Journal of Animal and Veterinary Advances 6 (1): 90-93, 2007

Quantitative Requirements of Dietary Ascorbic Acid Supplementation in the Diets of African Catfish *Clarias gariepinus* (Burchell 1822) Fingerlings

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Abstract: This study was undertaken to evaluate the quantitative requirement of dietary ascorbic acid supplementation in the diet of African catfish, *Clarias gariepinus*, using growth performance, nutrient utilization. *Clarias gariepinus* fingerlings weighing 6.02±0.4 g 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. Ascorbic Acid (AA) was supplemented in the diets as ascorbyl-2-polyphosphate. Each treatment had varying levels of Ascorbic Acid (AA) supplementation, 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 1600 h. Weekly weighing of fish was done and the data collected were subjected to statistical analysis. Biological evaluation of the fish was based on growth performance and nutrient utilization efficiencies. At the end of week 4, fish fed scorbutic diets (diets without Ascorbic acid supplementation) had significantly lower weight than fish fed AA supplemented diets ($p<0.05$). The results showed that the productivity indices, mean Body Weight Gain (BWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Feed Efficiency Ratio (FER) in all the treatment were significantly different from each other at ($p<0.05$). The best Feed Conversion Ratio (FCR) was recorded in treatment four as 0.94.±0.08 and the poorest FCR was obtained in treatment 1 (Control) fed scorbutic diet as 1.77±1.20. In all parameters considered, treatment 4 fed 150 mg AA kg^{-1} gave the best growth performance and nutrient utilization efficiencies, while treatment 3 fed 100 mg AA kg^{-1} showed the minimum dietary AA supplementation that prevented growth reduction in this study.

Key words: Ascorbic acid, quantitative, scorbutic

INTRODUCTION

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 (Dabrowski, 1990; Soliman *et al*., 1986). Ascorbic acid must therefore be supplied via feed especially in intensive fish farming. The symptoms associated with ascorbic acid deficiency are also well documented for many cultured fish (Halver *et al*., 1989; Sadnes *et al*., 1992). 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 immune systems in modern intensive fish farming is necessary. It is therefore prudent to investigate and establish the ascorbic acid requirement of catfish on species by species basis.

Many authors have established the ascorbic requirement of some species of commercial importance. This has been summarized by Halver (1989). Natural ascorbic acid is unstable. Recent studies indicated that inclusion of phosphate derivatives (Ascorbate-2-polyphosphate) were resistant to oxidation and retained ascorbic acid activity for fish (Abdelghany, 1996).

The clariid catfish *Clarias gariepinus* 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 (Balogun and Dabrowski, 1992). This fish grows rapidly, are resistant to disease and stress, sturdy and highly productive even in polyculture with many other fish species like Nile tilapia *Oreochromis niloticus* (Fagbenro *et al*., 1997). However, there is scarcity of work in both qualitative and quantitative ascorbic acid requirement of the African catfish *Clarias gariepinus*. This study was therefore aimed at determining the quantitative requirement of dietary ascorbic acid in the diets African catfish, *Clarias gariepinus* fingerlings.

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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 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 to 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 resistant mash was then passed without steam through a 0.9mm die to obtain five stands which were sun dried immediately. Diets were broken up and sieved to convenient sizes and stored in the refrigerator prior to feeding.

Experimental fish and management: *C. gariepinus* fingerlings with average weight of 6.0±0.4 g were randomly distributed into glass tanks (60×45×45 cm) 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 with their respective diets at 5% body weight twice daily at 9.00 and 16.00 hours 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.

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).

Performance evaluation: Fish performances during the experiment were based on productivity indices on growth performance and nutrient utilization efficiencies as described by Fasakin *et al.* (2003) as follows:

Mean Weight Gain (MWG): Mean final weight-Initial weight

Total Percentage Weight Gain (TPWG %)

Total weight gain / Initial weight × 100

Specific Growth Rate (SGR), loge Wt - Loge Wi) / T × 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 obtained were subjected to one-way analysis of variance (ANOVA). Where means were significantly different, they were compared with Duncan's multiple range test (Zar, 1984).

Table 1: The experimental diet composition in g 100 g^{-1} 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^{-1}	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: $MgSO_4$, 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

RESULTS

Results obtained in this study were as shown in Table 1-4. Table 3 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 diets, (without ascorbic acid supplementation) showed drastic reduction in weight and significantly different ($p< 0.05$) from other groups of fish fed ascorbic acid supplemented diets.

Fish fed 150mg/kg ascorbic acid showed the highest percentage weight gain and specific growth rate of 1057.12±1.5 and 3.50±0.05, respectively. The best feed conversion ratio (as fed basis) was also found in diet 4 which contained 150 mg kg^{-1} 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 mortality was recorded in Treatment 3, 4 and 5 which had 100, 150 and 200 mg kg^{-1}, respectively.

The results of the proximate composition of catfish carcass, at the beginning and the end of the experimental period were presented in Table 4. 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.

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^{-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[2] kcal $100g^{-1}$	431.30	429.00	429.40	429.70	427.50

[1]Itrogen free extract: Calculated as 100- (crude protein + ash + crude fibre + ether; [2]Gross energy (kcal 100 g^{-1}) based on 5.7 kcal protein; 9.5 kcal g^{-1} lipid; 4.1 kcal g^{-1} carbohydrate

Table 3: Cumulative growth performance, nutrient utilization and cost index of *Clarias gariepinus* fed varying levels of ascorbic acid

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Final weight	18.81±0.10^a	32.12±1.40^b	40.61±1.10^c	70.01±0.10^e	50.76±1.20^d
Initial weight (g)	6.06±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)	12.75±0.50^a	26.10±0.50^b	34.55±0.15^c	63..96±0.17^e	44..74±0.22^d
Daily weight gain (g)	0.18±0.02^a	0.37±0.02^b	0.49±0.02^c	0.91±0.60^e	0.64±0.03^d
Feed fed (g)	22.57±0.18^a	38.54±0.24^b	45.26±0.21^c	60.10±0.41^e	50.56±0.16^d
Daily feed int. (g)	0.32±0.18^a	0.55±0.24^b	0.70±0.19^c	1.20±0.38^e	0.87±0.15^d
FCR	1.77±0.02^a	1.48.±0.01^b	1.28±0.03^c	0.94±0.08^e	1.13±0.04^d
SGR (%)	1.61±0.06^a	2.38±0.04^b	2.72±0.05^c	3.50±0.02^e	3.03±0.15^d
Weight gain (%)	310.40±0.71^a	433.60±0.65^b	570.13±0.9^c	1057.20±1.5^e	743.19±0.89^d
Feed efficiency	0.56±0.12^a	0.68±0.13^a	0.76±0.10^b	1.06±0.14^c	0.88±0.11^c
Protein intake (g)	9.03±0.16^a	15.41±0.21^b	18.1±0.12^{bc}	24.04±0.12^c	20.22±0.10^c
PER	1.41.±0.01^a	1.69±0.01^b	1.91±0.02^c	2.66±0.01^e	2.21±0.02^d
Pellet water stability (%)	96.01^a	95.82^a	94.55^a	97.10^a	93.82^a
Cost index N kg^{-1}	30.60^a	55.20^b	70.00^c	90.00^d	124.25^e
Mortality %	30^a	10^b	0.00^c	0.00^c	0.00^c

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

Table 4: Proximate composition (% wet weight) of the carcass of *Clarias gariepinus* fed experimental diets containing varying inclusion levels of ascorbic acid

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

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

DISCUSSION

The result of this study showed the efficacy of ascorbic acid supplementation in the diets for the African clariid catfish *C. gariepinus*. Fish fed scorbutic diets showed dietary related mortality. The temperature (24.6±0.1) and dissolved oxygen (6.68±0.1 mg L^{-1}) values were within the range recommended for African Catfish culture Boyd (1986). Fish fed 150 mg kg^{-1} 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 Lovell (1985) 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.

Nutrient utilization indexes (BWG, SGR, FCR and PER) in fish fed scorbutic diets was poor as was also recorded by Baker *et al*., (1998) 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 utilizations of the catfish. The trend of performance agreed favourably with the findings of Sadnes *et al*. (1992) and Baker *et al*. (1998), that ascorbic acid, α-tocopherol, improved growth performance and nutrient utilization of *Clarias gariepinus*. A dose of 150 mg kg^{-1} ascorbic acid supplementation which gave the best nutritional performance is therefore recommended in the diet of African Catfish, *Clarias gariepinus*

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