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Growth and reproductive performance of African giant catfish, *Heterobranchus longifilis* Valenciennes 1840 broodstock on ascorbic acid supplementation

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ABSTRACT

This study was conducted to determine the effects of different levels of ascorbic acid supplementation on growth, reproductive performance and larval quality of *Heterobranchus longifilis* female broodstock fishes. Five diets were formulated incorporating ascorbic acid at levels of 0 (control), 50, 100, 150 and 200 mg kg^{-1}. Fish averaging 700 $\pm$ 2.84 g were randomly fed with the experimental diets for 8 weeks. Fish fed with control diet had lower weight gain than fish fed with ascorbic acid supplemented diets ($p>0.05$). The total percentage weight gain and specific growth rate in all treatments were significantly different ($p<0.05$). The best feed gain ratio and protein efficiency ratio was recorded in 150 and 200 mg kg^{-1} ascorbic acid. The percentage fertilization and hatchability in all the treatments were significantly different. The best percentage fertilization and hatchability was recorded in 200 mg kg^{-1} ascorbic acid diet. Diet supplemented with 150 mg kg^{-1} ascorbic acid performed best in terms of weight of eggs and fecundity than all other treatments. The percentage survival of the progeny was also highest in broodstock fed with diet supplemented with 150 mg kg^{-1} ascorbic acid. Based on the results of this study, we suggest that ascorbic acid needs to be supplemented at 150-200 mg kg^{-1} in the diet of female *Heterobranchus longifilis* broodstock.

Keywords: African giant catfish, Ascorbic acid, Broodstock, *Heterobranchus longifilis*, Reproductive performance

Introduction

Exogenous nutrition of broodfish provides the indispensable nutrients required for gonadal development of females and the performance of the seed produced (Gunasekera *et al.,* 1997). Inadequate food supply for broodstock fish will lead to poor reproductive performance and seed production (Gunasekera *et al.,* 1996; Gunasekera and Lam, 1997; Bhujel, 2000). Vitamins are often not synthesised by fish and need to be supplied in the diet. The inability of many fish species to synthesize vitamin C (ascorbic acid) which is essential for fish growth and reproduction (Dabrowski, 1990) is due to the lack of the enzyme L-gulonolactone oxidase which catalyses the conversion of L-gulonic acid to ascorbic acid (Landau, 1992; Elbaraasi *et al.,* 2004). Izquierdo *et al.* (2001) mentioned ascorbic acid as one of the major nutrients influencing various reproduction processes such as fecundity, fertilization, hatching and larval development. Reproduction appears to increase maternal demand for vitamin C in fish (Soliman *et al.,* 1986). Ascorbic acid reserves are rapidly depleted during embryonic and larval development of certain fish (Dabrowski, 1988).

Heterobranchus species are endemic to tropical Africa (Teugels *et al.,* 1990). The most widely distributed member of the genera in Africa is *Heterobranchus longifilis* while *Heterobranchus bidorsalis* is mainly restricted to the western coast of Africa, occurring in reservoirs, lakes and large rivers. *Heterobranchus* species is the second most important clariid catfish used for aquaculture in Nigeria (Vanden Bossche and Bernacsek, 1990). Even though there is increase in the demand for *H. longifilis,* there is scarcity for its fingerlings due to difficulties in production of seed in captivity (Tlusty, 2002).

Numerous authors have established ascorbic acid requirement of several fish species of commercial importance. Channel catfish, *Ictalurus punctatus* requires 51 mg ascorbic acid (AA) per kg diet for maximum weight gain and absence of deficiency symptoms (EL Naggar and Lovell, 1991). African catfish *Clarias gariepinus* requires 150 mg AA kg^{-1} diet of ascorbic acid (Gbadamosi *et al.,* 2006). However, there is little or no quantitative estimation of the dietary vitamin C or ascorbic acid requirement for *H. longifilis*. It is therefore important to establish the ascorbic acid requirement for *H. longifilis* broodstock based

on growth performance; nutrient utilization, reproductive performance and larval quality due to the multiple role of ascorbic acid in various metabolic pathways.

Materials and methods

The present study was carried out at the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology, Federal University of Technology Akure, Nigeria. The experiment consisted of five treatments, with each treatment representing different inclusion level of ascorbic acid, available commercially as ROVIMIX-STAY C (Roche-Istanbul, Turkey). The graded levels of ascorbic acid used were 0 mg kg^{-1} (control), 50 mg kg^{-1}, 100 mg kg^{-1}, 150 mg kg^{-1} and 200 mg kg^{-1} in diets 1, 2, 3, 4, and 5 respectively.

Experimental procedure and management

The feed ingredients (Table 1) were procured from Adedom Feed Mill Ondo Road, Akure, Nigeria and ascorbic acid from Dotak Nutrition and Feed Mill, Akure , Nigeria. Five experimental diets were formulated to provide 35% crude protein.

All dietary ingredients were weighed with top loading balance (Metler Toledo, PB8001 London). The ingredients were then pulverised to 0.1 mm particles size. Ingredients including vitamin/premix and ascorbic acid were thoroughly mixed in a Hobart A-200T pelleting and mixing machine (Hobart LTD London, England) to a homogeneous mass and cassava starch was added as binder. The resultant mash was then pressed without steam through a mixer with 4 mm die attached to the Hobart pelleting machine. Diets were immediately sun-dried at ambient temperature (26 °C) for two days. After drying, the diets were stored at 4 °C in air tight polyethylene bags prior to feeding.

Heterobranchus longifilis broodstock (700±2.84 g) were stocked in a concrete tank for acclimatization for a period of 2 months and fed with commercial diets containing 45% crude protein under intensive feeding before commencement of the study. The female fish were then transferred to the experimental tanks. Ten concrete tanks (1.4 m x1.4 m x1 m and water depth 0.75 m) were stocked with two fish per tank with three replications per treatment. The water was changed once in two weeks and the fishes were weighed every two weeks using beam balance (series model 700).

Table 1. Composition of the experimental diet containing varying inclusion levels of ascorbic acid and proximate composition

	Treatments				
Ingredients (%)	1 (control)	2	3	4	5
Soyabean meal	32.58	32.58	32.58	32.58	32.58
Groundnut oilcake	16.29	16.29	16.29	16.29	16.29
Fishmeal	8.14	8.14	8.14	8.14	8.14
Yellow maize	23.99	23.99	23.99	23.99	23.99
Wheat offal	11.99	11.99	11.99	11.99	11.99
Vitamin premix*	2.00	2.00	2.00	2.00	2.00
Vegetable oil	2.00	2.00	2.00	2.00	2.00
Starch	1.00	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00	1.00
Oyster shell	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
Proximate composition (% dry matter)					
Crude protein	35.38	35.12	35.23	35.32	35.18
Lipid	15.23	15.18	15.12	15.20	15.21
Crude fibre	8.03	8.18	8.11	8.08	8.05
Ash	9.12	9.14	9.17	9.22	9.20
Moisture content	7.70	7.50	7.80	7.20	7.50
Nitrogen free extract (NFE)	24.54	24.55	24.57	24.98	24.98

*Premix as supplied by Dotak Nutrition and Feed Mill, Akure, Nigeria
Vitamins/minerals supplied mg 100 g^{-1} diet: thiamine (B1) 2.5 mg, riboflavin (B2) 0.5 mg, pyridoxine 7, 36 mg, pantothenic acid 5.0 mg, inositol 3 mg, biotin 0.3 mg, folic acid 0.5 mg, chlorine chloride 150 mg, niacin 35 mg, cyanocobalamin 20 mg, tocopherol (E) 10 mg, antioxidant 50 mg, ascorbic (C) 1.0 mg,
Minerals: iron (Fe) 50 mg, manganese (Mn) 93 mg, copper (cu) 78 mg, zinc (Zn) 54 mg, cobalt (co) 1.0 mg, iodine (I)1.0 mg, selenium (se) 0.25 mg.

The female fish were fed with five different diets in triplicates: T1 {control} (0 mg AA kg^{-1}), T2 (50 mg AA kg^{-1}), T3 (100 mg AA kg^{-1}), T4 (150 mg AA kg^{-1}) and T5 (200 mg AA kg^{-1}), while the males to be used for fertilization were stocked in separate tank. The fish were fed twice daily at 0900 hrs and 1700 hrs with their respective diet at 5% body weight in split doses for 56 days. After every two weeks of intensive feeding, the female broodstock fishes were carefully examined for eggs.

Proximate composition of the experimental diets

Analysis of the proximate composition of diets was carried out to determine the moisture content, fat, fibre, crude protein and ash according to AOAC (1990). Ascorbic acid was also determined by analytical method (AOAC, 1990).

Growth performance evaluation of experimental fish

Growth performance and nutrient utilisation efficiencies of the experimental fishes were evaluated as per Fasakin *et al.* (2001):

Total weight gain (g) = Final weight – Initial weight

$$\text{Total percentage weight gain, TPWG (\%)} = \frac{\text{Total weight gained}}{\text{Initial weight}} \text{ X } 100$$

$$\text{Specific growth rate (SGR):} \quad \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1} \text{ X } 100$$

where W_2 = weight of fish at time T_2 (final) days

W_1 = weight of fish at time T_1 (initial) days

$T_2 - T_1$ = experimental period in days

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Fish weight gain (g)}}{\text{Protein fed (g)}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake}}{\text{Net weight gain}}$$

Induction of ovulation, egg fertilization and incubation

The catfish were collected from the tanks and examined after 56 days of feeding with the experimental diets. The males were selected for milt extraction. The gravid females were weighed and injected with synthetic hormone ovaprim^R (Syndel Laboratories Ltd. Vancouver, B. C Canada) at 1900-1930 hrs. The latency period for each fish was 12 h, after which the fish was stripped and eggs collected in clean bowls. After stripping, 1g of eggs was taken in an experimental bowl and was labeled according to the treatments. The males were dissected to obtain the milt, which was used to fertilize the eggs. Dry fertilization was used in the experiment. The spent female was weighed to determine the number of eggs per gram weight.

Reproductive performance evaluation of experimental fish

In order to analyse the reproductive performance, fertilization, hatching, survival and relative fecundity were calculated as shown below:

$$\% \text{ Fertilization} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs counted}} \times 100$$

$$\% \text{ Hatching} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs in a batch}} \times 100$$

$$\% \text{ Survival} = \frac{\text{No. of hatchlings alive up to larval stage}}{\text{Total no. of hatchlings}} \times 100$$

$$\text{Fecundity} = \frac{\text{Total no. of eggs}}{\text{Weight of fish (g)}}$$

Statistical analyses

Data in percentage were sine transformed before subjecting to statistical analyses. Comparison of various growth and reproductive parameters from different dietary treatments was carried out using one-way analysis of variance (ANOVA) and, where applicable, Duncan's Multiple Range Test was used to test significant differences. All statistical analyses were performed using SPSS 10 for Windows software package.

Results and discussion

Proximate composition of experimental diet

The proximate analysis of experimental diets revealed 35% crude protein. Lipid ranged from 15.12 in T_3 to 15.23% in T_1. This range is suitable for clariid fish growth and exceeds 8 – 14% fat content recommended by Jauncey and Ross (1984). There was slight difference between added AA in the diets and measured AA in diets (Table 2). This may be due to the fact that ascorbic acid is labile and its rate of destruction could be a function of time, temperature, oxygen, pH, and light (Halver, 1990).

Growth performance evaluation of experimental fish

The highest mean weight gain obtained in this study, 3.21g day^{-1} in T_5 was higher than 0.615 g day^{-1} for channel catfish *Ictalurus punctatus* fed varying ascorbic acid supplementation levels (Li and Lovell, 1985), 0.97 g day^{-1} for Atlantic Salmon (Waagbo *et al.,* 2003), and 0.91 g day^{-1} for *Clarias gariepinus* fingerlings (Gbadamosi *et al.,* 2006). This might be due to the differences in species, size/weight of fish, age, and dietary nutrient requirement of the fish (Halver, 1989).

The specific growth rate (SGR) and total percentage weight gain (TPWG) obtained in this study were lower than the report of Francalossi *et al.* (1998) for oscar *Astronotus ocellatus* (SGR 0.26 – 0.39 and TPWG 62.86 – 104); and Gbadamosi *et al.* (2006) for *Clarias gariepinus* fingerlings (SGR 1.61 – 3.50 and TPWG 21.40 – 1057.19) fed with varying levels of ascorbic acid. The differences may be attributed to species and size differences. The highest specific growth rate (SGR) in this study was recorded for fish fed with diet T_4 and lowest in fish fed with diet T_1. From the result of the percentage weight gain, it was obvious that fish fed on diet T_4 has the highest value followed by T_5, T_2, T_3 and least in T_1 which is the control. There was significant difference in all treatments ($p>0.05$).

Fish in T_1 had the lowest weight gain. This may be attributed to the absence of ascorbic acid in the diet fed to the fish. The growth rate of fish was highest in T_4 followed by T_5, T_2, T_3 and T_1. This can be attributed to the ascorbic acid level in the feed; which was the only variable factor in the experimental diets. In all the treatments, T_4 had the best growth performance. The better growth performance of fish in T_4 agreed with Liu *et al.* (1989) for channel catfish fed on elevated concentrations of dietary AA ranging between 100 – 150 mg kg^{-1} recommended for commercial feeds of channel catfish grow-out.

Nutrient utilisation of fish fed with experimental diets

Heterobranchus longifilis, fed on diets without AA supplementation performed poorly in terms of protein efficiency ratio (PER) and feed conversion ratio (FCR). There was no significant difference ($p>0.05$) in the nutrient utilization parameters. The protein efficiency ratio (PER) recorded for T_5 (200 mg kg^{-1}) was higher than the values obtained for T_1 (control), T_3, T_2, and T_4 (Table 3), and agrees with the work of Verlhac *et al.* (1996) on channel catfish *I. punctatus.*

Feed conversion ratio (FCR) obtained in this study varied among treatments. FCR values ranged from 1.13 in T_4 to 3.18 in T_1. The FCR for T_4 is within the range reported by Gbadamosi *et al.* (2006) for *C. gariepinus* fed 40% crude protein where FCR was between 0.94 – 1.71. T_4 had the best FCR followed by T_5 T_2, and T_3 while the poorest FCR was recorded in T_1 (control) as 3.18, which can be attributed to the absence of AA, and was in line with the report of Sadness *et al.* (1992) in farmed Atlantic salmon, *Salmo salar*.

Reproductive performance of H. longifilis broodstock fed on experimental diets

The percentage fertilization and percentage hatchability varied from 35 ± 28.28 to 90 ± 2.83 and 29.39 ±33.93 to 77.35 ± 8.94, respectively (Table 4); and there was no significant difference in the treatments ($p > 0.05$) having AA supplements. T_5 had the best performance in terms of % fertilization and % hatchability, followed by T_4, T_3, T_2, and least in T_1. This is in agreement with Sadness *et al.* (1984), who reported that reduced reproductive performance was observed in rainbow trout fed vitamin C deficient diets.

The survival rate of larvae was highest in T_5 followed by T_4, T_2, T_3 and T_1 (control), and there was no significant

Table 2. Ascorbic acid concentration in experimental diets and eggs of *H. longifilis* broodstock fed on diets with varying levels of ascorbic acid

Parameter \ Diet	T_1	T_2	T_3	T_4	T_5
Ascorbic acid supplemented in diets (mg kg^{-1})	0	50	100	150	200
Ascorbic acid measured in diets (mg kg^{-1})	1.56	42.30	102.70	132.40	187.20
Ascorbic acid present in eggs of fish fed with experimental diets (mg kg^{-1})	0.28	11.41	23.77	40.02	47.78

Table 3. Growth performance and nutrient utilisation in *H. longifilis* broodstock fed with varying levels of ascorbic acid

Parameters	T_1	T_2	T_3	T_4	T_5
Final weight (g)	765 ± 21.21^a	858 ± 353.55^a	800 ± 197.99^a	885 ± 134.35^a	880 ± 311.13^a
Initial weight (g)	700 ± 0.00^a	700 ± 282.84^a	700 ± 141.42^a	700 ± 141.42^a	700 ± 212.13^a
Weight gain (g)	65 ± 21.21^a	150 ± 70.71^a	100 ± 56.57^a	175 ± 21.21^a	180 ± 98.99^a
% Weight gain (g)	9.29 ±3.03^a	21.11 ± 1.57^{ab}	13.75 ± 5.30^{ab}	25.84 ± 8.25^b	24.71 ± 6.65^b
SGR (%)	0.07 ± 0.02^a	0.15 ±0.01^{ab}	0.10 ± 0.04^{ab}	0.19 ± 0.04^b	0.17 ± 0.04^b
FCR	3.18±1.03^a	1.47 ± 0.63^a	2.33 ±1.32^a	1.13 ± 0.14^a	1.28 ± 0.706^a
PER	1.86 ± 0.61^a	4.29 ± 2.02^a	2.86 ± 1.62^a	68.60 ± 0.00^a	5.14 ± 2.83^a
Protein intake (x 10^3)	68.60 ± 0.00^a	68.60 ± 0.00^a	68.60 ± 0.00^a	5.00 ± 0.61^a	68.60 ± 0.00^a

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

Table 4. Reproductive performance of *H. longifilis* broodstock fed with varying levels of ascorbic acid

Parameters \ Treatments	T_1	T_2	T_3	T_4	T_5
Weight of fish (g)	765 ± 21.21[a]	850 ± 353.55[a]	800 ± 197.99[a]	885 ± 134.35[a]	880 ± 311.13[a]
Weight of eggs (g)	75 ± 7.07[a]	105 ± 7.07[a]	100 ± 28.28[a]	145 ± 49.50[a]	140 ± 42.43[a]
Fecundity ($x10^3$)	29.92 ± 4.19[a]	45.69 ± 1.82[a]	37.32 ± 7.30[a]	62.25 ±20.67[a]	60.97 ±16.68[a]
% Fertilization	35 ± 28.28[a]	53.50 ± 6.36[ab]	79.00 ± 4.24[bc]	86.00 ± 1.41[bc]	90.00 ± 2.83[c]
% Hatchability	54.10 ± 4.14[ab]	29.39 ±33.93[a]	66.94 ± 0.02[ab]	75.69 ± 1.06[b]	77.35 ± 8.94[b]
% Survival	27.26 ± 1.76[a]	45.42 ±21.86[a]	28.08 ± 9.22[a]	35.24 ± 5.40[a]	49.54 ± 6.32[a]
Hatching time (h)	20.50 ± 0.71[b]	19.50 ± 0.71[ab]	19.00 ± 1.41[ab]	18.00 ± 0.00[a]	18.50 ± 0.71[ab]
Hatching period (h)	72.00 ± 0.00[a]	72 ± 0.00[a]	72.00 ± 0.00[a]	72.00 ± 0.00[a]	72.00 ± 0.00[a]

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

difference amongst treatments (p>0.05). The decrease in the survival rate can be attributed to depletion of AA reserves during embryonic development as reported by Sadness *et al.* (1992) that the requirement of AA during early life stage might be higher than for fingerlings or adult fish. T_4 had the highest weight of eggs and fecundity of 145 ± 49.50 and 62.25 ± 20.67, respectively, but there was no significant difference amongst treatments. This was in accordance with Eskelinen (1989), who reported that the effect of different diets on egg production and egg quality can be associated with vitamin inclusion. The hatching time observed in T_4 was the fastest among the treatments. Viveen *et al.* (1985) stated that hatching takes place between 20 – 57 h after fertilization depending on the water parameters. T_5, T_3, T_2 and T_1 showed considerable level of hatching within a short time.

The dietary vitamin C levels in the present study affected broodstock reproductive performance. The fecundity and vitamin C level in eggs increased as level of vitamin C increased in the diets (Table 2). In vertebrates, vitamin C functions in several reproductive processes such as spermatogenesis, oogenesis, embryonic growth and differentiation. Halver (1989) stated that active uptake of vitamin C seems to be very important at low doses while at high doses, uptake by passive diffusion also occurs.

Ascorbic acid levels in eggs of H. longifilis fed on ascorbic acid supplemented diets

The reduction in the AA found in eggs was due to uptake by tissue, cells and other organs. The highest AA levels in eggs was found in T_5, followed by T_4, T_3, T_2 and T_1 (Table 2). The presence of ascorbic acid in T_1 (control) was due to the premix added which contained about 100 mg AA. The factor responsible for the differences in ascorbic acid present in the eggs can be associated with the reproductive success, fecundity, percentage hatchability and percentage survival.

Based on the results of the study, it can be deduced that ascorbic acid is an essential nutritional element in the diet of *H. longifilis* broodstock. The diets with the best optimal growth, feed utilization, reproductive performance and larval quality in this study were T_4 and T_5 with 150 mg kg^{-1} AA and 200 mg kg^{-1} AA supplementation. Treatment 4 performed better in terms of growth, fecundity, weight of eggs and hatching time while treatment 5 performed better than T_4 in nutrient utilisation, percentage fertilization, hatchability and survival. It appears that the minimum dietary ascorbic acid requirement lies between 150 and 200 mg kg^{-1}. Therefore, good management, balanced ration and provision of adequate nutrients like ascorbic acid will help to achieve the production of healthy *H. longifilis* broodstock with larval quality.

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