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Basic Haematogical Parameters in African Catfish, *Clarias gariepinus* (Burchell, 1822) FED Ascorbic Acid Supplemented Diets

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Abstract: Basic haematogical parameters in African catfish, *Clarias gariepinus* fed ascorbic acid supplemented diets were evaluated in this study. *Clarias gariepinus* fingerlings weighing 6.02±0.4g were randomly distributed into glass tanks of 60×45×45 cm^3 dimension at ten fish per tank in a triplicate treatment. Five isonitrogenous and isocalorific diets containing 40% crude protein was formulated. Endogenous concentrations of Ascorbic Acid (AA) were monitored in the diet, this was supplemented with graduated concentration of ascorbyl-2-polyphosphate at 0 (Control) 50, 100, 150 and 200 mg AA kg^{-1}, in Treatment 1, 2, 3, 4 and 5, respectively. Fish were fed practical diets twice daily at 900 and 1600h. Weekly weighing of fish was done and two Fishes were removed from each aquarium for blood analysis, The blood was collected from cardiac puncture of fish using 2ml disposable heparinized syringes, with Tetra Acetic Acid (EDTA) usad as anti-coagulant, the haematological data collected were subjected to statistical analysis. Haematological studies on *Clarias gariepinus* conducted in this study after the feeding trials indicated that fish fed scorbutic diets had significantly lower ($p<0.05$) pack cell volume, haemoglobin concentration and red blood cells, while the highest white blood cells was recorded in this treatment as 4.25±0.14 ×10 mm^{-3} Among the derived haematological parameters only the mean corpuscular volume was significantly different ($p<0.05$).

Key words: Haematological, African catfish, *Clarias gariepinus*, ascorbic acid, supplementation, scorbutic

INTRODUCTION

Vitamins are essential organic compound and nutrient that are required in trace amount in feeds for the normal functioning of the body of animal. Situations that interfere with availability, dispersion of micro nutrients throughout feed mixture or increase the requirement of fish for vitamins can cause problem in fish performance (NRC, 1993). The inability of many fish species to synthesize Ascorbic Acid (AA), or Vitamin C, which is essential for fish growth, reproduction and health is well documented (Dabrowski, 1990). Ascorbic acid must therefore, be supplied via feed. Major clinical sign of ascorbate deficiency include anemia, reduced growth, scoliosis, lordosis, internal and fin heamorrhage, distorted gill filaments, fin erosion, abnormal pigmentation, increased capillary fragility, reproductive performance (Sadnes *et al*., 1992). Blood composition is usually altered during diseases or malnutrition conditions (Feist and Longshaw, 2000). Aletor and Egberongbe (1998) reported that Red blood cell count and Packed Cell Volume (PCV) are mostly affected by dietary treatment. Due to the multiple role of Ascorbic acid in various metabolic pathways, a better understanding of the mechanisms through which ascorbic acid as a nutritional element influences the immunlogical and haemolytical systems in modern intensive fish farming is necessary, to appreciate the many complex relationship and interaction between diet, stress and susceptibility to disease in fish. It is therefore, prudent to study the effect of ascorbic acid on the basic haematological profile of *Clarias gariepinus* .

The clariid catfish *Clarias gariepinus*, (Burchell, 1822) is the most important fish species cultured in Nigeria. This specie has shown considerable potential as a fish suitable for use in intensive aquaculture. This fish grows rapidly, are resistant, sturdy and highly productive were in polyculture with many other fish species. The dearth of information on the importance of vitamin C as an immuno modulator and haemolytical element in the African catfish, *Clarias gariepinus* and the need to establish the ascorbic acid requirements on a species by species basis prompted this study.

MATERIALS AND METHODS

The department of Fisheries and Wildlife research Laboratory, Obanla. Federal University of Technology Akure, Nigeria was used to carry out this experimental

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Table 1: The experimental diet composition in g/100g dymater containing various inclusion level of ascorbic acid supplementation for *Clarias gariepinus*

Ingredients	Treatment 1 control	2	3	4	5
Fish meal (70%)	22.00	22.00	22.00	22.00	22.00
GNC	28.00	28.00	28.00	28.00	28.00
SBM	24.00	24.00	24.00	24.00	24.00
Yellow maize	11.00	11.00	11.00	11.00	11.00
Vegetable oil	5.00	5.00	5.00	5.00	5.00
Oyster shell	2.00	2.00	2.00	2.00	2.00
Rice bran	4.00	4.00	4.00	4.00	4.00
*Vit/Min premix	2.00	2.00	2.00	2.00	2.00
Salt	1.00	1.00	1.00	1.00	1.00
Starch	1.00	1.00	1.00	1.00	1.00
Ascorbic acid mg kg^{-1}	0.00	50.00	100.00	150.00	200.00

Premix as supplied by Animal Care, Limited, Lagos, Nigeria

work. The graded level of ascorbic acid premix used are 0.00 mg kg^{-1} (control) 50, 100, 150 and 200 mg kg^{-1} in diet 1-5, respectively.

Experimental diet preparation: Five isocalorific and isonitrogenous diets containing 40% crude protein and 12% lipid were formulated for fingerlings catfish, *C. gariepinus* in a ten-week trial experiment (Table 1). Ascorbic acid, commercially available as ROVIMIX STAY C- (Roche, Istanbul, Turkey) was used. Scorbutic diets (without ascorbic acid supplementation) served as the control. Ascorbic acid supplementation in diets 2-5 were 50.0, 100.0, 150.0, 200.0 mg kg^{-1}, respectively. All dietary ingredients were first milled to small particle size, ingredients including ascorbic acid were thoroughly mixed in a Hobart A-200 pelleting and mixing machine (Hobart Manufacturing Ltd., London, England) to obtain a homogenous mixture, cassava starch was used as a binder. The resultant mash was then passed without steam through a 0.9 mm die and sun dried immediately. Diets were broken up and sieved to convenient sizes and stored in the refrigerator prior to feeding.

- Vitamins supplied mg 100 g^{-1} diet: Thiamine (B_1) 2.5 mg: Riboflavin (B_2), 2.5 mg Bridoxine.
- Pantothenic acid, 5.0 mg: Inositol, 3 mg: Biotin, 0.3 mg: Folic acid, 0.75 mg para-amino benzoic, 2.5 mg: Chlorine, 200 mg; niacin, 10.0 mg, cynicobalamin (B_{12}), 10.0 mg; menadione (k), 2.0 mg. Minerals: $CaHPO_4$, 727.8 mg: Mg SO_4, 1275 mg Nacl, 60.0 mg; kcl 50.0 mg; $FeSO_4$, 250 mg, $ZnSO_4$ 5.5 mg; Mn SO_4, 2.5 mg $CuSO_4$, 0.79 mg; $CoSO_4$, 0.48 mg: $CaClO_3$, 0.3 mg; Cr Cl_3.

Experimental fish and management: *C. gariepinus* fingerlings with average weight of 6.0±0.4 g were sourced from the Agricultural Development Programme Department, Akure, Ondo State, Nigeria and were randomly distributed into glass tanks (60×45×45 cm) at 10 fish per tank. Each treatment was in triplicate groups of fish. Tanks were supplied with water from a borehole powered by 1.5HP pumping machine in a flow through system. Water temperature was maintained at 24±0.5°C. Dissolved oxygen was kept at a saturation level of 6.00±0.1 mg L^{-1}. The fish were fed to satiation with their respective diets at 5% body weight twice daily at 9:00 h and 16:00 h GMT throughout the duration of the experiment. Fish responded by feeding well on the diets immediately. Fish weights were determined at the 7th day of each week by weighing all the fish in the tank and the quantity of feed adjusted based on the changes in body weight of fish for subsequent feeding.

Proximate composition: Proximate composition of diets and fish carcasses before and after experiment, were performed according to AOAC (1990) for moisture content, fat, fibre and ash. Ascorbic acid was determined by semi-automated flourometric method as described by AOAC (1990). Samples were weighed into a plastic cup containing 100 mL solvent extract, 4% matephosphoric acid, was added and the mixture filtered. The filterate was passed through an Autoanalyzer (Technicon corporation Urbana, Illnois.) Ascorbic acid was determined in mg 100^{-1}.

Haematological profile analysis: The blood analysis followed the methods described by Svobodova *et al.* (1991). Haemocytometer was used in blood cell counts. The counting chamber of the haemocytometer with the aid of compound microscope.

RBC = No. of cells counted×5×10×200 (10^6 mm^3).
WBC = No. of cells counted×0.25×10×20 (10^4 mm^3).

Haemoglobin estimation (hb):

Haemoglobinometer was used for haemoglobin estimation.
Haemoglobin = value obtained/100 × 17.2 (gm 100 mL^{-1}).

Packed Cell Volume (PCV): PCV was determined by drawing non-dotted blood by capillary action into microhaematocrit tubes: One end of the tubes was sealed

with synthetic sealant. The sealed tube was centrifuged in a microhaemotocrit centrifuge. Centrifugation lasted for five minutes at 10500 rpm. The PCV measured using microhaemotocrit reader and expressed as percentage.

Mean Cell Haemoglobin Concentration (MCHC):

$$\text{This was calculated as MCHC} = \frac{\text{HB}}{\text{PCV}} \times 1000\ \frac{\text{T}}{1}$$

$$\text{Mean Corpscular Volume (MCV)} = \text{PCV} \times \frac{1000}{\text{Er}} (\text{U3})$$

$$\text{Mean corpsular haemaglobin (mch)} = \frac{\text{Hb}}{\text{Er}} (\text{picogramme}) (\text{pg})$$

Statistical analysis: Biological data resulting from the experiment were subjected to one way Analysis of Variance (ANOVA) using the SPSS (Statistical Package Computer Software, 2000). Duncan's multiple range was used to compare differences among individual means. All percentages and ratio data were transformed. Differences were considered significant at (p-levels<0.05).

RESULTS AND DISCUSSION

Proximate composition of feed: The proximate composition of the experimental feed as shown in Table 2. The experimental feed used for the 5 treatments had varying levels of L-Ascorbic acid supplement T_1 (0) mg kg^{-1}, T_2 (50) mg kg^{-1}, T_3 (100) mg kg^{-1}, T_4 (150) mg kg^{-1} and T_5 (200) mg kg^{-1}.

The proximate analysis of experimental diets revealed that they contain a mean of 40% crude protein, Table 2. The result of the proximate composition of experimental fish in Table 3 showed that all the treatment (1-5) improved on their protein content over the initial which was 14.15. However, these results are in close agreement with work of Verlhac *et al.* (1996) which showed increase carcass protein value for Rainbow trout (Oncorhychus mykiss) fed ascorbic acid level higher than 100 mg kg^{-1}.

Table 4 showed the haematological parameters of fish fed the experimental diet, the highest PCV recorded in this study for *C. gariepinus* was 30.2%±0.97. WBC, (White Blood Cell) were highest in fish fed diet 1 without ascorbic acid concentration and there were significant difference ($p<0.05$) in the Heamoglobin estimation (Hb),RBC and WBC of fish fed this diet in this study with the control (T_1) having the least value of Hb and RBC while the high value of 4.25±0.14 $\times 10^3 mm^{-3}$ of WBC was recorded in the control fed scorbutic diets. There was no significant difference ($p>0.05$) in the MCH Mean Corpsular Heamoglobin and MCHC Mean Corpsular Heamoglobin Concentration values in the fish used in this study. Despite the fact that the RBC of fish were significantly different ($p<0.05$) the (MCH) Mean Corpsular Heamoglobin weight of RBC had no significance difference ($p>0.05$) with fish fed the control having and there was significant difference ($p<0.05$) in the MCV value of fish in this study.

Differences in blood parameters of fish in this study could be ascribed to differences in diet composition (Elbaraasi *et al.*, 2004). Under normal conditions the composition of blood is reasonably constant for any particular species of Fish, with changes falling with fairly narrow limits Banerjee *et al.* (2002).Changes in blood indices of fish fed the control (T_1) without ascorbic acid supplementation could be due to the reduction in the absorption and redistribution of iron and consequently

Table 2: Proximate composition of experimental diets % DM

	T_1	T_2	T_3	T_4	T_5
Crude protein	40.28	40.19	40.21	40.13	40.09
Crude lipid	12.39	12.21	12.33	12.17	12.03
Crude fibre	5.09	5.28	5.11	5.19	5.42
Ash	8.35	8.36	8.48	8.33	8.44
Moisture content	13.41	13.54	13.61	13.48	13.37
Nitrogen-free extract	20.48	20.42	20.26	20.70	20.65
Added ascorbic acid mg kg^{-1}	0.0	50.00	100.00	150.00	200.00
Measured ascorbic acid mg kg^{-1}	0.64	56.20	109.70	165.90	204.83
Gross energy kca $100g^{-1}$	431.30	429.00	429.40	429.70	427.50

(1) Nitrogen free extract: calculated as 100- (crude protein + ash + crude fibre + ether, (2) Gross energy (kcal/100g) based on 5.7kcal protein; 9.5kcal/g lipid; 4.1kcal/g carbohydrate

Table 3: The proximate composition of the experimental Fish

	Sample initial %	T_1	T_2	T_3	T_4	T_5	S.E.
Moisture	73.05	73.01^{c}	72.18^{b}	71.50^{a}	71.39^{a}	72.11^{b}	0.16
Protein	14.15^{a}	15.23^{b}	16.15^{c}	17.14^{d}	17.16^{d}	17.11^{d}	0.28
Fat	6.18^{b}	7.03^{a}	6.40^{a}	7.21^{a}	7.19^{a}	6.91^{b}	0.08
Ash	4.70^{a}	4.33^{b}	4.56^{ab}	4.24^{bc}	4.12^{cd}	4.07^{d}	0.93

Figures in each row having the same superscripts are not significantly different ($p<0.05$)

Table 4: Haematological parameters of fish fed experimental diet

Treatments	PCV %	Hb g moL^{-1}	WBC× 10^3 mm^{-3}	RBC× 10^6 mm^{-3}	Fentohtres MCV (H)	MCH (pg)	MCHC Hb 100 mL^{-1}
1	15.44±0.70^{a}	8.24±0.90^{a}	1.25±0.14^{a}	0.65±0.4^{a}	2368±41.01^{b}	21.75±2.23^{a}	57.42±9.85^{a}
2	24.54±10.64^{b}	12.07±1.30^{ab}	1.77±0.32^{ab}	1.28±0.2^{ab}	2018±373.9^{ab}	20.18±3.74^{a}	44.36±5.50^{a}
3	26.30±1.30^{bc}	13.01±1.00^{b}	2.40±0.21^{bc}	1.46±0.20^{bc}	1873±259.2^{ab}	18.73±2.59^{a}	49.51±3.45^{a}
4	30.19±0.97^{cd}	17.60±0.25^{c}	3.28±0.22^{d}	2.25±0.36^{d}	1411±235.0^{a}	14.14±2.40^{a}	58.43±2.20^{a}
5	27.53±0.4^{cd}	16.06±3.83^{bc}	3.28±0.22^{d}	2.11±0.27^{cd}	1348±176.5^{a}	13.48±1.76^{a}	58.48±8.43^{a}

Figures in each row having the same superscripts are not significantly difference ($p<0.05$)

a reduction in the synthesis of hemoglobin. *C. gariepinus* without dietary ascorbic acid had lower PCV, this is in concordance with the result recorded by Shiau and Jan (1992) where hybrid tilapia without dietary ascorbic acid had lower PCV than fish receiving dietary ascorbic acid supplementation. However, there was significant difference ($p<0.05$) in the hematocrit level of fish in this experiment.

Fish fed diet 4 of 150 mg kg^{-1} Ascorbic acid had the highest Pack Cell Volume (PCV). This could mean that this diet is adequate for growth and blood formation in *Clarias gariepinus*. Table 4 showed the haematological parameters of fish fed the experimental diet, the highest PCV recorded in this study for *C. gariepinus* was 30.2%±0.97 which was less than what was recorded for *Clarias isheriensis* 31.62±5.17% (Kori-Siakpere, 1985) more than and *Heterotis niloticus* which was 28.12±2.98% (Fagbenro *et al*., 2000). Haematological analysis in this study showed that when ascorbic acid was absent in the diets of *C. gariepinus*, there was obstruction of intestinal absorption of iron. This impaired erythrocyte synthesis leading into anaemia (as indicated by lowered levels of red blood cells, heamoglobin and heamatocrit).

Verlhac *et al.* (1996) stated that ascorbic acid acts as immunomodulator which reduce immune depression caused by stress, the highest value of WBC in treatment one could be as a result of the need of the fish to release more leucocytes to combat the stress hormone corticosteroids and increase the specific immune response, this results agreed with the result recorded by Kumari *et al*. (2005) that ascorbic acid supplementation level of 100 mg kg^{-1} increases the specific immune responses of Asian catfish *Clarias batrachus*. The variability of haemotological parameters obtained in this study was similar to those reported by Kori Siakpere (1985) in the study of the haematological parameters of Clariid fishes.

CONCLUSION

In this study, ascorbic acid contents of 150 mg kg^{-1} ascorbic acid supplementation kg^{-1} diet was sufficient to prevent the development of the clinical signs of scurvy/ascorbic acid deficiencies and at the same time gave the best heamatological profile performance.

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