African Journal of Biotechnology Vol. 6 (13), pp. 1559-1567, 4 July 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Vitamin C (ascorbic acid) requirements of *Heterobranchus longifilis* fingerlings

Ibiyo, L. M. O.[1], Atteh, J. O.[2], Omotosho, J. S.[3] and Madu, C. T.[1]

[1]National Institute for Freshwater Fisheries Research, New Bussa, Niger State, Nigeria.
[2]Department of Animal Production, Faculty of Agriculture, University of Ilorin, Nigeria.
[3]Department of Zoology, Faculty of Sciences, University of Ilorin, Nigeria.

Accepted 4 June, 2007

A feeding experiment was conducted to quantify the minimum dietary vitamin C requirement of *Heterobranchus longifilis* fingerlings. Fish were fed a basal diet with 42.5% crude protein for a conditioning period of 2 weeks. Following conditioning, fingerlings with initial mean weight, 2.3 ± 0.3 g were stocked as groups of 20 fingerlings into 30 litre tanks in a mini-flow through experimental system. Graded levels (0, 50, 100, 150, 200 and 250) mg of L-ascorbic acid kg^{-1} diet was included into the basal diet by replacing part of the silica component and fed to triplicate groups for 20 weeks. Fish fed the control (0 mg vitamin C kg^{-1}) diet exhibited deficiency signs including lordosis, caudal fin deformity, skin erosion and significantly ($P < 0.05$) suppressed weight gain and higher condition factor. Protein efficiency ratio and specific growth rate were significantly ($P < 0.05$) improved with increasing levels of vitamin C up to 200 mg kg^{-1} diets. Tissues (liver, kidney, gills and muscle) ascorbate concentration generally reflected dietary inclusion levels with significant ($P < 0.05$) lowest level occurring in the control groups. The dietary requirement based on least mean squares error regression analysis of weight gain and specific growth rate data on inclusion level of vitamin C was 82.2 ± 0.2 mg vitamin C kg^{-1} diet which corresponds to 100 mg of vitamin C kg^{-1} diet.

Key words: Vitamin C, requirement, *Heterobranchus longifilis*, tissue concentration.

INTRODUCTION

Vitamin C (ascorbic acid) belongs to the water-soluble group of vitamin. Its importance in fish diets and characteristics had been described by various authors (Lim and Lovell, 1978; De Silva and Anderson, 1995; Dabrowski, 2001). Ibiyo et al. (2006) also has a detailed account of its importance. Lack of gulonolactone oxidase responsible for the synthesis of vitamin C in many fish's liver and kidney (Dabrowski, 1990; Fracalossi et al., 2001) demands dietary inclusion to meet the nutritional requirement for optimum performance of the developing fish (Dabrowski, 1990; Ai et al., 2004).

Heterobranchus species is now widely cultured in Nigeria. Its protein requirements have been established as 40 and 42.5% (Fagbenro et al., 1992; Eyo, 1995) respectively. The preliminary work to test whether *H. longifilis* needs vitamin C in its nutrition revealed that vitamin C is a necessity in this fish species (Ibiyo et al., 2006) unlike *Sturgeons acipenser* that possess gulonolactone dehydrogenase and can synthesize ascorbic acid from glucose, therefore having little dependence on dietary sources for vitamin C (Dabrowski, 2001b). Nevertheless, there is a dearth of information on the actual level of vitamin C required by *Heterobranchus longifilis* for optimum growth and healthy performance. Although, the previous study revealed that 50 mg vitamin C kg^{-1} diet was able to eliminate the deficiency symptoms observed in the group that was devoid of vitamin C, it might not be the optimum level (Ibiyo et al., 2006). This work was designed to study the vitamin C requirements of *H. longifilis* fingerlings and the minimal dietary inclusion levels that will meet these requirements after degradation from feed processing and storage.

MATERIALS AND METHODS

Experimental diets

The basal experimental diets was formulated with the commonly available ingredients and vitamin C-free premix (Table 1) to meet

*Corresponding author. E-mail: oniviemercy@yahoo.com. Tel +2348059241879.

Table 1. Composition of the experimental diets.

Ingredients	% Inclusion levels					
	Diet 1*	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Vitamin C carrier	0.00	0.015	0.03	0.045	0.06	0.07
Silica	0.08	0.065	0.05	0.035	0.02	0.005
Clupeid meal (65%)	30.00	30.00	30.00	30.00	30.00	30.00
Soybean meal (45%)	35.00	35.00	35.00	35.00	35.00	35.00
Groundnut cake (40%)	13.82	13.82	13.82	13.82	13.82	13.82
Maize bran (12.5%)	13.35	13.35	13.35	13.35	13.35	13.35
Vegetable Oil	2.00	2.00	2.00	2.00	2.00	2.00
Starch	2.00	2.00	2.00	2.00	2.00	2.00
Bone meal	1.50	1.50	1.50	1.50	1.50	1.50
Vitamin C free Premix**	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.50	0.50	0.50	0.50	0.50	0.50
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100

*Basal diet and analysed proximate composition include crude protein = 42.6%; crude fat = 11.55%; crude fibre 2.2%; total ash = 8.89; nitrogen free extract = 27.91 and moisture = 7.19%. Calculated metabolizable energy is 375 Kcal Kg^{-1}.

**Provides per kg diet: Vitamin A, 50000 IU, Vitamin D_3 25000 IU, Vitamin E 160 mg, Vitamin K 8 mg; Vitamin B_1 12 mg; Vitamin B_2 22 mg; Vitamin B_6 20 mg; Vitamin B_{12} 220 mg; Biotin 4 mg; Zinc 320 mg; Iodine 6 mg; Calcium pantothenate 46 mg; Cupper 34 mg; Cobalt 1.2 mg; Selenium 0.48 mg; Antioxidant 480 mg; and Choline chloride 0.1 mg.

the predetermined 42.5% crude protein requirement of *Heterobranchus* species (Fagbenro, 1992; Eyo, 1995). Six graded levels of vitamin C (L-ascorbic acid, AA) obtained from Tuyil Pharmaceutical Industries Limited, Ilorin) at 0, 50, 100, 150, 200 and 250 mg Kg^{-1} diets were included in the basal diet. The inclusion was achieved through substitution of the silica content (Table 1). L-ascorbic acid was used due to its ready availability to farmers in this zone. The ingredients were grinded, milled, weighed, mixed and pelleted with meat mincer through a 2 mm die. After cold pelleting, the feeds were air dried and put in an air-tight container. Samples were taken for proximate and vitamin C analysis to determine percentage retention of vitamin C immediately after feed processing.

Experimental fish and feeding regime

Fingerlings of *H. longifilis* were obtained from National Institute of Freshwater Fisheries Research's Hatchery, where the study was performed and conditioned for 2 weeks prior to the start of the experiment. During acclamation they were fed the basal diet which finally served as the control diet (Table 1). Feed supply was stopped 2 days to the commencement of the feeding experiment. After acclimatization the seven weeks old fingerlings (with average initial weight of 2.3 ± 0.3 g) were weighed into 30 litre circular plastic tanks in a mini-flow through experimental system previously described by Madu (1989). The replicates with 20 fingerlings each were randomly allocated to the treatments in a completely randomised design set up. Some of the fingerlings were sacrificed and taken for analysis of initial vitamin C content and proximate analysis. Each of the six dietary treatments was fed for 20 weeks to the randomly assigned triplicate tanks.

A fixed feeding regime of 5% body weight per day divided into two and given between the hours of 800 – 900 and 1700 – 1800 was adopted except on sampling days, time of feeding was altered. The feed supply at 5% body weight continued in the first 8 weeks of the experimental period after which it was reduced to 3% body weight per day due to poor feed acceptability of the control group. Fish were fed daily, tanks scrubbed and drained at two days intervals to prevent microbial growth that could possibly alter the experiment. Feed preparation was carried out bi-weekly to prevent long storage.

Measurements and sample analysis

Sampling was carried out fortnightly with the fish bulk weighed per tank and feeding rate subsequently adjusted. During each weighing, time of draining and washing of tanks mortality and the condition of fish were observed and recorded. Photographs of fish with observed abnormalities were taken with a Digital still Camera mvc-FD92 with a zoom distance of 0.0 m at each time of occurrence. At the end of the experiment, length and weight were taken from individual fish per replicate. Blood samples were obtained using a heparinized syringe from the caudal vein and pooled for blood parameter analysis. Six (6) fish per replicate of diets 2 to 6 and three (3) in diet 1 (control treatment) were sacrificed for kidney, liver, gills and whole body samples which were pooled from fish of each replicate to determine vitamin C content and proximate analysis of the final fish. X-Ray of fish from control and vitamin C supplements groups were taken at the General Hospital New Bussa, Niger State, Nigeria. Micrographs of the gills were also taken with ZEISS-Stemi 2000-C and the film was developed and printed by Bob Shege Photo Laboratory, New Bussa.

The water quality parameters were monitored during the trial by the staffs of Liminology Division of National Institute for freshwater fisheries research and average value for temperature, dissolved oxygen, hydrogen ion concentration (pH) and conductivity were 29.5°C, 5.8 mg l^{-1}, 7.2 units and 220 µmhos cm^{-3} respectively.

Proximate composition was carried out using the Association of Analytical Chemists (AOAC, 2000) methods. Crude fat was determined using petroleum ether (40 – 60 Bp) extraction method with Soxhlet apparatus. Vitamin C concentrations were determined by

Table 2. Vitamin C retention of *Heterobranchus longifilis* fingerlings.

Diets	Vitamin C content immediately after feed processing		Vitamin C content after 1 week of storage	
	mg kg-1 of diet	%	mg kg-1 diet	%
1	0.01	0	0.0	0
2	39.5	79.0	37.1	74.3
3	82.2	82.2	77.3	77.3
4	122	81.3	114.7	76.5
5	162	81.5	152.3	76.1
6	206	82.4	193.6	77.5

dinitrophenylhydrazine (NDPH) spectrophotometric method as described by Bai and Gatlin (1992). One gram of tissue was added to 9 ml, 5% trichloroacetic acid (TCA) at 4°C on ice, homogenized with a homogenizer (Heidolph, Keiheim, Germany) for dispersion of the sample, centrifuged homogenates at 12,000 g at 4°C for 10 min and the supernatant extracts were analysed for vitamin C concentrations. The interfering substances in tissues were accounted for from the final absorbance (Dabrowski and Hinterleitner, 1989). The Pack cell volume (PCV) was determined by the microhaematocrit method and haemoglobin was determined using the cynomethaemoglobin method and the coulter haemoglobinometer (Coulter, U.K.).

Calculations and statistical analysis

The following variables were calculated:

Weight Gain (WG) = $W_t - W_0$
Specific growth rate (SGR) = (In W_t – In W_0) X 100 t^{-1}
Feed efficiency ratio (FER) = Wet WG in g dry feed fed in g^{-1} (Hardy and Barrows, 2002)
Condition factor = Wt X 100 L^{-3}
Survival = N_t X 100 N_0^{-1}

Where WG is weight gain; W_t and W_0 were final and initial fish weights, respectively; L is final length of each fish; N_t and N_0 were final and initial numbers of fish in each replicate, respectively; and t is the experimental period in days.

The data obtained from the trial were subjected to one-way analysis of variance (using Microsoft Excel programme) to test for effects of dietary treatments. When ANOVA identified significant difference among groups, multiple comparison tests among means were performed using Duncan's new multiple range test (SPSS 10.0 in a Computer). For each comparison, statistically significant differences were determined by setting the aggregate type I error at 5% ($P < 0.05$). Weight gain and specific growth rate data were also subjected to least square regression analysis to estimate the optimum dietary vitamin C requirement of *H. longifilis* fingerlings. The weight gain and survival were plotted against vitamin C levels their point of interception was used to estimate the minimum requirement of vitamin C by *H. longifilis* for an average survival. The average of vitamin C levels before and after the point of interception was taken for the estimate.

RESULTS

Vitamin C retention

The results of vitamin C retention analysis are presented in Table 2. The greater the level in the diet the more the percentage lost during feed processing.

Fish growth

The visual fish performance, weights gain (WG) and specific growth rates (SGR) were relatively similar in all the treatments in the first 7 weeks of the feeding trial. The reverse was the case at the end of the eighth week as differences in these parameters and others were apparent. Fish fed the diet devoid of vitamin C exhibited significantly ($P < 0.05$) poor growth (Table 3). Weight gain was significantly ($P < 0.05$) different between the control group and those with varying levels of vitamin C. Average WG and SGR increased significantly ($P < 0.05$) with increasing dietary ascorbic acid level and tend to reach a plateau at 100 mg AA kg^{-1} diet and above up to 200 mg AA kg^{-1} diet beyond which, a significant ($P < 0.05$) decline sets in. The fish that received the vitamin C free diet had significantly ($P < 0.05$) higher condition factor with slight differences within the group that received the diets supplemented with vitamin C (Table 3) which was not statistically significant ($P > 0.05$). The significant higher condition factor of the control group was probably due to the congestion of the vertebra which contributed to the overall significant poor growth and reduced length performance. Survival of the fish increased significantly ($P < 0.05$) with dietary vitamin C inclusion (Table 3). There was no significant difference ($P > 0.05$) between all the groups that received the vitamin C supplemented diets with respect to survival. Hepathosomatic index (HIS) showed no significant ($P > 0.05$) difference.

Nutritional performance

Generally, FER showed similar pattern to that of WG. Protein efficiency ratio (PER) was significantly ($P < 0.05$) higher in the group fed diet supplemented with 100 mg AA Kg^{-1}. The protein efficiency ratio significantly ($P < 0.05$) increased from 0.92% to 1.35% with increasing dietary level of vitamin C with the best achieved with 100 mg AA kg^{-1} diet supplementation. There were significant ($P < 0.05$) progressive improvement in feed conversion

Table 3. Effects of vitamin C on growth parameters, liver ascorbate concentration and haematology of *H. longifilis* fingerlings fed the diets with graded levels of vitamin C (0 – 20 weeks).

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	SEM
Growth indices							
Weight gain (g fish^{-1})	22.40^{d}	35.2^{c}	65.9^{a}	66.2^{a}	66.3^{a}	55.7^{b}	0.637
Feed conversion ratio	2.56^{a}	1.92^{c}	1.73^{d}	1.76^{d}	1.73^{d}	2.02^{b}	0.018
Feed Efficiency ratio (%)	38.95^{d}	51.98^{b}	57.47^{a}	56.49^{a}	57.53^{a}	49.34^{c}	0.511
Protein efficiency ratio	0.92^{e}	1.22^{cb}	1.35^{a}	1.25^{b}	1.14^{dc}	1.12^{d}	0.029
Specific growth rate (%)	1.66^{c}	2.11^{b}	2.37^{a}	2.38^{a}	2.43^{a}	2.20^{b}	0.042
Survival (%)	50.00^{a}	83.33^{b}	93.33^{b}	95.00^{b}	95.00^{b}	96.66^{b}	6.230
Condition factor	0.95^{a}	0.74^{c}	0.81^{ba}	0.79^{bc}	0.82^{ba}	0.81^{ba}	0.008
Tissue Ascorbic acid content							
Liver (µg g^{-1} tissue)	10.86^{d}	45.83^{c}	57.4^{b}	67.93^{a}	67.93^{a}	67.90^{a}	2.651
Kidney (µg g^{-1} tissue)	18.30^{a}	42.10^{d}	66.10^{c}	80.63^{b}	98.46^{a}	98.53^{a}	2.663
Gills (µg g^{-1} tissue)	26.00^{f}	41.50^{e}	47.53^{d}	57.00^{c}	69.33^{b}	82.50^{a}	0.063
Muscle (µg g^{-1} tissue)	5.23^{e}	19.10^{d}	27.5^{c}	29.23^{b}	37.86^{a}	38.30^{a}	0.301
Haematological indices							
Haematocrit (%)	22.55^{b}	27.14^{a}	26.86^{a}	26.45^{a}	21.60^{b}	22.42^{b}	0.238
Haemoglobin conc. (mg dl^{-1})	7.51^{b}	9.045^{a}	8.95^{a}	8.815^{a}	7.20^{b}	7.47^{b}	0.079

a-e: Means in each row with different superscripts are significantly different (P < 0.05).
Conc. = Concentration

Table 4. Proximate composition of fish prior and after the feeding trial (0 -20 weeks).

Parameters	Initial fish	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	SEM
Crude protein (%)	16.20	15.5^{d}	16.54^{c}	18.83^{a}	17.08^{c}	17.92^{b}	18.29^{b}	0.173
Crude fat (%)	4.65	3.45	3.65	1.85	3.55	4.15	4.05	
Crude fibre (%)	0.70	1.35	1.32	1.31	1.40	1.35	1.34	
Ash (%)	3.35	3.72	3.03	3.69	3.89	3.03	3.4	
NFE (%)	3.55	2.20	2.60	2.5	2.8	2.7	2.7	
Moisture content	71.55	73.78	72.86	71.82	71.28	70.85	70.22	

a-d: Means in each row with different superscripts are significantly different (P < 0.05).
NFE = Nitrogen free extract.

ratio (FCR) and protein efficiency ratio for fish fed diets with graded levels of vitamin C. With respect to FCR, the group that received 100, 150 and 200 mg AA kg^{-1} diet supplementation were not significantly (P > 0.05) different from each other while the vitamin C-free diet resulted in a significantly (P < 0.05) poor FCR and 50 mg AA kg^{-1} diet was significantly (P < 0.05) better than the 250 mg AA kg^{-1} diet supplementation (Table 3). Generally, there were significant (P < 0.05) differences in final carcass composition among treatments especially with respect to crude protein content. The fish fed diet devoid of vitamin C exhibited higher moisture content and a significantly (P < 0.05) depressed crude protein content whereas fish fed diet 3 (100 mg AA kg^{-1} diet) exhibited higher crude protein content (Table 4).

Clinical signs of vitamin C deficiency

Some deficiency symptoms of vitamin C were observed in the control treatment (diet 1) during the course of the experiment. The symptom of skin erosion (Figure 4) was visible at the 8th week of the study. Figure 5 shows the photographs of the skeleton of fish with symptom of lordosis (that is, twisting of the vertebra resulting into curvature of the fish) which was first observed in the 10th week and it continued to the end of the experiment with the affected fishes always seen lying prostrate at the bottom of the tank (Figure 6). Tail deformity (Figure 7) was also observed among the survivors of the fish fed control diet. The tail erosion was visible at the 14th week up till end of the study. These symptoms were visible in all the replicates of the control treatment while those with even the least level of vitamin C did not show any of these signs, though physical growth was not as encouraging as in those with higher levels. These symptoms resulted into deaths of most of the fish in the control group reflecting in the significant (P < 0.05) low survival. Figure 8 shows fish with and without vitamin C exhibiting disparity in growth. Figure 9 shows the gills of the fish with and without vitamin C. The X-Ray film was too dark

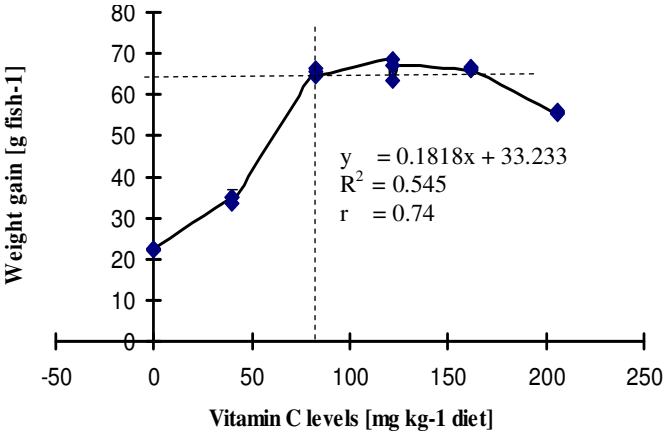

Figure 1. Effect of dietary vitamin C on the weight gain of *H. longifilis* fingerlings.

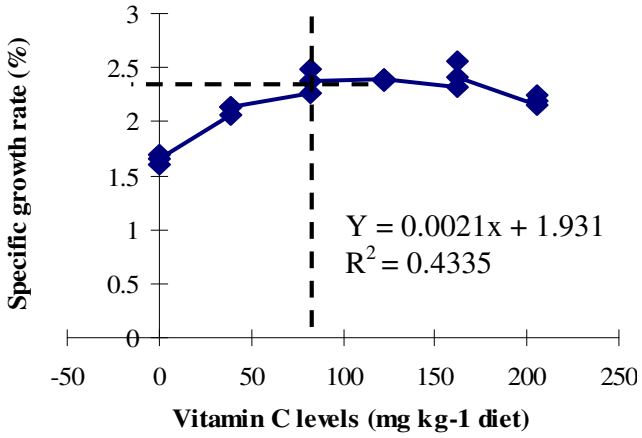

Figure 2. Effect of dietary vitamin C on the specific growth rate of *H. longifilis* fingerlings.

to be reproduced and so is not presented.

Tissue ascorbic acid concentration and haematological indices

There were significant ($P < 0.05$) difference in tissue ascorbate concentration and haematology of fish fed the graded levels of vitamin C. The vitamin C concentration in the liver, kidney, gills and muscle of fish fed graded levels of ascorbic acid were positively correlated with dietary levels of this vitamin. The liver tend to reach saturation at 150 mg AA kg^{-1} diet supplementation whereas the kidney and muscle tend to reach saturation at 200 mg AA kg^{-1} diet supplementation since there was no significant different between 200 and 250 mg AA kg^{-1} diet with respect to both tissues. However, there was still marginal increase as the level increases, while gills did not indicate any saturation effect as concentration significantly ($P < 0.05$) increased with dietary levels of vitamin C. The ascorbic acid analysis of whole body revealed that the initial fish contain 1.2 mg kg^{-1} carcass.

The haematocrit and haemoglobin improved significantly ($P < 0.05$) with increasing levels of dietary vitamin C. The 50, 100 and 150 mg AA kg^{-1} diet were significantly better than the 200, 250 and 0 mg AA kg^{-1} diet supplementation with respect to either of this parameter.

Estimation of vitamin C requirement

The responses of weight gain and specific growth rate to graded levels of dietary vitamin C were used to estimate the requirements. Due to the significant effect of this vitamin on these parameters, least mean square error regression analysis of the weight gain and specific growth rate data against graded levels of dietary vitamin C resulted in a requirement estimate of 82.2 ± 0.2 mg AA kg^{-1} diet which corresponds with the 100 mg AA kg^{-1} supplementation including that present endogenously before commencement of the trial (Figures 1 and 2). The plotting of weight gain and the survival on line graph with two axes indicates a minimum inclusion level of 60.85 mg AA Kg^{-1} to ensure 88.36% survival (Figure 3).

DISCUSSION

Majority of the fish species require a dietary supply of vitamin C to maintain normal growth because they lack the ability to convert L-gulonolactone to 2-keto-L-gulonolactone (NRC, 1993). In the present study, the survival and growth of *H. longifilis* fingerlings improved significantly with increasing supplementation of dietary ascorbic acid, and its growth reached a plateau at between 100 to 200 mg AA kg^{-1} diet beyond which a decline sets in. The decline might probably be due to hypervitaminosis (Table 3; Figure 1). These results further confirms that *H. longifilis* fingerlings needs adequate exogenous vitamin C to maintain normal growth and physiological functions as indicated in a prior trial by Ibiyo et al. (2006a, b) in an experiment to establish the necessity of an exogenous vitamin C in the nutrition of *H. longifilis* fingerlings. The significant differences also revealed that the supplemented groups would have performed better than that attained, if feed supply was not minimised after the 8^{th} week due to unacceptability of feed exhibited by the control group. These results agree with previous studies on some other fish (Eya and Mgbenka, 1990; Al-Amoudi et al., 1992; Gouillou-Coustans et al., 1998; Shiau and Hsu, 1999; Sealey and Gatlin, 1999; Wang et al., 2003; Ai et al., 2004). The reduction in growth performance of fish fed the control diet in the present study seems to indicate that AA has a specific effect on growth as first suggested by Ram (1966). Supplemental vitamin C levels above 100 mg Kg^{-1} diet resulted in no substantial additive effect on weight gain and specific growth rate (Table 3; Figures 1 to 3).

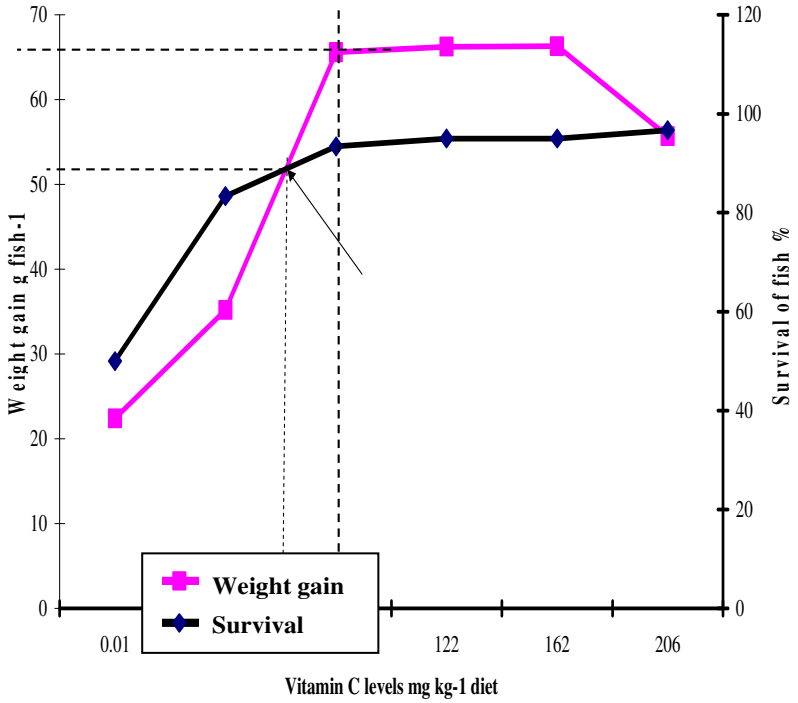

Figure 3. Effects of vitamin C levels on weight gain and survival of *H. longifilis* fingerlings for determination of minimum inclusion level for above 80% survival (arrow showing intersection point between the two parameters).

Figure 4. Fish showing opercula skin erosion.

The significant improvement of whole body crude protein composition of the fish is an indication of the importance of vitamin C in body protein metabolism. Such improvement was also observed in tilapia (Soliman et al., 1994). Vitamin C is an essential coenzyme in certain oxidative processes, including the oxidation of tyrosine and phenylalanine (Brander and Pugh, 1977). This probably explains the differences that occur in the weight gain and whole body crude protein content with respect to the vitamin C free and enriched groups.

The deficiency symptoms such as retarded growth (Figure 8), convulsive movement, opercula skin erosion (Figure 4), spinal deformity (Figures 5 and 6), tail deformity (Figure 7), are well known in many other fish species including salmonids (Dabrowski et al., 1996), ictalurids (Lim and Lovell, 1978; Miyasaki et al., 1985), cyprinids (Dabrowski et al., 1988), cichlids (Soliman et al., 1994; Shiau and Hsu, 1995), scophtalmids (Coustans et al., 1990), African catfish (Eya and Mgbenka, 1990) Korean rockfish (Lee et al., 1998) and red drum (Aguirre and Gatlin, 1999) that were fed vitamin C deficient diets. The time of occurrence of deficiency symptoms in the group devoid of vitamin C in this trial is an indication that *H. longifilis* fingerlings were able to depend on stored ascorbate for nine weeks (that is, 2 weeks of acclamation plus 7 weeks into the trial period) for normal physiological

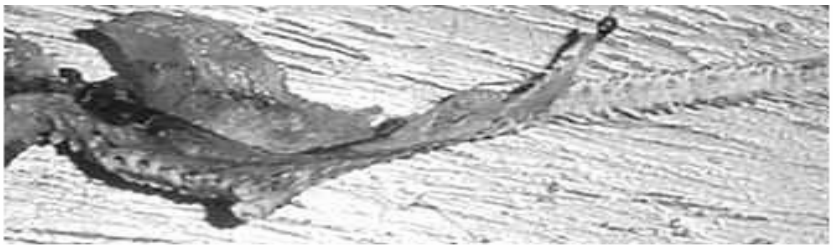

Figure 5. Vertebra colon of Fish showing deformity between 8th to 11th vertebrae.

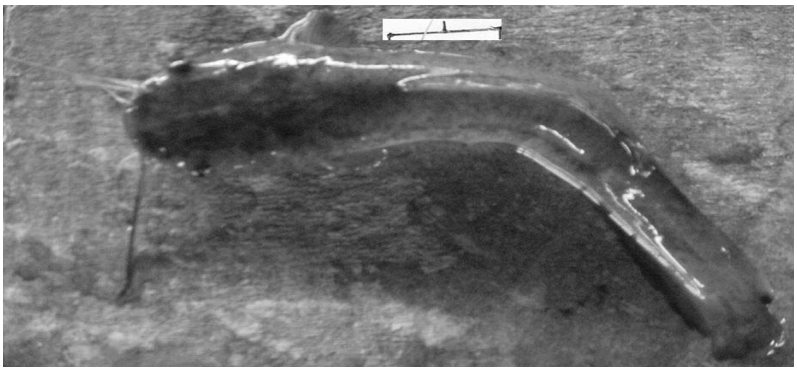

Figure 6. Fish fed vitamin C free diet showing symptoms of lordosis.

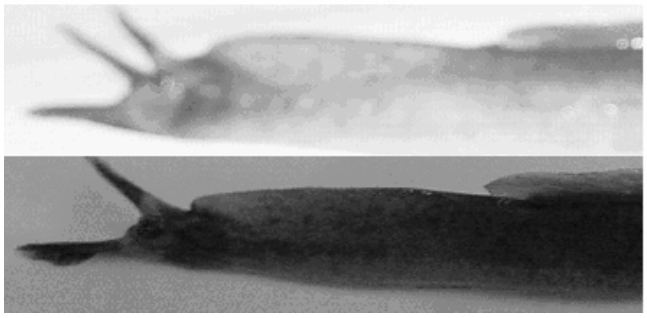

Figure 7. Fish showing tail erosion.

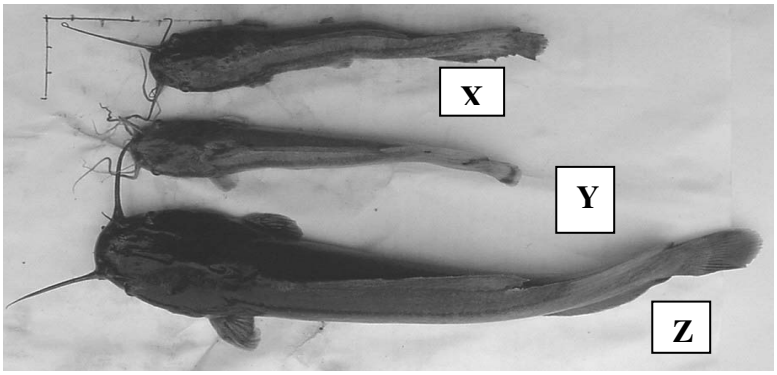

Figure 8. Fish showing disparity in growth and skin discolouration. X, Y is Fish fed vitamin C-free diet (stunted and deformed) and Z is fish fed vitamin C supplemented diet (normal). (Photographed at week 20; Scale: each space represent 1.0 cm).

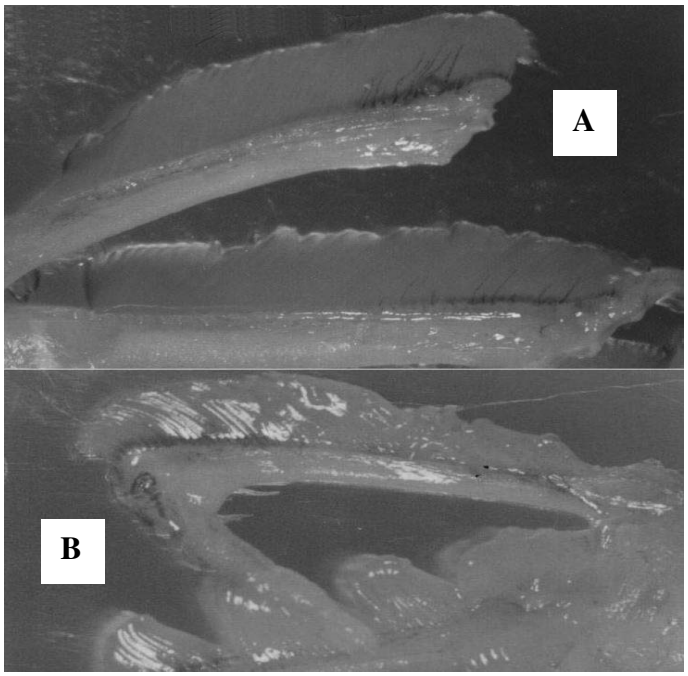

Figure 9. A and B are micrographs of gills with normal and clubbed filaments from fish fed diet supplemented with 50 and 0 mg vitamin C kg^{-1} respectively. (M = 10 x 123).

functions. This time is, however, longer than that observed in juvenile Olive flounder, *Paralichthys olivaceus* (Wang et al., 2002) which exhibited symptoms after 4 weeks into the trial period but similar to those observed in those fish mentioned above. The earlier occurrence of symptoms in Olive flounder was attributed to size of the fish used, the recirculating experimental system used which is more stressful and may require a higher amount of vitamin C than those reared in a flow through system used in some other experiment and the type of diet (Wang et al., 2002) flow through was also used in this present study. Clubbed gill filaments observed in some of the fish devoid of vitamin C (Figure 9) was an indication of the importance of vitamin C in the proper development and functioning of the gills. Gill is a very prominent organ in the wellbeing of the fish as it is the major interface with the environment where most of the exchange between the fish and its environment take place, the surface area of the gills being approximately twice that of the skin. As such, they are critical in mediating environmental effects (Muir, 1984). This might probably be one of the reasons why the group fed vitamin C deficient diet exhibited such a significantly pronounced poor growth in this present study.

The tissue ascorbate content showed a positive correlation with dietary inclusion of vitamin C. Although liver ascorbate concentration may not be a sensitive indicator of vitamin C deficiency, it has been widely used as an index of vitamin C status of fish (Matsusiewicz et al., 1995; Moreau and Dabrowski, 1996). Halver et al. (1975) preferred anterior kidney vitamin C (AA) content as sensitive index for vitamin C status. However, Lim and Lovell (1978) showed that the kidney vitamin C content of channel catfish did not reflect dietary vitamin C content. Liver ascorbate content in this trial showed a positive correlation (1.0) with dietary vitamin C supplementation.

The liver tend to reach saturation at 150 mg AA Kg^{-1} diet supplementation as it did not continue to increase in fish fed diets containing more than this level in this study. The kidney and muscle being saturated at 200 mg AA Kg^{-1} level as there is no significant difference between 200 and 250 mg AA Kg^{-1} levels with respect to these two tissues. However the gills ascorbate concentration tends not to be saturated as it significantly continued to increase with dietary inclusion levels. These are indications that the requirement to meet body tissues' saturation might be different from that needed for optimum growth. The significant improvement in the PCV and Hb with increasing levels of dietary vitamin C is an indication of the importance of this vitamin in blood formation. Ascorbic acid has been identified to be involved in the maturation of erythrocytes for maintenance of normal blood heamotology (Johnson et al., 1971 cited by Halver, 2005).

Due to the significant effect of dietary vitamin C on WG and SGR of *H. longifilis* fingerlings, these responses were used to estimate the dietary requirement. Regression analysis of these parameters against graded levels of dietary AA using the least square error method resulted in a vitamin C requirement estimate (± SE) of 82.2 (± 0.2) mg vitamin C Kg^{-1} diet. This corresponds to a dietary supplementation of 100 mg kg^{-1} diet plus the endogenous vitamin C of 0.01 mg Kg^{-1} that was present in the basal diet after preparation which must have resulted from the clupeid meal used as one of the protein sources. Also, in order to attain a survival rate above 80% the vitamin C inclusion level must be above 60 mg kg^{-1} diet. The requirement attained in this reported study seems to be higher than that for channel catfish and African catfish when L-ascorbate was used for the study. This might also not be unconnected with the fact that requirement for other nutrient (e.g. crude protein determined so far) for optimum performance of *H. longifilis* is higher than those of the earlier mentioned catfishes. Necessary precautions were also taken in this study to minimise the loss of vitamin C in the diet during feed preparation and before consumption due to its heat labile nature. This experiment was a necessity because some of the constraints that limit advancements in the intensive production of this fish remain, including the lack of optimized prepared diets. Results of this study can serve as a working tool for the practical fish farmers in this country who take *Heterobranchus* as favourite species since it was identified as fish with considerable potential for aquaculture in Nigeria. There is need to identify a natural source of vitamin C with high potential for inclusion into diets for easy handling by the practising fish farmers.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. S. I. Ovie, Mr. Lanre Ajayi and Mr. Badiru Adigun of Limnology Division, NIFFR for their assistance in the monitoring of water quality parameters during the experiment. We are equally thankful to Mr. John Agbontale and all other co-personnel for the assis-tance in the collection of blood and tissue samples for analysis without which the study could not have been completed.

REFERENCES

Aguirre P, Gatlin III, DM (1999). Dietary vitamin C requirement of red drum *Sciaenops ocellatus.* Aquacult. Nutr. 5: 247-249.

Ai Q, Mai K, Zhang C, Xu W, Duan Q, Tan B, Liufu Z (2004). Effects of dietary vitamin C on growth and immune response of Japanese seabass, *Lateolabrax japonicus.* Aquaculture 242: 489–500.

Al-Amoudi MM, El-Nakkadi AMN, El-Nouman BM (1992). Evaluation of optimum dietary requirement of vitamin C for the growth of *Oreochromis spilurus* fingerlings in water from the Red Sea. Aquacult. 105: 165 – 173.

Association of Official Analytical Chemists (AOAC) (2000). 17th Edition, A.O.A.C., Washington DC. 21: 447.

Bai SC, Gatlin III DM (1992). Dietary rutin has limited synergistic effects on vitamin C nutrition of fingerling channel catfish (*Ictalurus punctatus*) (ed. By BA Brown). Fish Physiol. Biochem. 10(3): 183–188.

Brander GC, Pugh DM (1977). Veterinary Applied Pharmacology and Therapeutics. Third Edition The English language Book Society and Bailliere Tindall London pp. 536.

Coustans MF, Guillaume J, Metailler R, Dygornay O, Messager JL (1990). Effects of an ascorbic acid deficiency on tyrosinemia and renal granulomatous disease in turbot (*Scophthalamus maximus*) interaction with a slight poly hypovitaminosis. Comp. Biochem. Physiol. 97A: 154-152.

Dabrowski K (2001). History, Present and Future of ascorbic acid research in aquatic organism. In: Dabrowski K (Editor), Ascorbic acid in aquatic Organisms–Status and Perspectives CRC Press, Boca Raton, pp. 255-277.

Dabrowski K (2001b). In “Ascorbic acid in aquatic organisms” (K Dabrowski ed.), CRC Press, New Yok p. 13.

Dabrowski K, Matusiewicz K, Matusiewicz M, Hopp PP, Ebeling J (1996). Bioavailability of vitamin C fro two ascorbyl monophosphate esters in rainbow trout *Onchorynchus mykiss* (Walbaum). Aquacult. Nutr. 2: 3–10.

Dabrowski K (1990). Absorption of ascorbic acid and ascorbic sulfate and ascorbate metabolism in common carp (*Cyprinus carpio L.*) Comp. Biochem. Physiol. 160: 549–561.

Dabrowski K, Hinterleitner S (1989). Applications of simultaneous assay of ascorbic acid, dehydroascorbic acid and ascorbic sulphate in biological materials. Analyst. 114: 83-87.

Dabrowski K, Hinterleitner S, Sturmbauer C, El-Fiky N, Wieser W (1988). Do carp larvae require vitamin C? Aquaculture 72: 295–306.

De Silva Sena S, Anderson TA (1995). Fish Nutrition in Aquaculture (Eds. SS De Silva & TA Anderson) Published by Chapman & Hall, London, pp. 319.

Eya JC, Mgbenka BO (1990). Ascorbic acid (vitamin C) requirement of African catfish, *Claria gariepinus* (Teugels 1984). J. Aqua. Sci. 5: 65-72.

Eyo AA (1995). Studies on the dietary protein requirement of *Heterobranchus longifilis* fingerlings. National Institute for Freshwater. Fish. Res. Ann. Rep. pp. 118-125.

Fagbenro OA, Balogun AM, Anyanwu CN (1992). Optimal dietary protein level for *Heterobranchus bidorsalis* fingerlings fed compounded diets. Isr. J. Aquacult. Bamidach 44(3): 87–92.

Fracalossi DM, Allen ME, Yuyama LK, Oftedal OT (2001). Ascorbic acid Biosynthesis in Amazonian fishes. Aquaculture 192: 321–332.

Gouillou-Coustans MF, Bergot P, Kaushik SJ (1998). Dietary ascorbic acid needs of common carp (*Cyprinus carpio*) larvae. Aquaculture 161: 453–461.

Halver JE, Smith RR, Tolbert BM, Baker EM (1975). Utilization of ascorbic acid in fish. In: Second Conference on Vitamin C (edited by CG King, JJ Burns) Ann. NY Acad. Sci. 258: 81–102.

Halver JE (2005). The vitamins. In: Halver J.E and Hardy R.W, editors. Fish Nitrition, 3rd edition, Academic press, San Diego, CA, p. 61-141.

Ibiyo LMO, Madu CT, Eze SS (2006a). Effects of vitamin C-supplementation on the growth of *Heterobranchus longifilis* fingerlings. Ach. Anim. Nutr. J. 60(4): 325–332.

Ibiyo LMO, Atteh JO, Omotosho JS, Madu CT, Okaeme AN, Ibiwoye TII (2006). Some pathological changes resulting from vitamin C deficiency in *Heterobranchus longifilis* fingerlings. Niger. J. Fish. 2(3): 2. In press.

Lee KJ, Kim KW, Bai SC (1998). Effects of different dietary levels of l-ascorbic acid on growth and tissue vitamin C concentration in juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Aquacult. Res. 29: 237 – 244.

Lim C, Lovell RT (1978). Pathology of vitamin C deficiency syndrome in channel catfish (*Ictalurus punctatus*). J. Nutr. 108:1137-1146.

Madu CT (1989). Hatchery Management and dietary protein utilization in the mudfish, *Clarias gariepinus* (L). Ph.D. Thesis. University of Jos, Nigeria. p. 218.

Matsusiewicz M, Dabrowski K, Volker L, Matusiewicz K (1995). Ascorbate polyphosphate is a bioavailable vitamin C source in Juvenile rainbow trout: tissue saturation and compartmentalization model. J. Nutr., 125: 3055 – 3061.

Miyasaki T, Plumb JA, Li YP, Lovell RT (1985). Hispathology of broken-back syndrome in Channel catfish. J. Fish Biol. 26: 647–655.

Moreau R, Dabrowski K (1996). The primary localization of ascorbate and its synthesis in the kidneys of acipenserid (*Chondrostaerei*) and teleost (*Teleostei*) fishes. J. Comp. Physiol. B 166: 178–183.

Muir JF (1984). Water quality and Aquaculture systems. In Disease and health control of farmed fish (including shelfish and crusteacea) in Europe. FAO Fiat Paris. p. 235.

NRC (National Research Council) (1993). Nutrient requirements of Fish. National Academy Press, Washington, DC. pp. 114

Ram MM (1966). Growth rate and protein utilization in vitamin C deficiency. Indian J. Med. Res. 541: 946–970.

Sealey WM, Gatlin III DM (1999). Dietary vitamin C requirement of Hybrid striped bass *Morone chrysops* X *M. saxatilis*. J. World Aquacult. Soc. 30(3): 297-301.

Shiau SY, Hsu TS (1999). Quantification of vitamin C requirements for juvenile hybride tilapia, *Orechromis niloticusX Oreochromis aureus,* with L-ascorbyl-2-monophosphate and L-ascorby-2-monophosphate-Mg. Aquaculture 175: 317–326.

Shiau SY, Hsu TS (1995). L-Ascorbyl-2-sulfate has equal antiscorbutic activity as l-ascorbyl-2-monophosphate for tilapia, *Oreochromis niloticus* X *O. aureus*. Aquaculture 122: 347–357.

Soliman AK, Jauncey K, Roberts RJ (1994). Water-soluble vitamin requirements of tilapia: ascorbic acid (vitamin C) requirement of Nile tilapia, *Oreochromis niloticus* (L.). Aquacult. Fish. Man. 25: 269-278.

Wang XJ, Kim KW, Bai SC, Huh MD, Cho BY (2003). Effects of the different levels of dietary vitamin C on the growth and tissue ascorbic acid changes in parrot fish (*Oplegnathus fasciatus*). Aquaculture 215: 21–36.

Wang, Xiaojie, Kanggwoong Kim, Sungchul C Bai (2002). Effects of different dietary levels of L-ascorbyl-2-polyphosphate on growth and tissue vitamin C concentrations in juvenile olive flounder, *Paralichthys olivaceus* (Temminck et Schlegel). Aquacult. Res. 33: 261-267.