# The Influence of Supplemental Vitamins C and E in Maternal Diets on Growth and Survival of *Heterobranchus longifilis* Fry Outdoor.

Ibiyo Lenient Mercy Onivie[1], Atteh Job Olutimilehin [2], Omotosho James Sunday[3] and Madu Chuka Titus[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.
oniviemercy@yahoo.com

**Abstract:** A 3 x 3 factorial design was used in a twelve weeks feeding experiment carried out to determine the residual effect of vitamin C and E in maternal diet on the survival and growth of fry fed a basal diet supplemented with 100 mg vitamin C and 50mg vitamin E $Kg^{-1}$ in outdoor tanks. In all the growth parameters measured, vitamin C levels uniquely indicated significant difference ($P<0.05$) except total length in which there was significant interactions ($P<0.05$). The group of fry from maternal parents fed on diets supplemented with required and excess vitamin C had significantly higher weight gain compared to the group of fry from the maternal parents fed on diet devoid of supplemental vitamin C. However, no significant difference ($P>0.05$) between required and excess groups on this parameter. Supplemental vitamin C levels improved survival of the fry outdoor ($P<0.001$) but not with vitamin E levels ($P>0.05$). Also there was no significant interaction on survival ($P>0.05$). [Nature and Science. 2010;8(1):75-80]. (ISSN: 1545-0740)

**Key word:** *H. longifilis* broodstock, fry, vitamin C and E, growth performance.

## 1. Introduction

The early development in fish fry is dependent on the internal compliment of essential nutrients present in the egg. (Palace and Werner, 2006). These nutrients are in turn determined by the maternal diet prior to and during oogenesis (Lavens *et al.*, 1999). Among the essential nutrients, dietary fatty acids are one of the strongest determinants of reproductive performance (Izquierdo *et al.*, 2001). Fish eggs are well known to contain high concentration of polyunsaturated fatty acid (PUFA) (Palace and Werner 2006). PUFA are essential for normal development of fish and are incorporated into cellular and subcellular membranes helping to maintain the fluidity of membranes. The structure of PUFA make them susceptible to reactive oxygen species (ROS) attack which are by-products of aerobic metabolism. These by-products can initiate lipid peroxidation which can lead to cell damage (Mourente *et al.*, 1999). To enable vertebrates to cope with the continuous generation of ROS from normal metabolism, cells and tissues contain a series of enzymatic and non-enzymatic cellular antioxidants (Palace and Werner, 2006). Enzymes such as catalase, glutathione peroxidase and reductase, superoxide dismutase and non-enzymatic factors such as vitamins A, C and E, glutathione, ubiquinone, iron chelators and thiol-containing proteins, all functioning to neutralize ROS by-products within the cell (Halliwell and Gutteridge, 1990). As long as the cell, tissue and whole organism can maintain antioxidant concentration above the level of ROS generation, cellular components are protected. Higher aerobic metabolism and generation of ROS also occurs during rapid tissue growth. Therefore early developmental stages of fish would be expected to increase production of ROS. The antioxidant enzyme systems that are present in the liver and other tissues of adult fish are not synthesized until late in the embryonic development of larval fish (Palace and Werner, 2006). This makes early antioxidant protection by maternally derived non-enzymatic antioxidant essential (Cowey *et al.*, 1985; Ciarcia *et al.*, 2000). The content of this lipid soluble vitamin in fish eggs has been suggested to permit larger initial egg size which in turn has been correlated with larger larval size, and better early survival (Lavens *et al.*, 1999). A lower ascorbic acid deposition in the eggs due to lower maternal intake may lead to signs of deficiency at both biochemical and morphological levels in newly hatched fish (Soliman, *et al.*, 1986). This experiment was designed to study the influence of broodstock fed on diets with varied vitamins C and E combinations on survival and growth performance of resultant fry.

## 2. Materials and Methods

The resultant fry from a 3 X 3 factorial design were subjected to a 42.5% crude protein basal diet supplemented with required levels of vitamins C and E (Table 1) for a twelve weeks period. The 3 X 3 factorial consisted of a combination of vitamins C and E. Vitamin C (L-ascorbic acid) obtained from Tuyil Pharmaceutical Industries, Ilorin, was supplemented to maternal diets at zero (0mg $kg^{-1}$ diet), adequate (100mg $kg^{-1}$ diet) or excess (1000 mg $kg^{-1}$ diet) while vitamin E (DL-α-tocopheryl acetate) obtained from Teva Pharmaceutical Industries, Petach Tikva, was supplemented at zero (0mg $kg^{-1}$ diet), adequate (50 mg $kg^{-1}$ diet) or excess (400 mg $kg^{-1}$ diet). Proximate analysis of the diet was carried out using the methods of AOAC (2000). After two weeks feeding on zooplankton indoor, the fry from each treatment were bulked and transferred outside in duplicate into 2m X 2m X 1m concrete tanks with a stocking density of 100 per replicate.

### 2.1 Feeding and Measurements

The fry were weighed before stocking and they were fed at 5% body weight throughout the experimental period. The diet was supplied to the fry three times in the first 4 weeks after which they were fed twice daily for the next 8 weeks. Sampling was carried out forthnightly to observe fish health while recording bulk weight and survival at each occasion. Also, those exhibiting differential growth characteristics (commonly called shooters) were separated. The weight was used to adjust feeding rate.

The physico-chemical parameters of the water were monitored. In the final sampling, weight, length measurements and total number of survivor were recorded. Twenty post-fingerlings were taken from each replicate for chemical analysis. Liver tissues were removed from ten fingerlings for determination of vitamins C and E concentration and liver thiobarbituric acid reactive substance (TBARS). The other ten were used for whole body TBARS concentration. Vitamin C was measured colorimetrically using the dinitrophenylhydrazine method, corrected for interfering substances (Dabrowski and Hinterleitner, 1989). Vitamin E concentration was determined with the procedure of Huo *et al.*, (1996) while TBARS was analyzed (Burk *et al.*, 1980). The data from physical measurements were used to calculate weight gain (WG), specific growth rate (SGR), feed intake (FI) and feed conversion ratio (FCR).

The data obtained were subjected to two-way analysis of variance and where significant differences were observed means were separated with Least significant difference test.

**Table 1: Composition of Basal Diet**

| Ingredients | Inclusion Levels |
|---|---|
| Clupeid meal (65%) | 25.00 |
| Soybean meal (48.5%) | 34.138 |
| Groundnut cake (40%) | 20.00 |
| Maize bran (12.5%) | 13.537 |
| Oil | 2.00 |
| Starch | 2.00 |
| Bone meal | 1.50 |
| Vitamin C free Premix* | 1.00 |
| Methionine | 0.50 |
| Salt | 0.25 |
| Vitamin C | 0.03 |
| Vitamin E | 0.045 |
| Total | 100.00 |
| **Analysed Proximate Composition** | |
| Crude protein % | 44.16 |
| Crude fat % | 14.55 |
| Crude Fibre % | 4.30 |
| Ash % | 6.89 |
| NFE % | 24.75 |
| Moisture % | 5.35 |

* Provides per kg diet: Vitamin A, 125000 IU, Vitamin $D_3$ 2500 IU, Vitamin E 40mg, Vitamin K 2mg; Vitamin $B_1$ 3mg; Vitamin $B_2$ 5.5mg; Choline chloride 500mg; Niacin 35mg; Vitamin $B_6$ 5mg; Vitamin $B_{12}$ 0.025mg; Folic acid 1mg; Biotin 0.08mg; Maganese 120mg; Iron 100mg; Zinc 80mg; Iodine 1.8 mg; Calcium pantothenate 11.5mg; Copper 8.5mg; Cobalt 0.3 mg; Selenium 0.12 mg; Antioxidant 120 mg;

## 3. Results

### 3.1 Growth Performance

The results of growth performance of the fry in outdoor rearing are presented in Table 2. In all the growth parameters measured, vitamin C levels indicated significant differences in most cases except total body length in which there were significant interactions between supplemental dietary levels of vitamins C and E ($P<0.05$). The group of fry from maternal parents that were fed on diets supplemented with required and excess vitamin C had significantly higher weight gain compared to the group of fry from the maternal parents fed diet devoid of supplemental vitamin C. However, no significant difference ($P>0.05$) between required and excess groups on this parameter. Vitamin C levels significantly affected survival of the fry outdoor ($P<0.05$) but not with vitamin E levels ($P>0.05$). Also there was no significant interaction on survival ($P>0.05$).

Vitamin E levels significantly improved SGR ($P<0.05$) but not with vitamin C levels ($P>0.05$). Increase in supplemental dietary level of both vitamins at zero level of the other caused a significant increase in lateral growth (P<0.05) (Table 3). The effect of increase in supplemental vitamin E at zero level of vitamin C was significantly greater than that of vitamin C at zero level of vitamin E (P<0.05). However the combination of the required supplemental levels of both vitamins resulted in a more elongation growth (P<0.05).

The rate of differential development was minimal and did not reveal treatment effect. There was relatively uniform growth within each group with few occurrences. No observable pathological symptoms of deficiency with respect to both vitamins C and E throughout the 12 weeks of outdoor growth experiment.

The results of physico-chemical parameters of the water monitored did not reveal any adverse condition for the fish growth. Temperature was between 26.5 - 30°C, pH 5.6 – 6.8, dissolved oxygen 6.5 – 6.8mgL$^{-1}$ and conductivity 220 μmhos cm$^{-1}$

Table 2: Growth Performance of Fingerlings 0 - 12 weeks.

| **Treatments** | **Parameters** | | | | | |
|---|---|---|---|---|---|---|
| | **Feed Intake (g/fish)** | **Feed Conversion Ratio** | **Weight Gain (g/fish)** | **Length (cm)** | **Specific Growth Rate (%/day)** | **Survival (%)** |
| Vitamin E | NS | NS | NS | * | * | NS |
| 0 | 11.26 | 1.26 | 9.09 | 10.62$^{b}$ | 7.90$^{a}$ | 84.7 |
| 50 | 11.37 | 1.35 | 8.65 | 11.27$^{a}$ | 7.80$^{b}$ | 77.8 |
| 400 | 11.41 | 1.33 | 8.74 | 11.13$^{a}$ | 7.80$^{b}$ | 82.8 |
| Vitamin C | NS | * | * | * | NS | * |
| 0 | 11.32 | 1.54$^{a}$ | 7.45$^{b}$ | 9.25$^{c}$ | 7.60 | 63.20$^{c}$ |
| 100 | 11.47 | 1.30$^{b}$ | 8.85$^{a}$ | 12.15$^{a}$ | 7.90 | 84.70$^{b}$ |
| 1000 | 11.26 | 1.11$^{c}$ | 10.19$^{a}$ | 11.62$^{b}$ | 8.10 | 94.70$^{a}$ |
| SED | 0.077 | 0.10 | 0.513 | 0.093 | 0.084 | 4.69 |
| Vit. E x C | NS | NS | NS | * | NS | NS |

a – c Means followed by different superscript within column are significantly different.

Table 3: Interaction of vitamin C & E on total length of fingerlings (0 – 12 weeks)

| **Vitamin E** | **Vitamin C (mg kg$^{-1}$ diet)** | | |
|---|---|---|---|
| | **0** | **100** | **1000** |
| **0** | 8.95$^{e}$ | 9.4$^{d}$ | 9.4$^{d}$ |
| **50** | 11.95$^{d}$ | 12.4$^{a}$ | 12.1$^{ba}$ |
| **400** | 10.95$^{c}$ | 12.0$^{b}$ | 11.9$^{b}$ |
| **SED** | 0.162 | | |

a – e Means followed by different superscripts significantly different (P<0.05)

**3.2 TBARS of Whole Body, Liver Vitamin C and E Content of Post-Fingerlings (0 – 12 weeks)**

There were significant interactions on TBARS of whole body of fish and concentration of vitamin E in the liver (P<0.05) but not on TBARS and vitamin C content in the liver of post-fingerlings (P>0.05) (Table 4). Increase in maternal supplemental dietary level of vitamin E at zero level of vitamin C significantly reduced the TBARS formation in the whole body of post-fingerlings (P<0.05). Also increase in dietary vitamin C at zero level of vitamin E caused a significant reduction in TBARS formation (P<0.05). Dietary supplementation of required and excess vitamin C had little significant effect on TBARS formation when maternal diet was supplemented with excess vitamin E (P<0.05). Also vitamin E had less effect on TBARS formation at excess level of vitamin C.

Increase in maternal dietary vitamin E at zero level of vitamin C significantly increase the concentration of vitamin E in the liver of post-fingerlings (P<0.05). The significant effect was more when either of the required or excess level of vitamin E was combined with excess level of vitamin C (P<0.05) (Table 5b).

Table 4: Effects of Maternal supplemental Dietary Vitamin C and E on Whole Body (WB) TBARS, Vitamin C and E Concentration in Liver of Fish (0 - 12 weeks).

| **Treatments** | **Parameters** | | | |
|---|---|---|---|---|
| | **WB TBARS ($\mu g\ g^{-1}$)** | **Liver TBARS ($\mu g\ g^{-1}$)** | **Liver VE ($\mu g\ g^{-1}$)** | **Liver VC ($\mu g\ g^{-1}$)** |
| Vitamin E | * | * | * | NS |
| 0 | $110.00^{a}$ | $0.20^{a}$ | $2.55^{b}$ | 104.90 |
| 50 | $63.66^{b}$ | $0.14^{b}$ | $3.17^{a}$ | 108.30 |
| 400 | $17.66^{c}$ | $0.10^{c}$ | $2.85^{b}$ | 113.10 |
| Vitamin C | * | * | * | * |
| 0 | $102.30^{a}$ | $0.27^{a}$ | $2.617^{b}$ | $3.70^{c}$ |
| 100 | $50.50^{b}$ | $0.10^{b}$ | $2.783^{b}$ | $58.00^{b}$ |
| 1000 | $38.50^{c}$ | $0.06^{c}$ | $3.167^{a}$ | $264.60^{a}$ |
| SED | 0.981 | 0.015 | 0.146 | 4.30 |
| Vit. E x C | * | NS | * | NS |

a – c Means followed by different superscript within column are significantly different (P<0.05).

NS = Not significant.

**Table 5 a&b: Interactions of Maternal Dietary Vitamin C and E on Whole Body TBARS and Vitamin E Concentration in Liver of Fish (0-12 weeks)**

**a) Whole Body TBARS**

| **Vit.amin E** | **Vitamin C (mg kg$^{-1}$ diet)** | | |
|---|---|---|---|
| | **0** | **100** | **1000** |
| **0** | $167.0^{a}$ | $104.0^{c}$ | $59.0^{d}$ |
| **50** | $126.5^{b}$ | $29.5^{f}$ | $35.0^{e}$ |
| **400** | $13.5^{h}$ | $18.0^{g}$ | $21.5^{g}$ |
| **SED** | | 0.054 | |

a-g Means followed by different superscript are significantly different (P<0.05).

**(b) Vitamin E Concentration in liver**

| **Vitamin E** | **Vitamin C (mg kg$^{-1}$ diet)** | | |
|---|---|---|---|
| | **0** | **100** | **1000** |
| **0** | $2.40^{d}$ | $2.70^{b}$ | $2.65^{c}$ |
| **50** | $2.70^{b}$ | $2.75^{b}$ | $3.55^{a}$ |
| **400** | $2.70^{b}$ | $3.05^{b}$ | $3.50^{a}$ |
| **SED** | | 0.133 | |

a-d Means followed by different superscript are significantly different (P<0.05).

## 4. Discussion

The early development of larval fish is dependent on the internal compliment of essential nutrients that are present in egg (Palace and Werner, 2006). These nutrients are also dependent on the maternal diet prior and during oogenesis (Lavens *et al.*, 1999). The significant difference shown by maternal supplemental vitamin C levels with respect to weight gain and FCR despite the non-effect on feed consumed is an indication of inherent growth performance quality that might have been acquired by the fry from the egg through the maternal parent. Vitamins C and E among other non-enzymatic factors present in the cell and tissues of all vertebrates to cope with continuous generation of reactive oxygen species (ROS) from normal aerobic metabolism (Halliwell and Gutteridge, 1990). The higher concentration of vitamin C in the eggs from the supplemented groups might have generally afforded the fry an upper hand of performance ability. It is an indication of extra ability to cope with the prevailing conditions in the culture medium, although there was no adverse environmental condition as revealed by the physico-chemical parameters. Feed intake was not significantly different but feed conversion ratio was different among the groups, which was particularly due to vitamin C levels (Dabrowski and Blom, 1994; Sealey and Gatlin, 2002). The differences observed in FCR manifested in weight gain too, which further revealed that utilization was actually different between the various groups despite feeding on the same type of diet. Vitamin C in particular had been identified as a very essential vitamin for fish since the only source of vitamin C in the fish's body is through the diet (Fracalossi *et al.*, 2001). It is possible that the presence of vitamin C in the diet in addition to the maternally derived together with what the fry was able to obtain from the secondary production in the tank enabled the fry of the vitamin C groups to perform better. It must be noted that the primary and secondary productions will only be available after been built up, so prio to build up, it is most likely that the group from the maternal parent devoid of supplemental vitamin C in its diet will be lacking the advantage of inherent reserves of the vitamin and there is likely to be a slow down of all the physiological reactions benefiting from such reserves in the body of the developing fish. This is likely going to happen at each occasion of draining the tank for sampling and replenishing the tank with freshwater. However fluctuation in primary and secondary production ought not to be a critical problem since the diet fed to the fry contained required amount of the vitamins for fingerlings. It is most likely that the fry require higher levels than fingerlings and if that is the case, the inherent reserves was an advantage to the groups from maternal parents that were fed vitamin C supplemented diets. The differences in performance with respect to weight gain and survival might be due to survival of the fittest, which is not unconnected with the imputed ability from the parent. This is in agreement with the results of Dabrowski and Blom (1994) who showed the positive effect of high ascorbate levels in embryos of rainbow trout on their survival. The lower concentrations of vitamins E and C in the liver of fingerlings derived from maternal parent that fed on diet devoid of the supplemental vitamins as revealed by the chemical analysis further confirmed this disadvantage in the zero groups. High content of vitamin C was observed to increase survival of egg and fry while a high dietary α-tocopherol did not in Atlantic salmon, *Salmon salar* (Eskelinen, 1989).

Although dietary vitamin E levels did not influence FCR and weight gain, it did affect the lateral growth of the fingerlings as significant interaction was indicated in length parameter. Therefore, it might not be wrong to say that vitamin E had a significant influence on growth since fish growth involves expansion and elongation of body tissues. Another likely reason why the influence of vitamin E on growth parameters was not pronounced could be due to the fact that minimum supplemental requirement level needed to prevent deficiency symptoms and suppressed growth is small (25-50 mg $kg^{-1}$) and the required level for fingerlings was included in the diet fed to the fry. In the previous growth experiments 25 mg vitamin E $kg^{-1}$ diet was able to prevent pathological problems (Ibiyo *et al*., 2008). It is most likely that the fish had a quick adjustment response from the low level acquired from the maternal parent to exogenous nutrients supply through feed hence the group devoid of supplemental maternal vitamins C and E was not significantly different from others with respect to vitamin E levels. Also vitamin E might not be a critical nutrient in the presence of dietary vitamin C, as it had no influence in some of the parameters under vitamin C's availability (Sealey and Gatlin, 2002). Dietary vitamin C improved feed efficiency and protected rainbow trout from anemia and mortality associated with a dietary deficiency of vitamin E (Frischknecht *et al*., 1994; Sealey and Gatlin, 2002).

The presence of one of the vitamins in the absence of the other reduced TBARS formation in the liver and whole body of post-fingerlings. Sealey and Gatlin (2002) also observed a reduction in TBARS in liver of hybrid striped bass fed marginal vitamin C at high level of vitamin E. These significant differences and interaction showed on the TBARS formation is an indication of the importance of vitamins C and E in tissue protection as non-enzymatic antioxidants.

It must be noted that the fry in this study were fed the same basal diet till post-fingerlings age but still exhibited varying responses in terms of the parameters studied. The differences observed could be attributed to the inherent performance ability due to individual differences, which was possibly acquired from the parents. Again the varying parent in this study was the maternal parent based on diet taken because the paternal parent was the same for all the groups. It might be concluded from the results of this study that maternal diets can possibly affect the performance of its offspring. There is need to look into the direct interactive effect of these antioxidants in the performance of fingerling of *H. longifilis* by incorporating combinations into their diets. It will be necessary to confirm whether vitamin C and E requirements for fry stage of *H. longifilis* is same as that of fingerlings.

**Correspondence to:**
Ibiyo Lenient Mercy Onivie
National Institute for Freshwater Fisheries Research,
New Bussa, Niger State, Nigeria.
Cellular phone: 08059241879
Emails: oniviemercy@yahoo.com

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