al.*, 2003). It has nutritional profile comparable to maize and other energy source feed ingredients.

Svobodova *et al.* (1991) opined that ichthyohaematology would be useful in the assessment of suitability of feeds and feed mixture, evaluation of fish condition, determination of toxic effect of substances, as well as diagnosis of disease. The use of haematological values, as indices of diagnosing diseases and stress induced condition as well as for feed assessment, are well documented (Fagbenro *et al.*, 1993; Adeparusi and Ajayi, 2000; George *et al.*, 2007; Yue and Zhou, 2008; Akintayo *et al.*, 2008). This study, therefore, seeks to study the haematological response of *Clarias gariepinus* fed diet containing *Chrysophyllum albidum* seedmeal.

MATERIALS AND METHODS

Seed collection and processing: Dried matured *Chrysophyllum albidum* seeds were obtained from Bodija Market, Ibadan, Oyo State and they were processed by boiling in water (100°C) for 30 min. They were prepared by grinding the samples in a laboratory mill, then mechanically defatted by the use of locally made screw press and sieved with a 200 mm mesh size sieve before putting in polythene bags and stored at 4°C. The cakes, therefore, were analysed for their proximate composition (AOAC, 1990). Fish meal, soybean meal and other feedstuffs obtained from commercial sources in Nigeria were separately milled screened to fine particle size and triplicate samples were analyzed for their proximate composition (AOAC, 1990). Based on the nutrient composition of the protein feed stuff (Table 1), a control diet and four test diets were formulated. The control diet contained maize which was replaced by cooked *Chrysophyllum albidum* seed meal. The rate of substitution was 0, 25, 50, 75 and 100% (Table 2).

Table 1: Proximate composition of the protein feed ingredients

Parameters	Fish meal	Soybean meal	CSM	Corn meal
Moisture	9.75	10.70	9.10	10.48
Crude protein	72.40	45.74	10.95	9.87
Crude lipid	10.45	9.68	2.94	4.28
Crude fibre	-	5.10	3.06	5.78
Ash	8.32	4.48	2.12	6.73
NFE	-	30.00	71.83	62.35

CSM: *Chrysophyllum albidum* seedmeal

Table 2: Gross composition of experimental diets (g/100 g) containing *Chrysophyllum albidum* seedmeal fed to *Clarias gariepinus*

Parameters	Experimental diets				
	D ₁	D ₂	D ₃	D ₄	D ₅
Fishmeal (72.4%)	52.78	52.78	52.78	52.78	52.78
Corn meal (10.48%)	20.00	15.00	10.00	5.00	-
CSM (10.95%)	-	4.56	9.13	13.69	18.25
*Vit/min premix	5.00	5.00	5.00	5.00	5.00
Fish oil	5.00	5.00	5.00	5.00	5.00
Starch	17.22	17.66	18.09	18.53	18.97
Total	100.00	100.00	100.00	100.00	100.00

CSM: *Chrysophyllum albidum* seedmeal. *Each killogram contains, Vitamin A: 4,000,000 IU, Vitamin B: 800,000 IU, Vitamin E: 16,000 mg, Vitamin K₃: 800 mg, Vitamin B₁: 600 mg, Vitamin B₂: 2,000 mg, Vitamin B₆: 1,600 mg, Vitamin B₁₂: 8 mg, Niacin: 16,000 mg, Caplan: 4,000 mg, Folic acid: 400 mg, Biotin: 40 mg, Antioxidant: 40,000 mg, Chlorine chloride: 120,000 mg, Manganese: 32,000 mg, Iron: 16,000 mg, Zinc: 24,000 mg, Copper: 32,000 mg, Iodine: 320 mg, Cobalt: 120 mg and Selenium: 800 mg manufactured by DSM Nutritional products Europe Limited, Basle, Switzerland

Culture condition: *Clarias gariepinus* fingerlings were acclimated to experimental condition for 7 days prior to the feeding trial. Groups of 15 catfish fingerlings were stocked into aquaria comprising 60 L capacity rectangular plastic tanks. Each diet was fed to the catfish in triplicate tanks twice daily (9 and 16 h) at 5% body weight for 56 days. Fish mortality was monitored daily, total fish weight in each tank was determined at two weeks intervals and the amount of diet was adjusted according to the new weight.

Haematological studies: The blood analyses were determined according to the method described by Svobodova *et al.* (1991). The following analyses were done:

- **Blood analysis:** The 5-10 mL blood samples were collected from cardiac puncture using 2 mL disposable heparinised syringe treated with EDTA as anti-coagulant
- **Blood cell count:** Haemocytometer was used in blood cell count. The blood diluting fluid was prepared as described by Svobodova *et al.* (1991). The blood cells were counted on the counting chamber of haemocytometer with the aid of compound microscope

RBC = No. of cells counted $\times 3 \times 10 \times 200$ (10^6 mm^3)

WBC = No. of cells counted $\times 0 \times 25 \times 10 \times 20$ (10^4 mm^3)

- **Haemoglobin estimation:** Haemoglobinometer was used for haemoglobin estimation based on acid haematin method (SAHLI):

$$\text{Haemoglobin} = \frac{\text{Value obtained} \times 17.2 \text{ mg} / 100 \text{ mL}}{100}$$

The packed cell volume was measured after placing sealed microhaematocrit tube in a centrifuge at 10,500 rpm using microhaematocrit reader and expressed as percentage.

- **Mean Corpuscular Volume (MCV):** It was calculated from the haematocrit value (PCV (%)) and the Erythrocyte count (Er mm^3):

$$\text{MCV } (\mu^3) = \frac{\text{PCV}}{\text{Er}} \times 10$$

- **Mean Corpuscular Haemoglobin Concentration (MCHC):** This was obtained using the following equation:

$$\text{MCHC } (\%) = \frac{\text{Hb}}{\text{PCV}} \times 100$$

- **Mean Corpuscular Haemoglobin (MCH):** This was expressed in picogrammes (μg) as follows:

$$\text{MCH } (\mu\text{g}) = \frac{\text{Hb}}{\text{Er}} \times 100$$

Statistical analysis: Data obtained from the experiment was expressed in Mean \pm SD and it was subjected to one way Analysis of Variance (ANOVA) using SPSS 16.0 version. Where, the ANOVA reveals significant difference ($p < 0.05$), Duncan multiple range test was used to compare differences among individual treatment means.

RESULTS

Proximate composition of the experimental diets: Table 3 shows the proximate composition of the experimental diets. It reveals the diets to be isonitrogenous and isolipidic as there was no significant difference ($p > 0.05$) in the crude protein and crude lipid content of the diet. The protein and lipid requirement of *Clarias gariepinus* was met by the 40 and 12% provided in the experimental diets. All the fish responded well to the dietary treatment given to them.

Haematological profile: Table 4 shows the haematological profile of *Clarias gariepinus* fed diet containing *Chrysophyllum albidum* seedmeal. The fish fed diet 1 had the highest PCV while the fish fed at diet 3 had the lowest PCV. There was significant different ($p < 0.05$) in the PCV of the fish fed various dietary treatments. However, there was no significant difference ($p > 0.05$) in the PCV of the fish fed diet 2, 3, 4 and 5. Similar trends, as observed for PCV, were also observed for Hb, RBC, MCV, MCH and MCHC. These was no significant difference in the WBC of the blood of the fish fed various dietary treatment so also were neutrophyl and lymphocyte.

Table 3: Proximate composition of experimental diets containing *Chrysophyllum albidum* seed meal fed to *Clarias gariepinus*

Parameters	Experimental diets				
	D ₁	D ₂	D ₃	D ₄	D ₅
Moisture	9.24 \pm 0.11	9.20 \pm 0.03	9.16 \pm 0.13	9.12 \pm 0.10	9.23 \pm 0.06
Crude protein	40.23 \pm 0.05	40.20 \pm 0.08	40.25 \pm 0.15	40.23 \pm 0.06	40.20 \pm 0.12
Crude lipid	12.17 \pm 0.09	12.20 \pm 0.05	12.15 \pm 0.12	12.16 \pm 0.08	12.20 \pm 0.13
Crude fibre	4.59 \pm 0.45	4.15 \pm 0.11	4.16 \pm 0.10	4.16 \pm 0.05	4.13 \pm 0.05
Ash	4.48 \pm 0.06	4.60 \pm 0.45	4.50 \pm 0.32	4.33 \pm 0.40	4.37 \pm 0.31
NFE	29.29 \pm 0.31	29.71 \pm 0.51	29.80 \pm 0.50	29.10 \pm 0.40	29.93 \pm 0.30

Mean in row without superscript are not significantly different ($p > 0.05$) from one another

Table 4: Haematological profile of *Clarias gariepinus* fed diet containing *Chrysophyllum albidum* seedmeal

Parameters	Experimental diets				
	D ₁	D ₂	D ₃	D ₄	D ₅
PCV	25.67 \pm 3.21 ^a	12.00 \pm 1.00 ^b	11.67 \pm 0.58 ^b	12.67 \pm 3.06 ^b	12.00 \pm 1.00 ^b
HB	8.60 \pm 1.13 ^a	4.00 \pm 0.20 ^b	3.93 \pm 0.12 ^b	4.23 \pm 0.97 ^b	4.00 \pm 0.20 ^b
RBC	2.25 \pm 0.28 ^a	1.05 \pm 0.09 ^b	1.04 \pm 0.05 ^b	1.11 \pm 0.27 ^b	1.06 \pm 0.11 ^b
WBC	142.93 \pm 6.22	140.57 \pm 8.26	177.00 \pm 61.82	190.23 \pm 78.30	133.83 \pm 12.91
Neutrophyl	64.33 \pm 4.04	60.00 \pm 5.00	65.67 \pm 11.60	70.33 \pm 10.50	31.70 \pm 2.90
Lymphocyte	35.67 \pm 4.07	40.00 \pm 5.00	34.33 \pm 11.59	29.67 \pm 10.50	31.67 \pm 2.89
MCV	99.10 \pm 12.44 ^a	46.33 \pm 3.85 ^b	45.03 \pm 2.19 ^b	48.90 \pm 11.82 ^b	46.33 \pm 3.85 ^b
MCH	30.77 \pm 4.05 ^a	14.30 \pm 0.70 ^b	13.97 \pm 0.35 ^b	15.17 \pm 3.48 ^b	14.30 \pm 0.70
MCHC	23.133 \pm 2.84 ^a	10.80 \pm 0.90 ^b	10.70 \pm 0.46 ^b	11.40 \pm 2.72 ^b	10.73 \pm 1.06 ^b

Mean in rows with the same superscript are not significantly different from each other ($p < 0.05$)

DISCUSSION

The result of the proximate analysis of the diets to be isonitrogenous and isolipidic. The protein and lipid requirement of *Clarias gariepinus* was met by the quantity provided in the diets. Uys and Hecht (1985) reported that the best growth rate and feed conversion efficiency in juvenile and sub-adult *Clarias gariepinus* are achieved with diets containing 38-42% crude protein and optimum liquid content of 10-11%.

The observed reduction in haematological parameters in *Clarias gariepinus* fed *Chrysophyllum albidum* meal, in this study, conform to the report of Tacon (1992) and Jimoh (2012) of nutritionally deficient diets can cause decrease in haemoglobin content, reduced PCV and red blood cell count. The decrease in haematological parameters with increasing level of incorporation of *Chrysophyllum albidum* meal agreed with other observation (Blom *et al.*, 2001; Dabrowski *et al.*, 2001; Rinchard *et al.*, 2003; Fagbenro *et al.*, 2010). However, the values recorded for RBC of the fish fed the dietary treatments were all within the range of normal haematology of a healthy fish (Fagbenro *et al.*, 1993; Rastogi, 2007). Erythrocyte count greater than $1 \times 10^6 \text{ mm}^{-3}$ is considered high and is indicative of high oxygen carrying capacity of the blood which is characteristic of fishes capable of aerial respiration and with high activity. The PCV values recorded in this study fall within the normal range of 20-38% for fish as reported by Clark *et al.* (1979) and Erondy *et al.* (1993).

Increase in white blood cell, as observed in the fish fed *Chrysophyllum albidum* diets, is attributed to increase in the production of leucocyte in the haemotopoietic tissue of the kidney and perhaps the spleen. Akinwande *et al.* (2004) reported that a measurable increase in WBC of fish is a function of immunity response to vulnerable illness and disease. Thus, it can be concluded that not much stress is placed on the health of *Clarias gariepinus* fed *Chrysophyllum albidum* seed meal replacing maize.

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