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Full Length Research

Haematological responses of replacement levels of fish meal with chicken offal in catfish (*Clarias gariepinus)* diets

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ABSTRACT: Fish demand by consumers keeps increasing, yet constraints to the production of fish are encountered, the commonest of which is the high cost and difficulty in acquiring fish feeds in Nigeria. The objective of this study was to determine the effect of the formulated test diets of 40% protein concentrate using varying levels of chicken offal diets on the haematological parameters of the African Catfish (*Clarias gariepinus)*. The chicken offal was used to replace fish meal at inclusion levels of 0% (control, treatment A), 25% (treatment B), 50% (treatment C), 75% (treatment D) and 100% (treatment E) respectively. Three hundred (300) fingerlings of average length, 8.12±0.48 cm, and average weight, 3.68±0.00 g, were stocked in concrete tanks. Fish were fed at 5 per cent body weight for 8 months. Physicochemical parameters including; water temperature (°C), pH, dissolved oxygen and ammonia were monitored every four days and maintained within acceptable ranges for fish culture. Data obtained were analysed using analysis of variance (ANOVA). Results showed haemoglobin (Hb), Packed cell volume (PCV), Mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) to be statistically different (ANOVA, $p<0.05$) between the treatments, although no significant difference (ANOVA, $p>0.05$) was observed in white blood cell (WBC), Mean Corpuscular haemoglobin (MCH) and erythrocyte sedimentation rate (ESR). Haematological parameters results were within the ranges for fish well-being. Chicken offal were suitable as partial replacement at (25, 50, 75 and 100%) for fish meal, it enhanced the hematological performance of *C. gariepinus* due to its mineral components.

Keywords: Chicken offal, *Clarias gariepinus,* fishmeal, growth, haematological parameters.

INTRODUCTION

Fish has long been a vital source of income and food for many people in developing countries because it is arguably the cheapest source of protein, certain minerals, vitamins and beneficial lipids as well as source of employment opportunities in these parts of the world. In many of the different ethnic groups and beliefs in Nigeria, most especially in the Southern part of Nigeria, a meal is never whole without some form of fish or fish's product (FAO, 2006, FAO, 2014, Edeghe and Ajah, 2018).

The experimental fish used was the African catfish (*Clarias gariepinus*). It is unarguably the most prominently farmed fish in Cross River State and Nigeria as a whole (Sogbesan and Ugwumba, 2006). This species has drawn attention of fish farmers because of its biological attributes that include faster growth rate, resistance to disease, ability to withstand low oxygen and pH in aquaculture systems. *C. gariepinus* grow on wide range of low cost artificial feeds and possibility of high stocking density (Saad *et al*., 2009).

Considering the rapidly growing market for fish and fishery products in Nigeria due to the high demand for fish protein resulting from increased human population density, aquaculture remains the most dependable, and the easiest way to ensure a sustainable supply of fish (Abareethan and Amsath, 2015). In aquaculture, the use of chicken offal as a source of animal protein to complement fishmeal and other livestock farming practices such as poultry, piggery, and fish farming is a good alternative as documented by

Ayim, *et al.* (2018).

Fish haematological studies according to Snieszko, (1960), Svobodova *et al.* (1991), Fagbenro *et al.* (2013), Adebayo and Daramola (2013) and Okorie-kanu *et al.* (2014) serves mainly for diagnostic purpose, Aside, this main purpose, they also noted that it can be used to appraise the suitability of feeds and to examine the effects of stress situation, owing to the fact that there can be wide variation in the quality of diet received by farmed fish. Certain factors such as availability of suitable ingredients, poor formulation and processing, lack of knowledge and understanding of dietary requirement could subject fish to some level of stress, and this can be monitored via studying certain haematological parameters.

The blueprint of this study was to appraise the suitability of the experimental feeds on the haematological parameters of the fish. Chicken offal is commonly available in the markets (chicken slaughterhouses) but are not commonly used as animal protein source in the formulation of fish feeds locally, despite their high protein content and low cost. Studies have been carried out to determine the growth performance of *C. gariepinus* fed with chicken offal diets (Ayim, *et al*., 2018). There is a need to determine the health effect of this proposed feed resource on the fish. Hence, this study aims to determine and compare the haematological indices of *C. gariepinus* fed with the test ingredient (chicken offal).

MATERIALS AND METHODS

Study area and human activities

This research work was carried out at Andem and Sons Fish Farm Limited (latitude 4.9340699, 4°56' 2.6514''N and longitude 8.3282381, 8°19' 41.65716°E) and at Planet fish farm limited (latitude 4.91726, 4°55'2.142''N and longitude 8.32593, 8°19'33.342''E), in Calabar South Local Government Area, Cross River State, Nigeria.

Study duration, procurement of fingerlings, source and processing of ingredients

This study was conducted for a period of thirty-two weeks. Fish fingerlings were purchased from the Institute of Oceanography Hatchery Complex, University of Calabar and transported to Andem and Sons fish farm in a 20 liter water storage plastic can, which was cut open at the top for free flow of oxygen. The feed ingredients [fish meal (FM), wheat offal, soybean meal (SBM), bone meal, lysine, methionine, wheat flour, vitamin premix, sodium chloride (NaCl), and Chicken offal (CO)] for this research were bought from aquaculture feed stores and other reputable stores in the experimental environ. Chicken offal was purchased from chicken slaughterhouses at the Market in Calabar, Cross River State, Nigeria. The freshly collected offal was thoroughly washed in water and par boiled for 20 minutes. It was allowed to cool before being oven-dried until it was required. The dried chicken offal was then weighed and ground to powder using an electrically driven grinder (Mill Grinding Machine-GX200-6.5HP, Nigeria).

Formulation and preparation of experimental diets

A control diet of fish meal, with fish meal serving as the only protein source was formulated. Five isonitrogenous test diets were formulated using Pearson square method to 40% crude protein level at Planet Fish Farm Anantigha. The five experimental diets were formulated such that chicken offal replaced fish meal in the diet by 0% (control), 25%, 50%, 75 % and 100 % inclusion levels of Chicken offal. The five experimental feed were labelled: Feed A (0 per cent CO), Feed B (25 per cent CO), Feed C (50 per cent CO), Feed D (75 per cent CO) and Feed E (100 per cent CO) (Table 1).

Culture condition

This study was carried out intensively using 15 square shaped concrete tanks of equal size measuring 120 by 90 by 120 cm. It consisted of five treatments of three replicates and 20 fish per replicate. Treatment 1, served as the control with the test organisms fed with a diet formulated without chicken offal, while the other sets of fish were fed with feeds formulated with local ingredients using chicken offal at varying levels, formed treatments 2, 3, 4 and 5 as described above. To aid replication of treatments, the tanks were labeled A1, A2, A3, B1, B2, B3, C1, C2, C3, D1, D2, D3, E1, E2 and E3. A total of 300 *C. gariepinus* fingerlings of 6 weeks old, were purchased from the University of Calabar fish farm and stocked in each of the 15 experimental units (20 fish per unit). Prior to the start of the feeding trials, the stocked fish were acclimated for fourteen days. The fish were fed twice daily to satiation during the acclimation time. The acclimated fish were fasted for 24 hours before the feeding trial began, during which the average initial wet body weight of the fish in each experimental unit was measured (METLAR MT-5000D electronic balance) to the nearest gram (Eyo and Ekanem, 2011).

Biweekly measurements of body parameters including total length (TL) and total weight (TW) were taken. Total length was measured using a measuring board from the snout to the base of the caudal fin rays to the nearest 0.1 cm, and fish bulk weight was measured (Metlar-5000D electronic weighing balance) to the nearest 0.1 g.

Feeding of experimental fish

The experimental fish were fed to satiation at the rate of

Table 1. Composition of experimental diets in percentages and in Grams Per Kilograms.

Ingredients	Diet A (0%)		Diet B (25%)		Diet C (50%)		Diet D (75%)		Diet E (100%)	
	Amount in g/kg	(%)	Amount in g/kg	(%)	Amount in g/kg	(%)	Amount in g/kg	(%)	Amount in g/kg	(%)
Chicken Offal (CO)	-	-	136	13.61	272	27.22	408	40.83	302	30.17
Fish meal (FM)	283	28.3	204	20.41	136	13.61	68	6.80	-	-
Soyabeans Meal (SBM)	283	28.3	204	20.41	136	13.61	68	6.80	302	30.17
Wheat Offal (WO)	192	19.9	203	20.27	203	20.27	203	20.27	173	17.32
Wheat Flour (WF)	192	19.9	203	20.27	203	20.27	203	20.27	173	17.32
Lysine	6	0.50	6	0.50	6	0.50	6	0.50	6	0.50
Methionine	6	0.50	6	0.50	6	0.50	6	0.50	6	0.50
Vitamin Premix	20	1.50	20	1.50	20	1.50	20	1.50	20	1.50
Bone ash/Calcium	8	0.50	8	0.50	8	0.50	8	0.50	8	0.50
NaCl	5	0.25	5	0.25	5	0.25	5	0.25	5	0.25
Palm Oil	5	0.35	5	0.35	5	0.35	5	0.35	5	0.35
Total		100		100		100		100		100

Experimental ingredients in g/kg and their per cent inclusion levels.

5% of their body weight, twice a day. The quantity of feed per day was determined using the formula:

$$\mathrm{F} = \mathrm{kg/tank\ /day} = \frac{\mathrm{W\ x\ S\ x\ P}}{1000\ X\ 100}$$

Where, F = weight of feed to be applied per tank daily, W = Average weight of fish obtained by random sampling, S = Stocking density (Total number of fish stock per tank), P = Percentage of body weight (5 %).

The total quantity of feed per tank was determined by multiplying the quantity of feed/day by the number of days fish were fed that quantity and the result was added up, (quantity of feed changed every 14 days as the fish in each tank attained new body weights). Initial length (L) and weight (g) measurements were taken before they were introduced into the culture tanks. The weight was determined by means of a weighing scale while the length was by means of a measuring board that has a total length of 1 m and whose surface was covered with a white formica.

Proximate analysis of experimental diets

Proximate analysis of the test diets, was performed according to AOAC (2000), in the Faculty of Agriculture Central Laboratory, University of Calabar, Calabar. The moisture content, crude protein, lipids content, ash and carbohydrate contents were analyzed following AOAC (2000).

Haematological test procedures

Blood samples were collected from the experimental fish at the end of the feeding trial, six (6) randomly selected catfish per treatment were respectively sacrificed and their blood collected using sample bottles containing ethylenediamine tetracetic acid (EDTA) anticoagulant. The stored blood samples were taken to the laboratory of the Federal Neuropsychiatric Hospital, Calabar, Cross River State, Nigeria for analyses.

Standard haematological methods described by Blaxhall and Daisly (1973), Svobodova *et al*. (1991) and Fagbenro *et al*. (2013) was applied to determine Packed cell volume (PCV), Red Blood Cell (RBC) and Erythrocyte sedimentation rate (ESR). Using a Microhaema-tocrit Centrifuge (Hermle model Z320), blood-filled heparinized microhaematocrit capillary tubes were centrifuged at 12000 for 5 minutes and the haematocrit (Hct) values were read directly. The cyan-methaemoglobin method (Svobodova *et al*., 1991) was used to determine the haemoglobin concentration at a wavelength of 540 nm. Simultaneously,

Table 2. Mean haematological parameters of *Clarias gariepinus* fed experimental diets.

Parameters	Diet A (0%)	Diet B (25%)	Diet C (50%)	Diet D (75%)	Diet E (100%)	P-values
Haemoglobin (Hb-g/l)	10.16±0.12^{a}	10.35±0.10^{ab}	10.44±0.23^{ab}	10.76±0.15^{b}	9.62±0.24^{a}	0.003
PCV (%)	34.92±1.94	32.79±0.24	31.69±2.08	33.20±0.50	36.21±0.60	0.155
White Blood Cell Counts (WBC X 10^3)	37.57±1.98	41.26±0.60	33.79±4.51	35.67±5.98	48.39±3.44	0.087
Red Blood Cell Counts (RBC X 10^6)	2.37±0.07^{a}	3.33±0.12^{b}	3.29±0.11^{b}	3.41±0.20^{b}	2.35±0.09^{a}	0.00
Mean Corpuscular Haemoglobin(MCH Pg cell^{-1})	35.79±1.52	36.54±33.20	28.40±1.21	31.18±1.40	36.12±0.54	0.544
Mean Corpuscular Haemoglobin Concentration (MCHC)	25.10±0.13^{a}	26.82±1.36^{a}	32.16±1.03^{b}	31.17±1.48^{b}	27.94±1.93^{ab}	0.004
MCV	151.37±3.08^{a}	154.92±1.07^{a}	139.45±2.07^{b}	146.72±2.98^{ab}	155.66±2.24^{a}	0.000
ESR	4.38±0.11	4.31±0.13	4.34±0.09	4.27±0.13	4.25±0.11	0.941

Means with the same superscript along the same row are not significantly different p>0.05) Legend PCV = Packed cell volume Hb = Haemoglobin RBC = Red Blood Cell WBC = White Blood Cell MCH = Mean corpuscular Haemoglobin MCV = Mean corpuscular volume MCHC = Mean corpuscular Haemoglobin concentration ESR=Mean erythrocyte sedimentation rate.

the Total Red Blood Cell (RBC) was determined using the Dacie and Lewis methods (1984). The Neubauer haematocytometer was used to count red and white blood cells under a light microscope. The following equations were used to calculate the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC):

$$\text{MCHC} = \frac{\text{Hb}}{PCV}\ x\ 100$$

$$\text{MCH} = \frac{\text{Hb}}{RBCC}\ x\ 102$$

$$\text{MCV} = \frac{\text{PCV}}{RBCC}\ x\ 100$$ (Wickham *et al*., 1990).

Statistical analysis

Data obtained from this study were subjected to one way analysis of variance (ANOVA) using SPSS 16.0 version to check for significant difference and the results were expressed in Mean ± SD.

RESULTS

Mean haematological parameters of *Clarias gariepinus* fed experimental diets

The haemoglobin (Hb) values throughout the five treatments in this study were substantially different (p<0.05), according to the haematological results (Table 2), the values varied from 9.62±0.24 to 10.76±0.15. White blood cells (WBC), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), and erythrocyte sedimentation rate (ESR) values were not significantly different across treatments (p>0.05). Red Blood Cell (RBC) values for treatments A, B, C, D, and E revealed a significant difference (p<0.05) across treatments, with treatment D having the greatest value (3.41±0.20) and treatment E obtaining the lowest value (2.35±0.09). A significant difference (p<0.05) between the treatments was also seen in the mean corpuscular haemoglobin concentration (MCHC). The values observed for mean corpuscular volume (MCV) also varied substantially (p<0.05) among treatments, with treatment E having the highest MCV (155.66±2.24) and treatment A recorded the lowest MCV (139.45±2.07) (Table 2).

DISCUSSION

Studies on haematological indices are helpful for checking the suitability of feeds, especially when it comes to feed components that have an impact on blood formation. The hematological indices reported in this study include; Hb, PCV, WBC, RBC, MCH, MCHC, MCV, and ESR. The results of the haematological analysis of this study varied slightly with the findings of Erhunmwunse and Ainerua (2013), who worked on characterization of some blood parameters of African catfish (RBC-2.88±0.7011, WBC - 41.1±11.048, PCV - 36.00±9.036, Haemoglobin - 6.044±25.572, MCV - 113±15.635, MCH - 23.381±6.238, MCHC - 26.881±30.332) but were all within the reference ranges reported by Owolabi (2011) in upside-down catfish (*Synodontis membranacea*) from Jebba Lake, Nigeria (PCV - 22.00 - 38.00%, HBC - 5.30 - 12.06 g/dl, RBC - 1.20 - 8.50 106/µl, MCV – 68.99 - 156.33 fl, MCH - 9.26 - 64.41 pg, MCHC - 6.94 - 34.17 g/dl). The findings of this study indicates that

the formulated diets were suitable for the experimental fish as reflected in their growth and good health. Though, no significant difference (ANOVA, p>0.05) was observed in PCV, WBC, MCH and ESR, the inclusion level of chicken offal may have had an impact on how suitable the diets were. This may be due to the substantial amount of offal present, which contained several advantageous minerals capable of raising the fish's blood level (Ayim *et al.*, 2018). The results of this study are consistent with that of Lawal *et al*. (2019) who observed similar leanings in terms of values to be within normal ranges for fish haematology. However, the present findings differ from those of Fagbenro *et al*. (2013) who research on the haematological changes of *C. gariepinus* fed diets containing raw sunflower and sesame seed meal instead of soya beans meal, where they found a statistical difference in the test fishes' hemological indices. Fish from all of the treatments fared well in the current investigations, though, some slight variations in the haematological indices were recorded. Haematological indicators, particularly PCV were observed to be normal and did not vary significantly between the treatments, and according to Hrubec *et al*. (2000), PCV can be used to assess the fish health state. Similarly, most fish blood characteristics have been studied to determine a standard value range, and deviation from it can suggest a disturbance in the fish physiological process (Rainza-Paiva *et al*., 2000; Joshi *et al*., 2002). The increased number of White Blood Cells (WBC) in the experimental fish fed varying amounts of chicken offal led to the experimental fish higher survival rate. This also improved the feed iron content, which is a major source of haemoglobin (Hb) in fish in the diet. The immunity of the experimental fish was high as reflected in the haematological indices. This result indicates high oxygen absorption and transportation capacity of the cells of the fish under study and is in line with the observation of Owolabi (2011), Okorie-kanu *et al.* (2014), Ayim *et al.* (2018) and Lawal *et al*. (2019).

Conclusion

Based on the results of this study, it can be established that the haematological parameter of *Clarias gariepinus* fed with the experimental diets was not negatively altered with varying dietary inclusion rate of chicken offal, the diets were suitable for fish. The fish were even observed to be better when fed at higher level of inclusion.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

Abareethan, M., & Amsath, A. (2015). Characterization and evaluation of probiotic fish feed. *International Journal of Pure and Applied Zoology*, *3*(2), 148-153.

Adebayo, O. O., & Daramola, O. A. (2013). Economic analysis of catfish (*Clarias gariepinus*) production in Ibadan metropolis. *Discourse Journal of Agriculture and food sciences*, *1*(7), 128-134.

AOAC (2000). Association of Official Analytical Chemist Official Methods of analysis. 15th edition, Washington, DC.

Ayim, E. M., Ivon, E. A., Ajang, R. O., & Joseph, A. P. (2018). Comparative assessment of growth performance and nutrients utilization of African catfish (Clarias gariepinus, Burchell 1882) fed chicken offal and shrimp-based diets. *Annual Research and Review in Biology*, 30(5), Article number 201.

Blaxhall, P. C., & Daisley, K. W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, *5*(6), 771-781.

Dacie, J. V., & Lewis, S. N., (1984). Practical haematology. 6th Edition. Edinburg, Churchill Livingstone.

Edeghe, A., & Ajah, P. (2018). Effect of feed and stocking density on the growth performance of Clarias gariepinus (Burchell, 1822) reared intensively in fiberglass and concrete ponds in Calabar. *South Eastern Nigeria*. *Global Scientific Journals,* 6(9), 589-612.

Erhunmwunse, N. O., & Ainerua, M. O. (2013). Characterization of some blood parameters of African Catfish (*Clarias gariepinus*). *American-Eurasian Journal of Toxicological Sciences*, 5(3), 72-76.

Eyo, V. O., & Ekanem, A. P. (2011). Effect of feeding frequency on the growth, food utilization and survival of African catfish (*Clarias gariepinus*) using locally formulated diet. *African Journal of Environmental Pollution and Health*, *9*(2), 11-17.

Fagbenro, O.A., Adeparusi, E.O., & Jimoh, W. A., (2013). Haematological profile of blood of African catfish (*Clarias gariepinus,* Burchell 1822) fed sunflower and sesame meal based diets. *Journal of Fisheries and Aquatic Sciences*, 8(1), 80-86.

FAO (2006). *The state of world fisheries and aquaculture*. FAO Fisheries Technical Paper. No. 500. Rome, Italy. Retrieved from http://www.fao.org.docrep/fao.

FAO (2014). The State of World Fisheries and Aquaculture. Retrieved from http://www.fao.org/3/a-i3720e.pdf.

Hrubec, T. C., Cardinale, J. L., & Smith, S. A. (2000). Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis hybrid*). *Veterinary clinical pathology*, *29*(1), 7-12.

Joshi, P. K., Bose, M., & Harish, D. (2002). Changes in certain haematological parameters in a siluroid cat fish *Clarias batrachus* (Linn) exposed to cadmium chloride. *Pollution Research*, *21*(2), 129-131.

Lawal, M. O., Lawal, A. Z., Adewumi, G. A., & Mudiaga, A. (2019). Growth, nutrient utilization, haematology and

biochemical parameters of African catfish (*Clarias gariepinus*, Burchell, 1822) fed with varying levels of Bacillus subtilis. *Agrosearch*, *19*(1), 13-27.

Okorie-Kanu, C. O., & Unakalamba, N. J. (2014). Haematological and blood biochemistry values of cultured *Heterobranchus longifilis* in Umudike, Abia state, Nigeria. *Animal Research International*, *11*(2), 1987-1993.

Owolabi, O. D. (2011). Haematological and serum biochemical profile of the upside-down catfish, Synodontis membranacea Geoffroy Saint Hilaire from Jebba Lake, Nigeria. *Comparative Clinical Pathology*, *20*, 163-172.

Saad, Y. M., Hanafi, M. S., Essa, M. A., Guerges, A. A., & Ali, S. F. (2009). Genetic signatures of some Egyptian Clarias gariepinus populations. *Global Veterinaria*, *3*(6), 503-508.

Snieszko, S. F. (1960). Microhaematocrit as a tool in fisheries management. Special scientific report –fisheries, No. 314. U. S Department International Fish and Fisheries Wildlife special Science Report. p.15.

Sogbesan, O. A., & Ugwumba, A. A. (2006). Bionomics evaluation of garden snail (*Limicolaria aurora*, Jay, 1937; Gastropoda: Limicolaria) meat meal in the diet of *Clarias gariepinus* fingerlings (Burchell, 1822). *Nigerian Journal of Fisheries*, *2*(3), 358-371.

Svobodova, Z., Pravda D., & Palackova J., (1991). Unified methods of haematological examination of Fish. Research Institute of Fish Culture and Hydrobiology, Vodnany, Czechoslivakia. Pp. 31-33.

Wickham, L. L., Costa, D. P., & Elsner, R. (1990). Blood rheology of captive and free-ranging northern elephant seals and sea otters. *Canadian Journal of Zoology*, 68(2), 375-380.