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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 growth response of C. gariepinus fed diets containing C. albidum seed-meal replacing maize. Five isonitrogenous diets containing maize which was replaced by C. albidum at a rate of 0, 25, 50, 75 and 100% were made. The diet without C. albidum seed-meal 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, diet 3, diet 4, diet 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 also were neutrophyls and lymphocytes.

Keywords: C. gariepinus, C. albidum, maize, dietary treatment.

Introduction

A review of the animal and aquafeed industries in Nigeria made by Fagbenro and Adebayo (2006) revealed that most catfish feeds are farm made, using locally available ingredients such as maize, soybean, fish meal, blood meal, rice bran, fish oil, etc. Olurin et al. (2006) reported that maize is the major source of metabolizable energy feed ingredient in most compounded diet for catfish species. 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. Moreso, that FAO (2005) reported insufficient quantities of maize that are produced in Nigeria were predominantly used for human consumption. Osuigwe (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, 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 and distributed throughout southern Nigeria (Idowu et al., 2006). Across Nigeria, it is known by several local names; in the southwest the fruit is called "agbalumo," "udara" in the southeast and is generally regarded as a plant with diverse ethno-medicinal uses (Amusa et al., 2003). Svobodova et al. (1991) opined that ichthyohemotology 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 hematological values as indices of diagnosing diseases and stress induced condition as well as for feed assessment is well documented (Fagbenro et al., 1993, Adeparusi and Ajayi, (2004), George et al., 2007 Yue and Zhou; 2008, Akintayo et al., 2008). This work therefore seeks to study the hematological response of *C. gariepinus* fed diet containing *C. albidum* seed-meal.

Materials and Methods

- **Seed collection and processing.** Dried matured *Chrysophylum albidum* seeds were obtained from Bodija Market, Ibadan Oyo State and they were processed by boiling in water (100°C) for 30 minutes. 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 200mm mesh size sieve, before putting in polyethylene bags and stored at 4°C. The cakes, therefore, were analyzed 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 *C. 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.

Parameter	Fish meal	Soybean meal	CSM	Corn meal
Moisture	9.75	10.70	9.10	10.48
Crude protein	72.4	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/100g) containing *C. albidum* seedmeal fed to *C. gariepinus*.

	D1	D2	D3	D4	D5
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	100	100	100	100

*CSM- Chrysophyllum albidum seedmeal. *Specification: each kg contains: Vitamin A , 4,000,000IU; Vitamin B, 800,000IU; Vitamin E, 16,000mg, Vitamin K_3, 800mg; Vitamin B_1, 600mg; Vitamin B_2, 2,000mg; Vitamin B_6, 1,600mg, Vitamin B_{12}, 8mg; Niacin,16,000mg; Caplan, 4,000mg; Folic Acid, 400mg; Biotin, 40mg; Antioxidant 40,000mg; Chlorine chloride, 120,000mg; Manganese, 32,000mg; Iron 16,000mg; Zinc, 24,000mg; Copper 32,000mg; Iodine 320mg; Cobalt, 120mg; Selenium, 800mg manufactured by DSM Nutritional Products Europe Ltd., Basle.*

- **Culture condition.** *C. 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 litre-capacity rectangular plastic tanks. Each diet was fed to the catfish in triplicate tanks twice daily (09.00h, 16.00h) 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.
- **Hematological studies.** The blood analyses were determined according to the method described by Svobodova *et al.* (1991). The following were done.
- **Blood analysis.** 5–10ml blood samples were collected from cardiac puncture using 2ml disposable heparinised syringe treated with EDTA as anticoagulant.
- **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 x 3 x 10 x 200 ($10^6 mm^3$)
- WBC = no of cells counted x 0 x 25 x 10 x 20 ($10^4 mm^3$)
- **Hemoglobin estimation.** Haemoblobinometer was used for hemoglobin estimation based on acid haematin method (SAHLI)

$$Hemoglobin = \frac{Value\ obtainedx\ 17.2\,mg/100ml}{100}$$

Packed Cell Volume

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

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

MCV (μ^3) = PCV x 10 Er

Mean corpuscular hemoglobin concentration (MCHC).

This was obtained using the formula $MCHC(\%) = \frac{Hb\ x\ 10^{2}}{PCV}$

Mean Corpuscular Hemoglobin (MCH) . This was expressed in picogrammes (ρg)

$$MCH(g) = \frac{H \times 0^{2}}{E}$$

- **Statistical analysis.** Data obtained from the experiment was expressed in mean ± 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 *C. 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.

Table 3: The proximate composition of experimental diets containing *C. albidum* seed meal fed to *C. gariepinus*

Parameter	D1	D2	D3	D4	D5
Moisture	9.24±0.11	9.20±0.03	9.16±0.13	9.12±0.10	9.23±0.06
Crude protein	40.23±0.05	40.20±0.08	40.25±0.15	40.23±0.06	40.20±0.12
Crude lipid	12.17±0.09	12.20±0.05	12.15±0.12	12.16±0.08	12.20±0.13
Crude fibre	4.59±0.45	4.15±0.11	4.16±0.10	4.16±0.05	4.13±0.05
Ash	4.48±0.06	4.60±0.45	4.50±0.32	4.33±0.40	4.37±0.31
Nfe	29.29±0.31	29.71±0.51	29.80±0.50	29.10±0.40	29.93±0.30

Row means without superscript are not significantly different ($p>0.05$) from one another.

- **Haematological profile.** Table 4 shows the haematological profile of *C. gariepinus* fed diet containing *C. albidum* seedmeal. The fish fed diet 1 had the highest PCV while the fish fed at diet3 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 diet2, diet3, diet4, diet5. 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 4: The haematological profile of *Clarias gariepinus* fed diet containing *Chrysophyllum albidum* seedmeal.

	D1	D2	D3	D4	D5
PCV	25.67 ± 3.21^{a}	12.00 ± 1.00^{b}	11.67 ± 0.58^{b}	12.67 ± 3.06^{b}	12.00 ± 1.00^{b}
HB	$8.60+1.13^{a}$	4.00 ± 0.20^{b}	$3.93+0.12^{b}$	4.23 ± 0.97^{b}	$4.00+0.20^{b}$
RBC	2.25 ± 0.28^{a}	1.05 ± 0.09^{b}	1.04 ± 0.05^{b}	1.11 ± 0.27^{b}	1.06 ± 0.11^{b}
WBC	142.93+6.22	140.57±8.26	177±61.82	190.23±78.30	133.83±12.91
Neutrophyl	64.33±4.04	60.00+5.00	65.67±11.60	70.33±10.50	31.70±2.90
Lymphocyte	35.67±4.07	40.00±5.00	34.33+11.59	29.67±10.50	31.67+2.89
MCV	99.10 ± 12.44^{a}	46.33 ± 3.85^{b}	45.03 ± 2.19^{b}	$48.90+11.82^{b}$	46.33 ± 3.85^{b}
MCH	30.77 ± 4.05^{a}	14.30 ± 0.70^{b}	13.97 ± 0.35^{b}	15.17 ± 3.48^{b}	14.30+0.70
MCHC	23.133 ± 2.84^{a}	10.80 ± 0.90^{b}	10.70 ± 0.46^{b}	$11.40+2.72^{b}$	10.73 ± 1.06^{b}

Rows means 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 *C. gariepinus* was met by the quantity provided in the diets. Uys and Hetch (1985) reported that the best growth rate and feed conversion efficiency in juvenile and sub-adult *C. gariepinus* are achieved with diets containing 38-42% crude protein and optimum liquid content of 10-11%.

The observed reduction in haematological parameters in *C. gariepinus* fed *C. albidum* meal in this study conform to the report of Tacon (1992) and Jimoh 2012that 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 *C. albidum* meal agreed with the observation of Blom *et al.* (2001); Dabrowski *et al.* (2001); Richard et al. (2003) and 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 $1 \times 10^{6}/mm^{3}$ 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 Clarke et al. (1979) and Erondu et al. (1993).

Increase in white blood cell as observed in the fish fed C. 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 C. gariepinus fed C. albidum seed meal replacing soybean meal

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