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Evaluation of Dietary Ascorbic Acid Supplementation in Pratical Diets for African Catfish *Clarias gariepinus* (Burchell, 1822) Fingerlings

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Abstract: The effect of dietary Ascorbic acid (Vitamin C) at inclusions levels of (0, 50, 100, 150 and 200 mg AA per kg of feed) on the growth performance and nutrient utilization of African catfish *Clarias gariepinus* (Burchell 1822) fingerlings were investigated in a study that lasted ten weeks. *Clarias gariepinus* weighing 6.02±1.3 were randomly distributed into 5 treatment groups. A treatment contained three replicates of 10 fishes each. Results showed that L-ascorbic acid inclusion in the diet improved weight gain in Treatment 2, 3, 4 and 5. The highest Mean Weight Gained (MWG) and protein efficiency ratio was recorded in treatment 4 fed a diet of 150 mg AA kg. Growth performance and nutrient utilization parameters such as specific growth rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Feed Efficiency (FE) in all the treatments were significantly different at ($p < 0.05$). After week 8, fish fed scorbutic diet in Treatment 1 (0 mg kg) began to develop clinical deficiency including lordosis, broken skull, pigmentation and feed refusal. In all parameters considered Treatment 4 fed 150 mg AA kg gave the best growth performance and nutrient utilization efficiencies, while fish in Treatment 1 fed scorbutic diet recorded the highest mortality of 30% and lowest growth performance and nutrient utilization efficiencies. However fish in Treatment 5 fed the highest level of ascorbic acid supplementation of-200 mg AA kg did not perform better than fish in Treatment 4 fed 150 mg AA kg in terms of growth and nutrient utilization efficiencies.

Key words: Ascorbic acid, *Clarias gariepinus*, scorbutic, mortality, supplementation

INTRODUCTION

The nutritional quality of the feed is a major factor in sustaining healthy fish. It has been shown that the immune system of fish can be enhanced by the used of immuno modulators and antioxidants vitamins like Ascorbic acid (AA)[1]. The combination of good management and nutritional prophylaxis will produce better survival rates and improve growth in Aquaculture [2].

The inability of many fish species to synthesize ascorbic acid (AA) or Vitamin C, which is essential for fish growth, health and reproduction, is well documented [3,4]. Ascorbic acid must therefore be supplied feed. The symptoms associated with ascorbic acid deficiency are also well documented for many cultured fish [5,6].

Due to the multiple role of ascorbic acid in various metabolic pathways, a better understanding of the mechanism through which ascorbic acid as a nutritional element influences the immunological systems in modern intensive fish farming is necessary. It is therefore prudent to investigate the growth performance, nutrient utilization and to establish the ascorbic acid requirement on species by species basis.

Many authors have established the ascorbic requirement of some species of commercial importance. This has been summarized by [5]. However because of disparities in methodology and assessment criteria, there are considerable differences both between and within species in term of proposed requirement. Natural ascorbic acid is unstable and feed manufacturer must add more than the requirement to ensure adequate levels in feed processing. Recent studies indicated that inclusion of phosphate derivatives (Ascorbate-2-polyphosphate) were resistant to oxidation and retained ascorbic acid activity for fish [7].

The clariid catfish *Clarias gariepinus* (Burchell, 1822) is the most important fish species cultured in Nigeria, where the study was undertaken. This specie has shown considerable potential as a fish suitable for intensive aquaculture[8]. This fish grows rapidly, are resistant to disease and sress, sturdy and highly productive even in polyculture with many other fish species like Nile Tilapia *Oreochromis niloticus* [9]. However, there is dearth of work in both qualitative and quantitative ascorbic acid requirement of the African catfish *Clarias gariepinus*.

This study is therefore aimed at evaluating the optimum growth performance and nutrient utilization efficiencies of African catfish, *Clarias gariepinus* fed dietary L-ascorbic acid supplemented diet.

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MATERIALS AND METHODS

Experimental diets: Five isocalorific and isonitrogenous diets containing 40% crude protein and 12% lipid were formulated for fingerlings catfish, *Clarias gariepinus* in a 10 week trial experiment (Table 1). Ascorbic acid, commercially available as ROVIMIX STAY C- (Roche, Istanbul, Turkey) was used. Diet without ascorbic acid supplementation served as the control. Ascorbic acid supplementation in diets 2 to 5 were 50.0, 100.0, 150.0, 200.0 mg/kg respectively. All dietary ingredients were first milled to small particle size (approximately 250 um), 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 resistant mash was then passed without steam through a 0.9 mm die to obtain five stands which were sun dried immediately. Diets were broken up and sieved to convenient sizes and stored at (-18°C) prior to feeding.

Experimental fish and management: *C. gariepinus* fingerlings with average weight of 6.0±0.4g were randomly distributed into glass tanks (60×45×45cm) at ten fish per tank. Each treatment was in triplicates group of fish. Tanks supplied water from a borehole powered by 1.5HP pumping machine. Water temperature was maintained at 24±0.5 dissolved oxygen was kept at a saturation level of 6±0.1. The fish were fed acclimated to the experimental conditions and their respective diets for two weeks prior to the start of the feeding trial. The fish were fed with their respective diets at 5% body weight twice daily at 9.00 and 16.00 h throughout the duration of the experiment. Fish weights were determined at the 7th day of each week and the quantity of feed adjusted based on the changes in body weight of fish for subsequent feeding.

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

	Treatments				
Ingredients	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	0.00	50	100	150	200

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

1. Vitamins supplied mg 100 g diet: thiamine (B_1) 2.5 mg: riboflavin (B_2), 2.5 mg pyridoxine 2.0 mg: 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, cycobalamin (B_{12}), 10.0 mg; menadione (k), 2.0 mg. Minerals: $CaHPO_4$, 727.8 mg: Mg SO_4, 1275 mg , 60.0 mg; kcl 50.0 mg; $FeSO_4$, 250 mg, $ZnSO_4$, 5.5 mg; Mn_4SO_4, 2.5 mg $CuSO_4$, 0.79 mg; $CoSO_4$, 0.48 mg: $CaClO_3$, 0.3 mg; Cr Cl_3

Proximate composition: Proximate composition of diets and fish carcasses before and after experiment, were performed according to [10] for moisture content, fat, fibre and ash. Ascorbic acid was determined by semi-automated flourometric method as described by [10].

Performance evaluation: Fish performances during the experiment were based on productivity indices on growth performance and nutrient utilization efficiencies: Mean Weight Gain (MWG): Mean final weight - Initial weight
Total percentage weight gain (TPWG %) Total weight gain/Initial weight×100
Specific growth rate (SGR), $\log_e Wt - \log_e W_1) / T \times 100$

- Where Wt = Final weight (g), W_1 = Initial weight (g) and T = rearing periods (days)
- Feed conversion ratio (FCR) = (dry weight gain (g) / fish weight gain (g)
- Protein efficiency ratio (PER = (fish weight gain (g)/ protein fed (g))
- Feed efficiency = Live weight gain (g) / feed supplied (g)

Data analysis: Biological data generated were subjected to one-way analysis of variance (ANOVA). Where means were significantly different, they were compared with Duncan's multiple range test Zara [11].

RESULTS

Results obtained in this study were as shown in Table 1, 2 and 3. Table 2 shows the performance evaluation indices of *C. gariepinus* fed on the test diets. The results show that the productivity rates of the fish (SGR, WG, FCR, PER, FE) were significantly different ($p < 0.05$). Comparatively, fish fed on scorbutic (control) showed drastic reduction in weight and significantly different ($p < 0.05$) from other groups of fish fed ascorbic acid supplemented diets.

Fish fed 150 mg/kg ascorbic acid shows the highest % weight gain and specific growth rate of 212.12% ±1.16 and 1.61±1.20 respectively. The best feed conversion ratio (as fed basis) was also found in diet 4 which contained 150 mg kg ascorbic acid supplementation. However, highest mortality was recorded in Treatment one (Control) fed no ascorbic and supplementation as 30%, followed by Treatment two (50 mg kg^{-1}) ascorbic acid supplementation while no

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
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 (NFE)[1]	20.48	20.42	20.26	20.70	20.65
Added ascorbic acid mg kg	0.0	50.00	100.00	150.00	200.00
Measured ascorbic acid mg kg	0.64	56.20	109.70	165.90	204.83
Gross energy[2] kcal 100g	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: Cumulative growth performance, nutrient utilization and cost index of *Clarias gariepinus* fed varying levels of ascorbic acid

Parameters	Treatment 0	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Final weight	8.81±0.10^a	10.12±1.40^b	12.61±1.10^c	17.01±0.10^d	14.76±1.20^d
Initial weight (g)	5.94±0.30^a	6.02±0.50^a	6.06±0.40^a	6.05±0.30^a	6.02±0.20^a
Weight gain (g)	2.87±0.50^a	4.10±0.50^b	6.55±0.15^c	10.96±0.17^e	8.74±0.22^d
Daily weight gain (g)	0.04±0.02^a	0.06±0.02^b	0.10±0.02^c	0.16±0.60^e	0.12±0.03^d
Feed fed (g)	12.89±0.18^a	17.50±0.24^b	19.39±0.21^c	21.70±0.41^e	20.13±0.16^d
Daily feed int (g)	0.18±0.18^a	0.21±0.24^b	0.28±0.19^c	0.31±0.38^e	0.29±0.15^d
FCR	4.49±0.02^a	4.27±0.01^b	2.96±0.03^c	I.98±0.08^e	2.30±0.04^d
SGR	0.56±0.06^a	0.74±0.04^b	1.05±0.05^c	1.48±0.02^e	1.28±0.15^d
% Weight gain	48.32±0.71^a	68.11±0.65^b	108.09±0.9^c	181.16±1.5^e	145.18±0.89^d
Feed efficiency	0.22±0.12^a	0.23±0.13^a	0.39±0.10^b	0.51±0.14^c	0.43±0.11^c
Hepatosomatic index	0.05±0.13^a	0.08±0.05^a	0.26±1.03^b	1.39±0.05^d	1.01±0.04^c
Protein intake (g)	5.19±0.16^a	7.03±0.21^b	7.80±0.12^{bc}	8.71±0.12^c	8.07±0.10^c
PER	0.55±0.01^a	0.58±0.01^b	0.84±0.02^c	1.26±0.01^e	1.08±0.02^d
Pellet stability %	96.01^a	95.82^a	94.55^a	97.10^a	93.82^a
Cost index N/ kg	170.50^a	172.50^a	175.0^a	177.50^a	180^a
Mortality %	30^a	10^b	--^{c}	-^{c}	-^{c}

Figures in each row having the same superscripts are not significantly different (p<5)

mortality was recorded in Treatment 3, 4 and 5 which had 100, 150 and 200 mg kg respectively.

The results of the whole body proximate composition of the fish at the beginning and the end of the experimental period is presented in Table 3. The protein and lipid contents of fish showed a marked increase over the initial whole body composition, although, the protein values of fish were similar in all the treatments, there were significant difference ($p < 0.05$) in protein values of fish in Treatment one fed scorbutic diet which showed the lower values of protein than fish fed on ascorbic acid supplemented diet.

DISCUSSION

The result of this study shows the efficacy of ascorbic acid supplementation in the diets for the African clariid catfish *C. gariepinus*. Fish fed scorbutic diets showed dietary related mortality and morphological symptoms such as impaired collagen formations. The temperature (24.6±0.1) and dissolved oxygen (6.68± 0.1mg L) values are within the range recommended for African Catfish culture Boyd (1986).

Fish fed 150 mg kg AA supplemented diets showed the best growth profile during the feeding period. Fish in treatment one fed scorbutic diet showed reduction in growth rate and weight gain from the 8th week, this agreed with the results recorded by Li and Lovel [12] for Channel catfish fed scorbutic diets. This could be associated to the ascorbic acid variation in the diets, which was the only heterogeneous factor in the experimental diets. However fish in treatment 5 fed 200 mg kg Ascorbic acid did not perform better than treatment 4, which was fed 150 mg kg Ascorbic acid supplemented diets this agreed with the Halver *et al*., [5] that there was no effect of feeding a megadose of Ascorbic acid supplemented diet in Channel catfish than required by the fish. The mechanism behind this effect is postulated to be decrease synthesis of bile from cholesterol in Ascorbic acid deficiency through inhibition of the rate of limiting enzyme cholesterol-7-a-hydroxylase.

Nutrient utilization indexes (BWG, SGR, FCR and PER) in fish fed scorbutic diets was poor as was also recorded by Baker *et al*., [13] in diets of Channel catfish fed ascorbic acid supplemented diets, the nutrient utilization of fish in treatment 2 to 4 increased with increasing level of ascorbic acid supplementation resulting in an improved nutrient utilisations of the catfish. the trend of performance agrees favourably with the findings of [6,9,14], that ascorbic acid, a-tocopheroland niacin supplementation improves growth performance and nutrient utilization of Clarias gariepinus.

CONCLUSION

A dose of 150 mg kg ascorbic acid supplementation is therefore recommended in the diet of African Catfish, Clarias gariepinus, this is higher than the NRC [15] recommendation of 100 mg kg for Channel catfish. However the role of ascorbic acid in free radicals protection and its interaction with other antioxidants like vitamin E should be studied in fish nutrition.

REFERENCES

1 Verlhac, V. and Gabaudan, 1997. The effect of Vitamin C on fish health. www.roche.com /nutrition.

2. Fasakin, E.A., 1997. Studies on the use of Azolla africana DESV (WATER FERN) and spirodela polyrrhiza L. schleiden (Duckweed) as feedstuffs in production diets for *Orechromis niloticus* and *Clarias gariepinus* fingerlings. Ph.D thesis, Dept. Fisheries and Wildlife, Federal University of Technology, Akure, Ondo State, Nigeria.

3. Dabrowski, K., 1990. Ascorbic acid status in the early life of white fish (*Coreyonus lavaretus* L.). Aquaculture, 84: 61-70.

4. Soliman, A. K., K. Jauncey and R.J. Roberts, 1986.The effect of varying forms of dietary ascorbic acid on the nutrition of juvenile tilapias (*Oreochromis niloticus*) Aquaculture, 52: 1-10.

5. Halver, J.E., 1989. The Vitamins. In: Fish Nutrition, 2nd (Edn.) (Ed. By J. E. Halver). Academic Press New York. NY, pp: 32-102.
6 Sadnes, K., O. Torrisen and R. WaagbΦ, 1992. The minimum dietary requirement of Vitamin C in Atlantic salmon (Salmon salar) fry using Ca ascorbate-2-monophosphate as dietary source. Fish phiscol. Biochem., 10: 315-319.
7. Abdelghany, A. E., 1996. Growth response of Nile tilapia *Oreochromis niloticus* to dietary L-ascorbic acid, L-ascorbnyl-2-sulfateand L-ascorby1-2-polyphosphate. J. World Aquaculture. Soc., 27: 449-455.
8. Balogun, A.M. and K. Dabrowski, 1992. Improvement in the nutritive quality of soybean meal by co-ensiling with under-utilized fish discards. Proc. Fish Soc. Nig., 10: 75-86.
Boyd, C.E., 1986. Water quality in warm water fish ponds. Agricultural experiment J. Aubum University, pp: 35.
9. Fagbenro, O.A., A.M. Balogun and E.A. Fasakin, 1997. Dietary methionine requirement of the African catfish *Clarias gariepinus*. J.Applied Aquaculture (In Press).
10. AOAC, 1990. Association of Official Analytical Chemists. Official method of analysis. (15th Edn) vol. 1. (K. Heltich Ed.), Arlington, Virginia.
11. Zar, J.H., 1984. Biostatistical analysis.Prentice-Hall, Englewood Cliffs, New Jersey. USA.
12. Li, Y. and R.T. Lovell, 1993. Vitamin C and disease resistance in channel catfish (*Ictalurus punctatus*), Can. J. Fish Aquat. Sci., 39: 948-951.
13. Baker, R.T.M., P. C. Morris and S.J. Davies, 1998. Nicotinic acid supplementation of diets of the African catfish, *Clarias gariepinus* (Burchell). J. Aq. Res., 29: 791-799.
14. Odubanjo, A.B., 2001. The utilization and growth Response of African Catfish (*Clarias gariepinus*) fingerlings fed diets with varying levels of livestock vitamin premix (Growers) inclusions. A project submitted to Department of Wildlife and Fisheries Management, University of Ibadan, Nigeria.
15 NRC., 1993. (National Research Council). Nutrient Requirement Fish. National Academy Press.Washington D.C., pp: 114.