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Effect of Fish Gro® on Haematology of *Clarias gariepinus* Fingerlings

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Abstract: The present study evaluates the effect of Fish Gro® a commercial growth booster on the haematological parameters of *Clarias gariepinus* fingerlings. Fish Gro® was included in 35% crude protein basal diet at inclusion levels of 0.0% (control), 0.5% (DT_1), 1.0% (DT_2), 1.5% (DT_3) and 2.0% (DT_4). The test diets were applied in duplicates in 70 L plastic bowls; each was stocked randomly with 20 *Clarias gariepinus* fingerlings with average initial body weight of 13.26±0.20 g. The experiment lasted for 56 days. Haematological parameters of fish fed experimental diets showed significant difference ($p<0.05$). White Blood Cells (WBC) ranged from 184.00×10^3 μL^{-1} (DT_3)-209.70×10^3 μL^{-1} (control), Red Blood Cells (RBC) from 1.71×10^6 μL^{-1} (DT_3)-2.16×10^6 μL^{-1} (control). Hemoglobin (HGB) ranged from 7.50 g dL^{-1} (DT_3)-9.10 g dL^{-1} (DT_1), Haematocrit (HCT) from 24.10% (DT_3)-32.40% (control) and Mean Corpuscular Volume (MCV) ranged from 140.90 μm (DT_3)-162.60 μm (DT_2). Mean Corpuscular Hemoglobin (MCH) had the highest value recorded in DT_2 (48.00 pg) and lowest value recorded in the control (39.8 pg), Mean Corpuscular Hemoglobin Concentration (MCHC) ranged from 26.5 g dL^{-1} in the control to 31.10 g dL^{-1} in DT_3. Platelets count (PLT) ranged from 4.00×10^3 μL^{-1} (DT_2)-29.00×10^3 μL^{-1} (control).

INTRODUCTION

Diet supplementation is an important aspect in aquaculture management especially in intensive and semi-intensive fish culture, and is promising for increasing fish production. According to FAO (2007), feed accounts for up to 70% of the variable cost of commercial aquaculture operation for many fish species. This cost depends on several factors such as protein level, the source and type of ingredients that could be derived from plant or animal resources, and manufacture practices.

Fish nutrition requires high quality feeds, which should contain not only necessary nutrients but also complementary feed additives to keep organisms healthy, enhance fast growth and environment-friendly aquaculture. Some of the utilized growth-promoting feed additives include hormones, antibiotics, ionospheres and some salts (Fuller, 1992; Klaenhammer and Kullen, 1999).

Knowledge of haematological characteristics is used as an effective and sensitive index for monitoring physiological and pathological changes in fishes (Zhou *et al*., 2009). The analyses of blood indices have

proven to be valuable approach for analyzing the health status of farmed animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status (Bahmani *et al*., 2001). This study seeks to investigate the haematological changes that occur in *Clarias gariepinus* fed diets supplemented with Fish Gro® booster.

MATERIALS AND METHODS

Study area: Feeding of fish with experimental diets was carried out at the hatchery unit, Department of Fisheries, Ministry of Agriculture and Natural Resources, Makurdi, Benue State, Nigeria. Haematological analyses were carried out at the medical laboratory of the Federal Medical Centre, Makurdi, Benue State, Nigeria.

Formulation of experimental diets: Commercial feed additive (Fish Gro®) were obtained from fish feed store in Makurdi, Nigeria. The feed additive were mixed separately at rates of 0% (control), 0.5, 1.0, 1.5 and 2.0% with a basal feed (35% C.P.) comprising standard amounts of fish meal, yellow maize, soybean meal, rice bran, vegetable oil, vitamin premix, mineral premix, salt and starch formulated using pearson square method (Table 1).

All dietary ingredients were milled to fine particle size. The ground feedstuffs were thoroughly mixed, and wet mixed using hot water (60°C) to form dough then pelleted using an electric motor pelleter. The dough was then forced through a dice of 3mm dimension, the pellets collected in tray, sun dried, packaged and stored until required to be used.

Proximate analyses for experimental diets was carried out to determine moisture content, crude protein content, lipid content, ash content, crude fibre content and nitrogen free extract using AOAC (2000) standard method.

Experimental fish and management: Three hundred fingerlings of *Clarias gariepinus* were obtained from Aqua Haven Farms in Makurdi and acclimatized in indoor tanks for 2 weeks. They were then distributed into plastic bowls of 70 L each at the rate of 20 fingerlings (mean weight 13.25±0.20 g) per bowl. The dietary treatments for each inclusion level (0.5% (DT_1), 1.0% (DT_2), 1.5% (DT_3) and 2.0% (DT_4) as well as 0.0% (the control) were used in duplicates.

Table 1: Composition of the experimental diets in parentage

		Fish		Gro®	
Ingredients	Control	TD_1	TD_2	TD_3	TD_4
Fish meal	20.00	20.00	20.00	20.00	20.00
Yellow maize	19.63	19.63	19.63	19.63	19.63
Soybean meal	36.24	36.24	36.24	36.24	36.24
Rice bran	19.63	19.63	19.63	19.63	19.63
Veg. oil	0.50	0.50	0.50	0.5	0.50
Vit. premix	1.50	1.50	1.50	1.50	1.50
Min. premix	1.50	1.50	1.50	1.50	1.50
Starch	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Fish Gro®	0.00	0.50	1.00	1.50	2.00

The total weight of the fish in each bowl was taken prior to commencement of feeding. Fishes were fed 5% of body weight twice daily at 09.00 h and at 16.00 h. All the fish were weighed and counted weekly and feeding rates adjusted accordingly. Water quality parameters such as temperature, pH and Dissolved Oxygen (DO) were determined using standard methods (APHA *et al*., 1988).

Haematological analyses of blood from fish samples: At the end of eight weeks feeding with the experimental diets, The Red Blood Cells (erythrocyte) count (RBC), White Blood Cell (leucocyte) count (WBC), platelet (thromobocyte) count (PLT) and Hemoglobin cotent (HGB) in all the treatments were estimated following the methods of Blaxhall and Daisley (1973). Thus 1.0 mL of blood was drawn from the caudal blood vessels of a specimen from each treatment using 2 mL disposable syringe and immediately transferred into 5 mL test tube containing 0.1 g of Ethylene-Diamine-Tetracetic Acid (EDTA) as anticoagulant. By the use of a hemoglobin pipette, 0.02 mL blood was drawn from each extracted blood sample into 5.0 mL of formol citrate (sodium citrate, 3.0 g; formaldehyde, 1.0 mL and distilled water 100 mL) in a bijou bottle to give a dilution ratio of 1:250. A few drops of mixture were loaded into the Improved Neubauer haemocytometer.

The red cell number was estimated with the aid of a light microscope. A second 0.02 mL of blood was drawn from each extracted blood sample into 0.4 mL of Turk's solution (1% glacial acetic acid) in a bijou bottle to give a dilution ratio of 1:20. Some drops of the mixture were again loaded into the same improved Neubauer haemocytometer and white cell number was determined by using a light microscope.

A third 0.02 mL of blood was drawn from each extracted blood sample using a haemoglobin pipette into 0.4 mL of 1% ammonium oxalate in a bijou bottle to give another dilution ratio of 1:20. The mixture was loaded into the Improved Neubauer haemocytometer. The blood platelet number was estimated with a phase contrast microscope (Biostar B4, Exacta and Optech) by employing methods described by Blaxhall and Daisley (1973) as well as Okafor and Chukwu (2005).

Finally, 0.02 mL of blood was drawn from the remainder of each extracted blood sample using a haemoglobin pipette into 5.0 mL of Drabkin's fluid (potassium cyanide 0.2 g; potassium ferricyanide 0.2; sodium bicarbonate 1.0 g and one litre of distilled water) of pH 7.0 contained in a bijou bottle. This gave a dilution ratio of 1:250. Then 1.0 mL of this mixture was poured

into a cuvette and it's Optical Density (OD) read in a photoelectric colorimeter at absorbance of 540 nm, zeroing with blank. The Haemoglobin (Hb) content of each blood sample was calculated as:

$$Hb = \frac{T \times C \times D \text{ g } 100 \text{ mL}^{-1}}{A \times 1000}$$

Where:

T = Test absorbance
A = Standard absorbance
C = Concentration of cyanmet haemoglobin standard (mg 100 m L^{-1})
D = Dilution factor = 250
100 = Converts mg 100-g 100 m L^{-1}

The mean values of haematocrit (%) were measured with a micro-capillary reader. Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) was calculated by using:

$$MCV = \frac{\text{Hematocrit } (\%) \times 10}{\text{RBC count } (\text{million/mm3 blood})} \quad (1)$$

$$MCH = \frac{\text{Hemoglobin } (\text{g } 100 \text{ mL}^{-1}) \times 10}{\text{RBC number in millions}} \quad (2)$$

$$MCHC = \frac{\text{Hemoglobin } (\text{g } 100 \text{ mL}^{-1}) \times 100}{\text{Hematocrit } (\%)} \quad (3)$$

The hematological indices were calculated from the equations given above by Svobodova *et al*. (1991).

Data analyses: The data obtained from the study were analyzed using Genstat®, descriptive statistics were done and mean gotten were subjected to Analyses of Variance (ANOVA). Where significant differences were obtained ($p>0.05$), means were separated using Duncan's Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Proximate composition of experimental diets: Table 2 shows the proximate composition of experimental diets with different levels of inclusion of Fish Gro® Booster. There was no significant difference ($p>0.05$) in the proximate value among the feed used in this study, which indicates that the feed additive used at various percentage inclusion levels had no effect on the proximate value of the feed.

Crude Protein (CP) content was within the acceptable range recommended for commercial feed (NRC, 1983). Wilson (2000) reported that most of the commercial catfish feeds contain 32% CP. Boonyratpalin (1988) estimated the protein requirement for tropical catfish to be 35-40, 25-35 and 28-32% for fry, grow out and brood stock, respectively. The observed lipid levels were lower but close to that reported by Wilson (2000) that lipid level in catfish feed should be 5-6%.

De Silva and Anderson (1995) noted that it was not desirable to have Crude Fiber (CF) content above 8-12% in diets for fish, thus CF in the experimental feeds were above the recommended range.

Hematological parameters for blood extracted from fish fed the experimental diets: The result for hematology shows that all parameters taken among the various diet concentrations of Fish Gro® showed significant difference ($p<0.05$). This implies that there was effect on blood chemistry of fish fed Fish Gro® at the various inclusion levels. When the results for WBC, RBC, HGB and HCT are compared with the control as seen in Table 3, it is observed that the control had higher concentrations when compared to DT_1, DT_2, DT_3 and DT_4. This indicates that high concentrations of Fish Gro® when included in fish diets will lower WBC, RBC, HGB and HCT counts in *Clarias gariepinus* juveniles. MCV recorded higher concentrations in DT_1 and DT_2 while lower or nearly equal concentrations with the control were recorded in DT_3 and DT_4, implying that lower concentrations of Fish Gro® increase MCV. MCH was

Table 2: Proximate composition of the different experimental diet containing Fish Gro® in parcentage

Parameters	Control	DT_1 (0.5%)	DT_2 (1.0%)	DT_3 (1.5%)	DT_4 (2.0%)	p-values
Moisture	8.52±0.04	8.51±0.08	8.32±0.05	8.23±0.02	8.67±0.41	0.221
Protein	35.55±0.01	34.89±0.01	35.02±0.01	34.76±0.01	34.96±0.04	0.131
Lipid	3.80±0.04	4.19±0.02	4.56±0.02	4.22±0.03	4.19±0.02	0.231
Ash	12.66±0.02	12.56±0.00	12.62±0.02	12.73±0.02	12.59±0.01	0.130
Fibre	15.12±0.05	15.82±0.00	12.62±0.01	15.34±0.04	15.32±0.04	0.091
NFE	24.35±0.02	24.03±0.08	26.86±0.07	24.72±0.01	24.27±0.37	0.073

Mean in the same row do not differ significantly ($p>0.05$)

Table 3: Mean water quality parameter of the culture Units with fish fed different experimental diets containing Fish Gro®

Parameters	Control	DT_1	DT_2	DT_3	DT_4	p-values
pH	7.50±0.01	7.47±0.01	7.46±0.00	7.47±0.00	7.51±0.00	0.221
Temp. (°C)	23.80±0.00	23.80±0.00	23.75±0.05	23.80±0.00	23.75±0.05	0.588
DO (mg L^{-1})	3.15±0.11	3.12±1.02	3.14±1.91	3.10±0.10	3.11±1.02	0.932

Mean in the same row do not differ significantly ($p>0.05$); DO = Dissolved Oxygen

Table 4: Hematological Parameters of fish fed different experimental diets containing Fish Gro®

Parameters	Control	DT_1 (0.5%)	DT_2 (1.0%)	DT_3 (1.5%)	DT_4 (2.0%)	p-values
WBC	209.70±0.08^{a}	200.30±0.08^{b}	191.6±0.06^{c}	184.00±0.03^{d}	188.40±0.03^{cd}	0.005
RBC	2.16±0.34^{a}	1.96±0.34^{b}	1.79±0.77^{d}	1.71±0.29^{d}	1.87±0.29^{c}	0.002
HGB	8.60±0.29^{b}	9.10±0.29^{a}	8.60±0.83^{b}	7.50±0.29^{c}	8.60±0.20^{b}	0.003
HCT	32.40±0.01^{a}	31.6±0.01^{a}	29.10±0.15^{b}	24.10±0.01^{b}	28.60±0.004^{b}	0.003
MCV	150.00±0.04^{b}	162.60±0.04^{a}	162.60±0.16^{a}	140.90±0.06^{c}	152.90±0.02^{b}	0.007
MCH	39.80±0.34^{c}	46.40±0.34^{ab}	48.00±0.94^{a}	43.90±0.42^{b}	46.00±0.17^{ab}	0.001
MCHC	26.5±0.11^{d}	28.80± 0.11^{c}	29.60±0.46^{bc}	31.10±0.32^{a}	30.10±0.21^{ab}	0.006
PLT	29.00±0.85^{a}	6.00±0.85^{c}	4.00±2.55^{d}	7.00±1.54^{c}	11.00±0.85^{b}	0.050

Mean in the same row with different superscripts differ significantly ($p<0.05$); WBC = White Blood Cell Count ($\times10^3$ µV); RBC = Red Blood Cell Count ($\times10^6$ µL^{-1}); HGB = Haemoglobin Content (g d L^{-1}); HCT = Mean Haematocrit (%) MCV = Mean Corpuscular Volume (µm) MCH = Mean Corpuscular Haemoglobin (pg); MCHC = Mean Corpuscular Haemoglobin Concentration (%) and PLT = Platelet Count ($\times10^3$ µL^{-1})

higher in all the diets with Fish Gro® inclusion when compared with the control diet; the same trend was also recorded for MCHC implying that MCH and MCHC increased as diets with Fish Gro® concentrations increased. PLT was much lower in all the Fish Gro® diets than the control, indicating that PLT counts dropped when Fish Gro® booster was used (Table 4).

Observed differences in haematology of *Clarias gariepinus* fed the control diet and the various inclusion levels of Fish Gro® could be as a result of the presence of toxic substances. This was similar to the report of Adeyemo (2005) and Osuigwe *et al.* (2005) that ascertained the reduction in values of hematological parameters such as PCV, HB, and RBC were due to the presence of toxic substances in the diet of fish. It could therefore be inferred from this study that fish with lower growth booster inclusion levels were of better health status than those with higher growth booster inclusions based on the submissions of Svobodan *et al.* (1991) and Ncha *et al.* (2015). Physiologically, HGB is crucial to the survival of fish being directly related to the oxygen binding capacity of blood. However the reduction observed in this study may not have had a deleterious effect on *Clarias gariepinus*, given that the values are within the normal range recorded for African catfish (Erondu *et al.* 1993; Musa and Omoregie 1999; Sowunmi, 2003). So also were the values obtained for HCT, RBC and WBC. The HCT values also fall within the normal range of 20-35% and rarely >50% for fish (Clark *et al.*, 1979).

CONCLUSION

It is concluded that Fish Gro can be used as a feed additive in fish feed to enhance growth since all the haematological parameters fall within normal range, though it is recommended it should be used in low percentages of inclusion in fish feed.

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