Haematological Characteristics of *Clarias gariepinus* Exposed to Graded Levels of Dexamethasone

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Abstract

A feeding trial was conducted for seventy days in order to evaluate the effect of dexamethasone on the haematological parameters of *Clarias gariepinus* juveniles. The fish were fed with diets containing four different levels of inclusion of dexamethasone which were: 3mg/kg, 6mg/kg, 9mg/kg and 12mg/kg. However, the diet fed to the fish as the control had no inclusion of the additive. At the end of the experiment, blood samples were obtained from all the treatments and were analyzed to determine their haematological parameters and electrolyte composition. The study revealed that Erythrocyte sedimentation rate (ESR) and packed cell volume (PCV) were not significantly different from those of the control except in the treatment fed with diet containing 6mg/kg of dexamethasone ($p<0.005$). Also, red blood cell counts (RBC) in all treatments differed significantly from the control except in the one fed with a diet containing 12mg/kg of the additive ($p<0.005$). The white blood cell counts (WBC) in all treatments differed significantly ($p<0.005$) from and were higher than that of the control except in the one fed with a diet containing 9mg/kg of the additive. Haemoglobin concentrations were also not significantly different ($p<0.005$) from that of the control in all the treatments. In addition to that, blood sodium levels in all treatments were significantly different from that of the control, while calcium concentrations only differed significantly from the control in fish fed with diets having 3mg/kg, 6mg/kg and 12mg/kg inclusions of dexamethasone respectively ($p<0.005$). Moreover, blood magnesium and potassium levels in all treatments were not significantly different from that of the control ($p<0.005$). This study has therefore shown that the doses of dexamethasone included in the diets of *C. gariepinus* juveniles induced some changes in the haematological characteristics and ionic composition of their blood and could consequently induce negative changes in their physiology and health status.

Keywords: *Clarias gariepinus*, Dexamethazone, Electrolyte Composition, Feeding Trial, Haematology.

1.0. INTRODUCTION

Fish farming (or Aquaculture) is an effective way of generating food and income from dwindling land space, as fish supplies from the natural waters continue to fall and human population, as well as fish demand increase (Adebayo and Adesoji, 2008). Aquaculture involves cultivating freshwater and saltwater aquatic organism populations under controlled conditions (FAO, 2019). To reduce pressure on natural fish stock, aquaculture has been identified as a viable option that can bridge the gap between fish demand and supply as it has the capacity to expand (Obe *et al*., 2018). The challenge therefore is for the aquaculture industry in Nigeria to grow in order to be able to meet this growing demand for fish.

The African cat fish *Clarias gariepinus* is the most popular and widely cultivated fish in Nigeria. The rearing of catfish in Africa stated in the early 1970s in Central and Western Africa as it was realized to be a very suitable species for aquaculture (Anon, 2014). The African catfish *Clarias gariepinus* (Family *Clariidae*) has also gained wide spread recognition as a promising species in aquaculture production (Taiwo *et al*, 2008), it is an economically important food fish, cultured primarily in freshwater pond. Fagbenro *et al.* (2005) and Fasakin *et al.* (2006) also reported that *Clarias gariepinus* is suited to low-technology farming system because of its ability to consume supplementary feed and natural aquatic food, good conversion of feed to flesh, resistance to diseases, ability to reproduce in captivity, fast growth rate, good flesh quality or palatability, tolerance to wide range of environmental conditions, high dressing percentage and high market value.

Dexamenthasone is a synthesis of glucocorticoid class of steroid used for growth in humans (Bibbi, 2011). It function as anti – stress and an immunosuppressant. Its biology action includes growth promotion, energy mobilization, appetites stimulation and social behaviour enhancement (Provan *et al*, 2010).

Haematology is the branch of Medicine concerned with the study of blood, the blood forming organs and blood pressure. The word "haeme" comes from the Greek word for blood (Marco *et al*, 2003). Blood tissues of fish give clues about physiological and environmental condition of the fish (Ramaway *et al*, 2008). In recent years, blood parameters have been commonly used to observe and monitor fish health (Bhaskar, 2005) since variation in blood tissue of fish are caused by feeds (Hickey, 2002) gender, fish size, seasonal differences and breeding (Wohlschlong, 2001). In order to determine haematological value of fish, haematological characteristics of fish blood should be considered.

2.0. MATERIALS AND METHODS

The dietary feed ingredients were: fish meal, maize meal, soybean meal, blood meal, fish premix, methionine, starch and dexamethasone. The ingredients were purchased at Metrovet Feed Mill, Ado-Ekiti except the blood meal and dexamethasone. Blood meal was processed by obtaining fresh cattle blood at an abattoir, pouring it in a large frying pan and boiling on fire using firewood while stirring constantly. When moisture was sufficiently reduced, it was spread on clean polythene bags on a clean cemented surface and then sun-dried for three days at atmospheric temperature of 28^0C.

Dexamethasone was purchased from Kascot Pharmaceutical, along Old Garage, Ado – Ekiti, Ekiti State.

2.1. Processing of Dexamethasone into Powder Form

The drug which comes in tablet form of 1.0ml per tablet and 12 tablets per sachet was purchased at a pharmaceutical store. The tablets were counted out according to the number (mg) required per treatment i.e. 3, 6, 9, 12mg for treatment 2, 3, 4 and 5 respectively. The dexamethasone was grounded separately per treatment into powdery form by a pestle in a small mortal so that no part of the dexamethasone poured away.

2.2. Feed Formulation and Preparation

The control diet (TR_1) contained no Dexamethasone, diets TR_2, TR_3, TR_4 and TR_5, were formulated with Dexamethasone which served as a growth hormone enhancer (Table 1). The dexamethasone was administered at different concentration levels of 3mg, 6mg, 9mg and 12mg for treatment TR_2, TR_3, TR_4 and TR_5 respectively. The ingredients were mixed thoroughly together in a bowl and warm water was added. Starch was also added to act as binder before it was pelleted using a pelleting machine with a die size of 2.0mm, the pellets were then sun dried and packaged in a plastic container and stored in a cool and dry condition.

Table 1: Gross Composition of Experimental Diets

Ingredients	**TR_1**	**TR_2**	**TR_3**	**TR_4**	**TR_5**
Fish Meal (72%)	28.10	28.10	28.10	28.10	28.10
Soybean Meal (45%)	28.10	28.10	28.10	28.10	28.10
Blood Meal (80%)	4.62	4.62	4.62	4.62	4.62
Maize (10%)	34.18	34.18	34.18	34.18	34.18
Fish Premix	4.0	4.00	4.00	4.00	4.00
Methionine	0.3	0.30	0.30	0.30	0.30
Starch	0.7	0.70	0.70	0.70	0.70
Total	100	100	100	100	100
Dexamethasone (g)	-	3.00	6.00	9.00	12.00

2.3. Experimental set-up

Two hundred and twenty five juveniles of *C. gariepinus* (average weight 11g) were purchased from Federal University of Technology, Akure, (FUTA) Research Farm. The fish were acclimated for seven days in plastic tanks (dimension 70cmX45cmX40cm) before the commencement of the experiment. The fish were randomly stocked at the rate of fifteen fish per tank. Each treatment was replicated thrice and the experiment lasted for 70 days.

The water quality parameters, temperature, dissolved oxygen and pH were measured every two weeks. Mercury – in – glass thermometer was used for the temperature, dissolved oxygen meter for dissolved oxygen and the pH was also monitored by the pH meter.

The five experimental diets were fed to the fish in each corresponding tank within the period of the experiment. The treatments were replicated thrice to minimize experimental errors. The fish were fed twice at 3% of the body weight at 9:00 – 10:00am and 5:00 – 6:00pm daily for the entire period of the experiment. Feeding rate was adjusted every 2 weeks according to increase in body weight. Feeds and faeces were siphoned out every day from the tanks before feeding. The growth weights recorded were used to evaluate the performance of each diet. Water was changed at four days interval during the experimental period.

2.4. Haematological Evaluation:

This was carried out as described by Svobodova *et al*. (1991). Blood samples were collected for each treatment from fish randomly selected from each replicate using 2mL heparinized plastic syringe, treated with Ethlyne Diamine Tetra-Acetic Acid (EDTA) as anti-coagulant.

Packed cell volume: The packed cell volume or haematocrit value was determined by placing sealed micro-haematocrit tube with blood sample in a centrifuge and allowed to spin for 5 minutes at 15,000rpm. Haematocrit percentage was then read directly on Hawskey micro haemotocrit reader and the value was expressed as percentage.

Red Blood Cell (RBC) count: This was carried out by preparing the blood diluting fluid according to the method of Svobodova *et al*. (1991). Haemocytometer was used in blood cell count. The blood cells were counted on the counting chamber of haemocytometer with the aid of compound microscope. RBC was calculated as:

RBC = No of cells counted x3x10x200 (10^6 mm^3)

White Blood cell (WBC) count: The same procedure for RBC count was carried out. WBC was then calculated as:

WBC = No of cells counted x0x25x10x20 (10^4 mm^3)

Haemoglobin estimation: Hemoglobin was measured using the standard cyanomethaemoglobin method.

Erythrocyte sedimentation rate (ESR):

The volume of ESR with the given time interval is the difference between 100% and the percentage part presented by the corpuscle volume.

2.5. Use of Blood Electrolytes (Cations and Anions) to Assess Fish Chemistry

The use of blood electrolytes such as Cl, Mg, Pb could serve as physiological indicators to evaluate the fish chemistry.

Cations and Anions Sampling

Fresh blood was drawn and collected in a 5ml syringe using a needle of not smaller diameter than 19 gauge. The needle was removed from the syringe and the blood was expelled gently into a bijou bottle containing the EDTA. The bottle was capped and was mixed for 1 minute so that the blood will not coagulate. It was kept away from sun and stored at 4°C. This was done on each treatment.

3.0. RESULTS

3.1. Haematological Parameters

Haematological profiles of control and experimental groups are shown in Table 2.

No significant difference (P>0.05) in PCV compared with the control group was observed in fish exposed to dexamethasone except in TR_3 with 6mg concentration of the drug. The highest values of PCV (43.5 ± 0.50) in this study was obtained in specimen without dexamethasone exposure, while the lowest was in TR_3. There was no significant difference (P>0.05) between the RBC values of the control group and the fish fed TR_5 (fish exposed to 12.0mg of dexamethasone), whereas significant differences (P<0.05) were observed between then and all the other treatments. TR_3 had the least value of RBC and the highest was recorded in TR_4.

The highest value of WBC (184 ±1.41) was recorded in TR_3 (fish exposed to 6.0mg of dexamethasone) while the least was recorded in TR_4. For Hb, the highest value was recorded in TR_4 (11.95 ±0.71) while the lowest was recorded in TR_3 (10.55 ±0.77). For ESR, highest value was recorded in TR_3 while TR_2, TR_4 and TR_5 had the same and lowest values.

Table 2: Haematological Parameters

	Experimental diets				
Parameters	TR_1 (control)	TR_2	TR_3	TR_4	TR_5
PCV (%)	43.5±0.50[b]	34.0±1.00[b]	31.0±1.00[a]	36.5±0.50[b]	34.0±0.00[b]
RBC($x10^6/mm^3$)	2.340±2.83[b]	2.415±2.12[c]	2.250±2.12[a]	2.590±1.41[d]	2.345±2.12[b]
WBC($x10^4/mm^3$)	1.660±1.41[a]	1.700±2.83[b]	1.840±1.41[d]	1.640±2.83[a]	1.745±2.12[c]
ESR	3.0±0.00[ab]	2.5±0.71[a]	4.0±0.00[b]	2.5±0.71[a]	2.5±0.71[a]
Hb (d/gL)	11.35±.0.07[ab]	10.70±.0.0[a]	10.55±.0.77[a]	11.95±.0.71[b]	11.40±0.14[ab]

Mean and Standard deviation on the same row and followed by the same superscript are not significantly different (p < 0.005).

3.2. Electrolyte Analysis

The result for the electrolyte sampling is shown in Table 3. There were significant differences (P<0.05) in the values recorded in all the treatments for zinc (Zn), copper (Cu), chlorine (Cl) and iron (Fe), but no significant differences (P>0.05) in the values recorded for magnesium and potassium. There were also no significant differences (P>0.05) in TR_2 and TR_3 for sodium (Na) values, no significant differences (P>0.05) in TR_2 and TR_4 for calcium (Ca) values, but they differed significantly (P<0.05) from that of other treatments.

Table 3: Electrolyte Analysis

	Experimental diets				
Electrolytes	TR_1	TR_2	TR_3	TR_4	TR_5
Zn	0.69±0.03[a]	1.00±0.09[b]	1.06±0.04[c]	1.45±0.03[d]	1.82±0.42[e]
Cu	1.03±0.1[a]	1.23±0.11[b]	1.28±0.01[c]	1.56±0.01[d]	1.60±0.28[e]
Cl	30.78±1.65[c]	36.01±0.83[b]	20.85±2.83[a]	43.19±0.84[d]	103.23±2.12[e]
Fe	0.57±0.07[a]	0.67±0.01[b]	0.75±0.04[c]	0.85±0.05[d]	1.08±0.04[e]
Na	128.67±1.36[b]	112.04±1.41[a]	113.56±2.21[a]	146.88±2.44[c]	325.73±3.47[d]
Ca	35.00±2.83[a]	120.64±1.56[c]	219.00±1.41[d]	33.18±6.99[a]	46.13±4.29[b]
Mg	15.50±2.87[a]	18.77±1.89[a]	16.93±3.52[a]	14.37±1.07[a]	16.87±0.93[a]

K	279.09±7.09[a]	310.69±77.78[a]	227.00±3.34[a]	288.19±2.69[a]	320.47±2.13[a]

Mean and standard deviation on the same row and followed by the same superscript are not significantly different ($p<0.05$).

4.0. DISCUSSION

Haematological parameters are useful in determining the health (Duman and Sahan, 2017) and evaluating the physiological status of fish (Vazquez and Guerrero, 2007). Packed cell volume (PCV), which is also known as haematocrit, is the percentage of red blood cells in a volume of blood (Basten, 2010). In this study, the PCV values in all treatments were lower than that of the control. The differences in the PCV value of the control and those of other treatments were, however, not statistically significant ($P< 0.05$) except in those exposed to 6mg/L. Exposure of *C. gariepinus* juveniles to dexamethasone might have led to the shrinkage of blood cells, thus causing the lowering of their PCV values (Gabriel *et al*., 2009). The non-concentration dependent changes in RBC counts in *C. gariepinus* juveniles exposed to dexamethasone in relation to the control indicate that the toxicant induced haphazard alteration of the physiology of the fish. This is similar to the findings of Gabriel *et al*. (2009) who reported that non-concentration decline in PCV occurred in *C. gariepinus* injected with aqueous extracts of *Lepidagathis alopecuroides*. Nevertheless, it is not in agreement with the result obtained by Ayotunde *et al*. (2011) where there was concentration-dependent reduction in PCV in adult *C. gariepinus* exposed to aqueous extracts of *Carica papaya* seeds.

Apart from haematological parameters, blood electrolytes are used as indicators of health status of fish (Kulkarni, 2015). Generally, they play a major role in the retention and distribution of body water (Kulkarni, 2015). Electrolytes such as sodium, potassium and calcium are indicators of the state of certain homeostatic mechanisms in fish (Kulkarni, 2015). Sodium is an electrolyte that is required for osmoregulation and acid-base balance (Muralidharan, 2014). In this study, there were changes in blood sodium concentrations in treatments that were exposed to dexamethasone. Therefore, it can be inferred that exposure of *C. gariepinus* juveniles to the drug brought about the disruption of osmoregulatory and homeostatic mechanism. Calcium is required for blood clotting, muscles contraction and nerve excitability (Latif and Ali, 2014). Besides, it helps to regulate mitochondrial function and osmoregulation (Kulkarni, 2015). Exposure of *C. gariepinus* juveniles to dexamethasone, in this study, was found to have significantly induced elevation of blood calcium ion concentrations. Srivastav *et al*. (2013) reported that degenerative changes occur in fish gills after exposure to toxicants. It is therefore suggested that gill deterioration might have affected electrolyte permeability in this study (Srivastav *et al*., 2013), thus leading to indiscriminate and excessive absorption of calcium from water. Apart from that, the drug might have impaired muscle contraction and nerve excitability in *C. gariepinus* juveniles used in this study. The fish could have responded by absorbing more calcium ions from the environment in order to ensure that muscle and nerve functions are maintained. Magnesium ions are required by erythrocytes for maintaining their functional entity (Latif and Ali, 2014). There was no significant change in blood magnesium ion concentration of *C. gariepinus* exposed to dexamethasone. Potassium is involved in the regulation of physiological functions such as: osmotic pressure maintenance, acid-base balance and proper functioning of muscles as well as nerves (Muralidharan, 2014). Potassium migrates from blood into the gut when gut epithelium has not been damaged (Muralidharan, 2014). In this study, exposure of *C. gariepinus* to different concentrations of dexamethasone did not have any significant effect on blood potassium levels. This indicates that the drug did not cause any infraction on gut epithelium, thus ensuring that the normal pattern of potassium ion migration was not impaired. Chlorine is a monovalent electrolyte in intracellular fluids and is involved in acid-base balance (Jadhav, 2009). Concentration of blood chloride ions was observed to have changed significantly ($P< 0.05$) in *C. gariepinus* juveniles exposed to dexamethasone in this work. This can be an indication that the drug affected the mechanism of acid-base balance in the fish.

5.0. CONCLUSION

The results obtained in this study have shown that the tested concentrations of dexamethasone induced some changes in the haematological characteristics and ionic composition of the blood of *Clarias gariepinus* juveniles. It can therefore be concluded that the exposure of the fish to the drug can impair their physiology and health status.

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