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# Possible Improvement in Income with Vitamin C Fortified Diets in Practical Farming of *Heterobranchus longifilis* Fingerlings

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**Abstract:** A completely randomised design with three replicates was used for an experiment conducted to study the profitability of vitamin C inclusion into the ration formulation of *Heterobranchus longifilis*. Graded levels (0, 50, 100, 150, 200 or 250) mg vitamin C $kg^{-1}$ diet as ascorbate polyphosphate was included in a 42.5% crude protein basal diet and fed to triplicate groups of twenty fingerlings. The groups fed diets with vitamin C had statistically significant ($p<0.05$) higher final weight than the group fed the control diet without vitamin C at the end of twenty weeks study. Feed consumed was significantly ($p<0.05$) lower in the group fed diet without vitamin C. There was a significant difference ($p<0.05$) between the groups fed 0 and 50 mg vitamin C $kg^{-1}$ diets but no significant difference ($p>0.05$) between the groups fed diets with 100, 150, 200 and 250 mg vitamin C $kg^{-1}$ diet on this parameter. Mortality was significantly ($p<0.05$) higher in the groups fed diet devoid of vitamin C while there was no significant difference ($p>0.05$) between the fish fed the diets with the graded levels of vitamin C. The Thiobarbituric Acid Reactive Substance (TBARS) of the liver and whole body of fish was significantly ($p<0.05$) higher in fish fed diet devoid of vitamin C. Cost per kilogram of fish base on feed input was significantly ($p<0.05$) higher in the group fed diet without vitamin C than those enriched with vitamin C. However within the groups enriched with vitamin C cost per kg of fish was significantly ($p<0.05$) decreasing in the following order: diet 2>diet 6>diets 4, 5 and 3. There was no significant difference ($p>0.05$) between fish fed diets with 100, 150 and 200 mg vitamin C $kg^{-1}$ with respect to cost per kilogram of fish. The results of this study suggest that there is no alternative to the use of a complete diet with vitamin C in a sustainable intensive practical farming of *H. longifilis*.

**Key words:** Vitamin C, profitability, practical farming, *Heterobranchus longifilis*

## INTRODUCTION

The success of aquaculture production depend on adequacy of nutrition therefore the main challenge to improve aquaculture nutrition in Nigeria is the development of an economic adequate rations for the culture of the farmed species in Nigeria (Adikwu, 2003). Catfish diets must provide adequate energy, protein, vitamins and minerals in the proper proportions for fast, efficient growth and health maintenance. Using the right feed plays an important role in determining the productivity and profitability of aquaculture operations. In fact, producers aren't the only people who are interested in diet quality. Certain characteristics of the diet influence the quality of catfish products during processing and storage. As a result, catfish processors, wholesale marketers and retailers also depend on proper feed quality to yield desirable results. Vitamin C is essential for normal fish growth and has some properties that allow food products to resist oxidation. The major beneficial actions of vitamin C and E are due to their antioxidant properties that scavenge reactive oxygen species in biological fluid (Frei *et al*., 1990) and membranes (Burton *et al*., 1983).

The major task of fish nutritionists has been to select feedstuffs and levels (i.e., feed formulation) that would meet the nutrient requirements of the cultured species at the most economic cost (Eyo, 1990; Adikwu, 2003). This economic cost must also relate to the output of the fish consuming such economic diet. If complete diets are needed for intensification of any of the culturable freshwater fish species in Nigeria, the vitamins and mineral premixes in required amount must be included in the feed along with other ingredients before pelleting (Adikwu, 2003). Most farm practices in this part of the world do not include vitamins and mineral premixes in the

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feed outside what naturally occurs in the feedstuffs used. Primary and secondary producers in the pond are also assumed to naturally provide some nutrients in-situ for the growing fish in addition to whatever supplemental diets that might be provided. However, the secondary producers are always not in sufficient supply in an intensive pond culture involving catfishes. Complete diet is essential for optimum growth and productivity of the fish. Optimum growth and productivity determines the overall output of an intensive pond culture. The anatomical and physiological importance of vitamin C in the nutrition of different fish species have been shown by various studies in fish nutrition (Mahajan and Agrawal, 1980; Eya and Mgbenka, 1990; Ishibashi *et al.*, 1992; Mac Connell and Barrows, 1993; Soliman *et al.*, 1994; Aguirre and Gatlin, 1999; Sealey and Gatlin, 1999; Bloom *et al.*, 1999; Fracalossi *et al.*, 2001; Wang *et al.*, 2002; Ai *et al.*, 2004; Halver, 2005; Ibiyo *et al.*, 2006). It has been noted that vitamin C is essential in the nutrition of *H. longifilis*, that its absence leads to poor growth and broken-back syndrome (Ibiyo *et al.*, 2006). Though there is a resultant improvement in growth performance with the supplementation of vitamin C in the diet of *Heterobranchus longifilis* fingerlings, it will be meaningful if viewed with respect to input-output relationship. There is a possibility that such improvement is not productively economically important. Such comparison can aid the fish farmers' decision to either neglect or include vitamin C in any supplemental feed that might be provided for the growing fish. However, there is a paucity of information on the practical profitability of the inclusion of vitamin C in the diet of *Heterobranchus longifilis* fingerlings. This study was undertaken to establish the possible enhancement of economic gains from fish farming venture by the use of vitamin C with the commonly used feed ingredients in *H. longifilis* diets and its effect on thiobarbituric reactive substance of the final carcass and stored products. It is expected to further create awareness on the necessity of vitamin C in this fish's nutrition. Then the Nigerian fish farmers will be able to say no to the use of diets without vitamin C and always make use of vitamin C fortified premixes in practical diets for the sustainability of profit maximization in the production of Nigerian indigenous catfish species.

## MATERIALS AND METHODS

**Experimental animal, system and diets:** Fingerling mudfish, *Heterobranchus longifilis* with approximately 2.3 g mean weight were obtained from National Institute for Freshwater Fisheries Research (NIFFR) hatchery and subjected to 6 graded levels of vitamin C in a mini-flow through experimental system in the NIFFR. Basal diet formulated with readily available ingredients to contain 42.5% crude protein was used as the control for this experiment (Table 1). L-Ascorbyl-2-Polyphosphate (LAPP) (25% vitamin C equivalent, SAMP, China) was supplemented into the basal diet at the expense of silica to obtain the graded levels of 0, 50, 100, 150, 200, or 250 mg vitamin C $Kg^{-1}$ diet. The cold pelleted dried feeds were kept in polyethylene bags and stored at -20°C after preparation until used.

**Experimental procedure:** Five fingerlings were randomly selected for vitamin C analysis after acclimatization. The fish were weighed and randomly assigned in triplicates of 20 fingerlings each to the treatments. The feeding trial lasted twenty weeks after two weeks acclimatization period in which the fish were fed control diet. The fingerlings were fed 5% body weight supplied twice (morning and evening) daily for the first 8 weeks and was later reduced to 3% body weight due to loss of appetite observed in the control group. The fish were bulked weighed fortnightly and used to adjust feeding rate. The tanks were scrubbed at two days intervals despite flow through to minimize microbial growth that could possibly alter the results of the experiment. The conditions of fish were observed and recorded at the time of cleaning the tank. The water quality parameters as monitored by the staff of Liminology Division of NIFFR were within the range reported to be conducive for the growth of freshwater fishes.

Table 1: Composition of the basal diet

| Ingredients | Inclusion levels |
|---|---|
| Clupeid meal (65%) | 30.00 |
| Soybean meal (45%) | 35.00 |
| Groundnut cake (40%) | 13.82 |
| Maize bran (12.5%) | 13.35 |
| Oil | 2.00 |
| Starch | 2.00 |
| Bone meal | 2.00 |
| Vitamin C-free Premix* | 1.00 |
| Methionine | 0.50 |
| Salt | 0.25 |
| Silica | 0.08 |
| Total | 100.00 |
| Proximate composition | |
| Crude protein % | 42.26 |
| Crude fat % | 11.55 |
| Crude fibre % | 1.20 |
| Ash % | 8.89 |
| NFE % | 27.91 |
| Moisture content % | 8.19 |
| Metabolizable energy (Kcal 100 $g^{-1}$) ** | 375.00 |

** Metabolizable energy (Kcal 100 $g^{-1}$) is based on standard physiological values of 4.5, 3.3 and 8 Kcal $g^{-1}$ for protein, carbohydrate and fat, respectively (Brett and Grooves, 1979; Panaflorida, 2002). * Provides per kg diet: Vitamin A, 50000 IU, Vitamin $D_3$ 25000 IU, Vitamin E 160mg, Vitamin K 8mg; Vitamin $B_1$ 12mg; Vitamin $B_2$ 22mg; Vitamin $B_6$ 20mg; 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; Choline chloride 0.1 mg

**Sample collection and analysis:** At the end of the experiment, 6 fish from each replicate were randomly selected and sacrificed to collect liver and whole body of fish. Fractions of the pooled replicate samples were analysed immediately and some were stored to observe whether there is any difference in storage of fish from different levels of supplemental vitamin C (mg $kg^{-1}$) with respect to oxidative stability of mudfish fillets. The fish were stored for 15 days and analysis was subsequently carried out at day 0, 5, 10 and 15 of storage. The Thiobarbituric Acid Reactive Substance (TBARS) of the pooled liver and fish were determined using a method adopted from that of Burk *et al.* (1980). Another three fish from each replicate were also randomly sampled for determination of vitamin C concentration of liver and muscle. Vitamin C concentrations in fish and diets samples were measured colorimetrically by the dinitrophenylhydrazine method, corrected for interfering substances (Dabrowski and Hinterleitner, 1989). Sample of the basal diet was dried to a constant weight at 105°C to determine moisture content. Protein was determined by measuring nitrogen (N×6.35) using the Kjeldahl method and crude fat by ether extraction using Soxhlet extractor (AOAC, 2000).

Measurements were also taken for the calculations of the variables below:

| | | |
|---|---|---|
| Mean final weight | = | Total Weight of fish per replicate ÷ $N_t$. |
| Feed efficiency ratio | = | Wet WG in g ÷ dry feed fed in g (Hardy and Barrows, 2005) |
| Percent mortality | = | 100 ($N_0$ . $N_t$) ÷ $N_0$. |

Where WG is Weight Gain; $W_t$ and $W_0$ were final and initial fish weights, respectively; $N_t$ and $N_0$ were final and initial numbers of fish in each replicate, respectively; t is the experimental period in days.

**Cost to benefit analysis:** The cost analysis was carried out with the following considerations while silent in other expected input costs which are also mandatory in a fish farming venture. The results will determined whether they could be taken care of in the enterprise by the farmer conveniently as feed constitutes over 60% of production cost.

| | | |
|---|---|---|
| Cost per kg of feed | = | Total cost of ingredients to produce 1 kg feed. |
| Cost of feeding | = | Cost of Feed Consumed (FC) by each group of fish (as fed basis). |
| Additional cost due to vitamin C | = | Cost of supplemented diets cost of control diet. |
| Cost per kg of fish due to feeding | = | Total cost of feed needed to gain 1 kg weight by the fish. |
| Reduction in cost | = | Cost of feeding on control diet cost of feeding on vitamin C enriched diet. |
| Economy of WG | = | Total cost of FC/Unit of WG (Igbinoson, 1982). |

**Statistical analysis:** The data obtained from the trial were subjected to one way analysis of variance. When ANOVA identified significant difference among groups, multiple comparison tests among means were performed using Duncan's new multiple range test in SPSS 10.0 for Windows. The data from TBARS analysis through the days of storage were subjected to repeated measure ANOVA in GraphPad Instat analysis, with Turkey used to separate the means. The differences were considered statistically significant at 5% level ($p<0.05$).

## RESULTS

There was no significant difference ($p>0.05$) in the initial mean weight of the fish. The final weight of the group fed diet without vitamin C was significantly lower ($p<0.05$) than those fed the respective diets enriched with vitamin C (Table 2; Fig. 1). Within the groups fed diets enriched with vitamin C, final weight showed a significant ($p<0.05$) progressive increase up to the 200 mg $kg^{-1}$ diet supplementation after which final weight significantly ($p<0.05$) decreased, in the subsequent level (Table 2). The groups fed 50 mg vitamin C $kg^{-1}$ diet had significantly ($p<0.05$) lower final weight and was significantly ($p<0.05$) followed by the group that received 250 mg vitamin C $kg^{-1}$ diet whereas, there was no significant difference ($p>0.05$) between the groups fed 100, 150 and 200 mg vitamin C $kg^{-1}$ diet (Table 2). The feed consumed per fish was significantly higher ($p<0.05$) in the groups fed the vitamin C enriched diet (Table 2). The highest feed efficiency was achieved with 100, 150 and 200 mg vitamin C $kg^{-1}$ diet supplementation. Mortality was significantly higher ($p<0.05$) in the control group while there was no significant difference between the groups fed diets enriched with vitamin C irrespective of the level with respect to mortality. The vitamin C content of fish liver after acclimatization was 32.5 μg $g^{-1}$ tissue. The vitamin C concentration of the liver and muscle was significantly

Table 2:Effects of vitamin C on the production, TBARS concentration of liver and stored fish and cost analysis of *H. longifilis* (0– 20 weeks)

| Parameters | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Diet 6 | SEM |
|---|---|---|---|---|---|---|---|
| Vitamin C levels mg $kg^{-1}$ diet | 0 | 50 | 100 | 150 | 200 | 250 | - |
| Mean initial weight (g $fish^{-1}$) | 2.4 | 1.8 | 2.4 | 2.4 | 2.3 | 2.7 | - |
| Mean final weight (g $fish^{-1}$) | $24.80^{d}$ | $37.03^{c}$ | $68.30^{a}$ | $68.27^{a}$ | $68.60^{a}$ | $58.37^{b}$ | 0.637 |
| Feed consumed (g $fish^{-1}$) | $57.5^{c}$ | $67.7^{b}$ | $114.7^{a}$ | $117.23^{a}$ | $115.23^{a}$ | $112.87^{a}$ | 1.44 |
| Feed efficiency ratio (%) | $38.95^{d}$ | $51.98^{b}$ | $57.47^{a}$ | $56.49^{a}$ | $57.53^{a}$ | $49.34^{c}$ | 0.511 |
| Mortality (%) | $10.0^{a}$ | $3.3^{b}$ | $1.3^{b}$ | $1.0^{b}$ | $1.0^{b}$ | $0.67^{b}$ | 1.25 |
| 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 |
| 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 |
| Liver TBARS nmoles $g^{-1}$ | $68.57^{d}$ | $44.30^{c}$ | $42.70^{cb}$ | $39.83^{b}$ | $29.83^{a}$ | $29.00^{a}$ | 1.04 |
| TBARS of stored Fish * | $2.3^{e}$ | $1.5^{d}$ | $0.9^{c}$ | $0.8^{b}$ | $0.58^{a}$ | $0.57^{a}$ | 0.025 |
| Cost per Kg of feed (N $Kg^{-1}$) | 110.00 | 110.50 | 111.00 | 111.50 | 112.00 | 112.50 | - |
| Cost per Kg of fish on feed basis [N /Kg fish} | $282.4^{d}$ | $212.78^{b}$ | $193.16^{a}$ | $197.38^{a}$ | $194.62^{a}$ | $227.81^{c}$ | 1.99 |

a-dMeans in each row with different superscripts are significantly different ($p<0.05$). *mg malonaldehyde per kg muscle

lower in the group fed diet devoid of vitamin C and it generally reflected supplementation levels. The clinical symptoms of broken-back syndrome, anorexia and convulsive movement observed by Ibiyo *et al.* (2006) was also apparent from the eighth week to the end of this study.

The increase in cost of feed due to inclusion of vitamin C was minimal but reduction in cost of producing a kilogram of fish due to feeding was significantly ($p<0.05$) encouraging when cost analysis with respect to feed was carried out (Table 2). The cost of producing a kilogram of fish with respect to feed cost was significantly higher in the control group while there was no significant difference ($p>0.05$) between diets 3, 4 and 5. Although the highest output was obtained with diet 5 the least cost of production due to feed as a v ariable input was achieved with diet supplemented with 100 mg vitamin C $kg^{-1}$ (Table 2).

The TBARS of the liver was significantly ($p<0.05$) affected by the levels of vitamin C. The TBARS of the group fed vitamin C deficient diet was significantly ($p<0.05$) higher than those fed with vitamin C supplemented diets. However within the groups fed vitamin C supplemented diets the significant ($p<0.05$) lowest TBARS occurred in the groups fed 200 and 250 mg vitamin C $kg^{-1}$ diet. There was no significant difference ($p>0.05$) between the groups fed 50 and 100 mg $kg^{-1}$ diet with respect to TBARS of the liver (Table 2). The TBARS of the stored fish samples also follow the same trend with that of the liver. There was significant difference ($p<0.05$) between the levels of vitamin C but there was no significant ($p>0.05$) difference between days of storage, hence there was no significant interaction ($p>0.05$) between days of storage and vitamin C level with respect to this parameter.

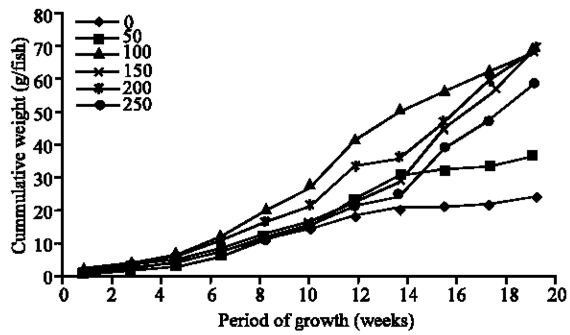


Fig. 1: Effects of vitamin C levels on the cumulative weight of *H. longifilis* fingerlings

## DISCUSSION

This study reveals that the practices of intensive aquaculture require feeding fish with complete diets either as a supplement to abundant natural food in the culture system or as a complete diet where natural fish food is in short supply (Eyo, 2001). The fact that fish require all the essential nutrients (protein, lipids, energy, vitamins and minerals) in their diets to meet the physiological needs for normal growth, reproduction and health maintenance (NRC, 1993) was manifested in the *H. longifilis* fingerlings in this study. Proper and improper meeting of the physiological needs of the fish resulted in the significant variation in total output obtained with the different levels of vitamin C. Fish feed constitute an important item in any fish culture operation (Akinyama, 1988). The resultant improvement in output with respect to vitamin C supplementation (Fig. 1) showed that provision of a qualitative complete diet is essential in an intensive production system with the objective of profit maximization. This supports the point that an economic diet formulation is important in practical fish farming (Eyo, 2001; Adikwu, 2003). Reduced cost of feeding, with good feed conversion efficiency positively affect the profitability of an intensive aquaculture system (Crampton, 1985; Sadiku, 2003) as observed in this study.

Dietary vitamin C supplementation level significantly reduced hepatic TBARS concentration in *H. longifilis*. The higher the level of supplementation the less the TBARS concentration in the liver and whole body of fish (Table 2) suggesting that vitamin C can suppress lipid peroxidation, thus protecting the cells from oxidative stress. There was an observation that liver vitamin E showed a large drop when vitamin C became deficient in Atlantic salmon (Hamre *et al.*, 1997). The TBARS response of *H. longifilis* in this study tends to encourage higher supplementation level for a sustainable effective protective strength against oxidative stress in the liver and muscle. There by preventing degeneration process occurrence in the liver of the fish and in the final stored fish product to some extent. The result of the TBARS analysis suggest that vitamin C tend to reduce oxidative spoilage that could occurred in stored products of this nature. Although, there are some other interactive and synergistic action of vitamin C with some other nutrients like vitamin E and selenium depending on the levels and presence of those nutrients (Shiau and Hsu, 2002; Sealey and Gatlin, 2002). The TBARS value has a relationship with product quality especially in terms of storage of fleshy products. There is an indication that TBARS could signify the product quality in that it tends to measure the extent of oxidative damage that has occurred. Therefore vitamin C supplementation will also be of interest to the fish product marketers and consumers.

The high mortality that occurred in the group fed diet without vitamin C (Ibiyo *et al.*, 2006) is also an added source of possible loss in a production system since the number of survivors was significantly reduced. The number of survivors invariably determined the final total output in a fish production process. The economic analysis showed that vitamin C is essential for economical diet formulation and profit maximization as there was N89.24 cost reduction in the feeding of *H. longifilis* fingerlings to gain 1.0 kg weight when vitamin C was added to the diet at 100-200 mg $kg^{-1}$ compared to the diet that was not supplemented. The results of this study showed that the use of premixes with vitamin C fortification should be encouraged to reduce a unit cost of production for profit maximization. This is due to the fact that no alternative to an economic complete diet in a sustainable practical farming of *H. longifilis* geared towards optimum profit maximisation in terms of feed as one of the variable input. Feeding takes the lion share of cost of production in any fish farming operation (NRC, 1993). The farmers of *H. longifilis* should note that, if the nutrient requirements of fish is not met or compensated for in the diet, the farmers cannot actualize the desired objective, which is usually profit oriented.

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