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Hematological Indices of *Clarias griepinus* (Burchell, 1882) Fingerlings Fed Diet Containing Graded Levels of Calabash (*Lagenaria vulgaries*) Seed Meal

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Abstract
The effect of graded levels of calabash *(Lagneria vulgaries*) seed meal (CSM) on hematological response of *Clarias gariepinus* juveniles was investigated. Five isonitrogeneous (45% crude protein level) diets at 0, 15, 30, 45 and 60% inclusion levels of CSM designed as diets I, II, III, IV and V were fed to 15 groups of *Clarias gariepinus* juveniles for 56 days. There was no clear trend in the Packed Cell Volume, Hemoglobin, Red Blood Cell and Mean Cell Volume of the fish fed the experimental diets. However, there was decreased in Parked Cell Volume (24.00±1.00%), Hemoglobin (8.27±0.06g/dl), Red Blood Cell ($2.09±0.0910^{12}$/ml) and Mean Cell Volume (114.52±6.08) with significant ($p<0.05$) difference between the treatments on the fish fed the highest inclusion level (60%) than the control. White Blood Cell ($2.09±0.1010^{9}$ml) and Mean Corpuscular Hemoglobin Concentration (34.48±1.23%) exhibit a significant ($p<0.05$) difference at higher inclusion level (60%) than the control and other dietary treatments. The study recommends that *Clarias gariepinus* fingerlings can be fed up to 29.69(60%) inclusion level of calabash seed meal with out negative effect on the health status of the fish.
Keywords: Calabash seed meal, *Clarias gariepinus* fingerlings*,* Heamatological parameters

Introduction

Fish feeds account for about 70% of aquaculture operations, and most fish farmers in Nigeria do not make use of standard fish feed due to high cost of production (Anderson *et al*.,1997; Eyo *et al*., 2004). Feed cost constitutes one of the major problems of aquaculture in Nigeria (Tobor, 1990; Eyo *et al*., 2004). However, with the advent of non- conventional feed resources found abundantly in the country, this problem can be addressed. Non-Conventional Feed Resources (NCFRs) are feeds that are not usually common in the market and are not the traditional ingredients for commercial fish feed production (Devendra, 1988; Madu *et al*., 2003) and these include host of plants and animals by-products.

Blood cells responses are important indicators of changes in the internal or external environment of fish, and these changes depend on fish species, age, sexual maturity and diseases (Golovina, 1996: Luskova, 1997). Blood is a good indicator to determine health condition of an organism (Joshi, *et al*., 2002). Calabash seed has been considered to have nutritional values containing about 35% crude protein and 42% lipids (Mercy *et al.,* 2005: Chinyere *et al*., 2009).

Plants used in livestock production as sources of plant protein may contain different kind of anti nutritional factor (Mercy *et al.,* 2005; Erickson *et al.*, 2005; Sotolu and Faturoti (2009). The use of calabash seed meal may be limited due to the presence of cucurbitacin, an anti – nutritional factor (Erickson *et al.,* 2005; Chinyere *et al*., 2009). Therefore, the study of hematological indices will be paramount in the overall assessment of the nutritional value of the plant.

Previous study has proved the efficiency of calabash seed meal on growth, and nutrients utilization of *Clarias gariepinus* fingerlings (Mamman, 2012). Sotolu and Faturoti (2009) fed *C. gariepinus Leucaena leucocephala* seed meal with different results on the hematological parameters of the fish. There was paucity of information on the effect of calabash seed meal on the hematological parameters of *C. gariepinus*, a cultivable omnivorous fish species that can utilize both animal and plant protein. The aim of this study therefore was to assess the effect of calabash seed meal on the hematological parameters of *C. gariepinus* fingerlings.

Materials and Methods

Experimental Site

The experiment was conducted at the Department of Forestry and Fisheries Hatchery, Usmanu Danfodiyo University, Sokoto, on latitude 13^{0} 07’ 78’’ N and longitude of o5’ 12’ 25’’ E at 275m above sea level Nigeria (Mamman, 2000), between September, 2011 to November,2011. Annual rainfall in the area ranged from 500 to 724 mm, and the mean relative humidity was 14.9% and 40% between March and June and 41^{0} C maximum (Mamman, 2000). The culture medium was fifteen (15) 40 liters round bottom plastic tanks, for the period of ten weeks (70 days). Twenty five liters level was maintained through the duration of the study, and the tanks were covered with mosquito net to prevent fishes from jumping.

Calabash Seed Collection and Oil extraction

Calabash (*Lagenaria vulgaries*) seed was bought from Gayari in Gummi Local Government Area of Zamfara State. They werer identified as Lagenaria vulvarie at the Botanical Unit of the Deparment of Biological Sceince, Usmanu DanfodiyoUniversity, Sokoto. The seed wee processed mechanically at Ummul Khairi Oil mill, Birnin Kebbi, Kebbi State oil mill processing plant for oil extraction and production of the meal. The process of calabash seed oil extraction was achieved by the used of an oil expeller which has a rotating screw inside a horizontal cylinder that is capped at one end. The oil extractor screws the seeds through the cylinder with gradual increasing pressure. The seeds were heated by friction and electric heaters, once the cap was removed the oil escapes from the cylinder through small holes and the press cake, or calabash seed meal emerged from the end of the cylinder. The procedure was as described in (Herz, 1997).

Diet Formulation

Five diets of 45% crude protein were formulated after the proximate composition of the running ingredients was determined (AOAC, 2000). Calabash seed meal was incorporated in the diets at varying substitution levels at 0 (control), 15, 30, 45 and 60% for substitution calabash seed meal for groundnut cake. The feed ingredients and the tested diet (calabash seed meal) were ground in powdery form by the aid of a grinding machine, and weight individually then properly mixed with an electric mixer with water to enhance gelatinization and palliating processes. The feeds were then later dried and parked in air tied container and stored under refrigeration before the commencement of the experiment.

Experimental Management

The fishes were fed the tested diets for ten (8) weeks at 3% body weight with the average initial weight of 2.32g in each plastic bowl. The daily ration was split in three times daily and fed at 9.00-, 10am 1.00-2.00pm and 5.00- 6.00pm respectively. Two hundred and sixty (260) fingerlings of *Clarias gariepinus* were bought from Barak fish farm, Sokoto. The fishes were distributed randomly in ffteen (15) 40 liters round bottom plastic tanks, with ten (10) fishes in each plastic bowl, after acclimatization for two weeks.

Blood Collection and Analysis

At the end of the experiment blood samples of 45 fish samples (three from each replicate in separate containers) from all the dietary treatments were collected by puncturing the caudal artery at the pedicle. Blood samples were collected by using micro-capillary and sampling tubes treated with anticoagulant. The blood samples collected were taken immediately to hematology Laboratory at the Faculty of Vetenary Medicine, Usmanu Danfodiyo University, Sokoto. Hemoglobin concentration was estimated by cyenomethemoglobin and White Blood Cells (WBL) were counted by Neubaurs improved hemocytometer using Hyem's and Turk,s solution as diluting fluid as described in (Stoskopf, i993). Red Blood Cell (RBC) and Packed Cell Volume (PCV) were estimated as in Blaxhall and Daisley (1973). Mean Cell Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentrate (MCHC), and Mean Cell Volume (MCV) were calculated using standard formulae as described in Dacie and Lewis (1991), Joshi *et al*. (2000).

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) and, the treatment means were separated for significant ($p<0.05$) differences following the procedure of Duncan Multiple Range Test (Steel and Torrie, 1980) .All the analyses were carried out using the computer soft ware Statistical Package for the Social Sciences (SPSS) version 9.0 windows (SPSS, 2007).

Table 1: Gross composition of experimental diets (%)

	Diets				
Ingredients	I (0%)	II (15%)	III (30%)	IV (45%)	V (60%)
Maize	14.82	13.47	12.72	12.48	11.74
Fish meal	19.80	20.07	20.44	20.07	21.03
Groundnut cake	49.49	42.74	35.77	28.82	21.03
Calabash seed cake	-	7.42	14.85	22.27	29.69
Blood meal	9.89	10.3	10.22	10.36	10.51
Palm Oil	2.5	2.5	2.5	2.5	2.5
Mineral premix	0.5	0.5	0.5	0.5	0.5
Vitamin premix	0.5	0.5	0.5	0.5	0.5
Bone meal	2.25	2.25	2.25	2.25	2.25
Salt	0.25	0.25	0.25	0.25	0.25
TOTAL	**100**	**100**	**100**	**100**	**100**
Calculated Crude Protein %	45.00	45.00	45.00	45.00	45.00
Calculated Metabolizable Energy(Kcal/kg)	3446.1	3587.1	3737.4	3945.4	4052.9

Results

Table 2, shows the hematological parameters of the experimental fish after the feeding trial. The highest value (28.33±0.58) of Packed Cell Volume was recorded in fish fed diet I containing 0.00% CSM and was significantly ($p>0.05$) different from those fed the other dietary treatments. Fish fed diet containing 29.69 (60%) CSM recorded the lowest PCV of 24.00±1.00 with significantly ($P < 0.05$) lower value than those fed diets containing varying levels of CSM. Fish fed diet IV containing 22.27 (45% %) CSM recorded the highest hemoglobin value of 9.37±0.15 but not significantly higher than those fed diets containing 7.42 (15%) CSM and 22.27(45%) CSM and those fed the control diet. Fish fed diets containing 22.27 (45%) CSM recorded the highest Red Blood Cell value of 2.59 ± 0.31 but was not significant ($P > 0.05$) different from those fed the control. Fish fed diet 7.42 (15%) CSM had the lowest values for RBC (2.02±0.03) and was not significantly ($p > 0.05$) different from those fed diet containing 14.85 (30%) and 29.69 (60%) CSM but significantly ($P > 0.05$) from those fed he control. The values for White Blood Cell (2.09 ± 0.10) which was significantly ($p > 0.05$) higher in fish fed diet containing the highest level of CSM than those fed the other diets, the Mean Corpuscular Hemoglobin Concentration follows the same trend. The highest Mean Cell Hemoglobin values of 46.7±2.75 was recorded in fish fed diet containing 7.42 (15%) CSM but was significantly ($P > 0.05$) different from the control and other dietary treatments, the lowest value of 36.73± 0.35 was recorded in those fed diet containing 22.27 (45%) CSM. Fish fed diet II containing 7.42 (15%) CSM recorded the highest MCV (159.85±12.04) which was significantly ($P < 0.05$) higher than those fed the other diets.

Discussion

The results of the Packed Cell Volume (PCV), Hemoglobin (Hb), Red Blood Cell (RBC), and White Blood Cell (WBC) are presented in Table 2. The values are within the acceptable range, indicating that calabash seed meal is not toxic to *Clarias gariepinus*, and are safe to feed up to 29.69 (60%) CSM level of inclusion. These values were higher than those reported by Omitoyin (2006) when the same fish species was fed with poultry litter. However fish fed diet containing 22.27 (45%) CSM have highest Packed Cell Volume and Hemoglobin and were significantly different from those of dietary treatment III. The dietary treatment V recorded the highest WBC and MCHC than the fish fed diets containing other dietary treatments including the control. The initials values of PCV, RBC and MCV were similar to those reported when *C. gariepinus* were fed with different non conventional feeds (Oyelese *et al*., 1989; Omoniye *et al*., 2002; Sotulo and Faturori, 2009; Ayoola, 2011 ; Ayoola and Maduekwe, 2012).

The Red Blood Cell (RBC) and Hemoglobin (HB) are highest in fish fed diet IV 22.27 (45%) CSM level of inclusion, but there were no significant different from that of the control diet. The increase in hematological parameters such as Hb and RBC in fish fed diet containing 22.27 (45%) CSM and other dietary treatments diets was an indication that blood was not lost in fish fed those diets compared with what was reported when *Leucaena leucocephala* was fed to *Clarias gariepinus* (Sotulo,and Faturori, 2009). The values obtained although there was significant differences of both control and tested diets, were within the range of healthy juvenile's cat fish (Oyelese *et al.*, 1999; Omoniye *et al.,* 2002).

The highest white blood cell WBC recorded in fish fed diet V containing 29.69% CSM could be attributed to increased production of leucocytes in the hematopoietic tissue of the kidney and perhaps the spleen (Omoniye, 2002; Ayoola, 2011). Lymphocytes are the most numerous cells comprising the leucocytes which function in the production of antibodies and chemical substances serving as defensives against infection (Golovina, 1996; Joshi *et al.,* 2000). Therefore, with regard to the findings of the present study, Increase in Red Blood Cell (RBC) and Hemoglobin (HB) as recorded in fish fed diet IV dffers from values obtained when fed the same fish with poultry litter (Omitoyin, 2006). The study concluded that, *Clarias gariepinus* fingerlings can be fed up to 29.69 (60%) inclusion level of calabash seed meal with out negative effect on the health status of the fish.

Table 2: Hematological parameters of *Clarias gariepinus* fed diets containing varying level of calabash seed meal

Parameters		Diet			
	I (0%)	II (15%)	III (30%)	IV (45%)	V (60%)
PCV (%)	28.33 ± 0.58^{a}	26.00 ± 0.58^{c}	26.00 ± 0.12^{c}	27.33^{c} ± 0.58^{a}	24.00 ±1.00^{d}
HB (g/dl)	9.27 ± 0.31^{a}	9.30 ± 0.10^{a}	8.43 ± 0.12^{b}	9.37 ± 0.15^{a}	8.27 ± 0.06^{b}
RBC (10^{12}/ml)	2.29 ± 0.31^{a}	2.02 ± 0.03^{c}	2.11 ± 0.02^{c}	2.59 ± 0.31^{a}	2.09 ± 0.09^{c}
WBC (10^{9}/ml)	1.34 ± 0.80^{d}	1.12 ± 0.02^{c}	1.82 ±0.02^{c}	1.38 ± 0.04^{d}	2.09 ± 0.10^{a}
MCHC (%)	32.70 ± 0.50^{b}	32.45 ± 0.39^{b}	32.44 ± 0.45^{b}	32.16 ± 0.21^{b}	34.48 ± 1.23^{a}
MCH (pg)	39.71 ± 4.60^{b}	46.71 ± 2.75^{a}	39.84 ± 0.28^{b}	36.73 ± 0.35^{b}	39.45 ± 1.75^{b}
MCV (fl)	122.85 ± 9.63^{bc}	159.85 ±12.04^{a}	130.86 ±0.60^{b}	133.39 ±1.05^{b}	114.52 ±6.08^{c}

Means in rows with the same letters are not significantly different ($P > 0.05$)

PCV - Packed Cell Volume
HB - Hemoglobin
RBC - Red Blood Cell
WBC - White Blood Cell
MCHC - Mean Corpuscular Hemoglobin Concentration
MCH - Mean Cell Hemoglobin
MCV - Mean Cell Volume

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