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Response of *Heterobranchus longifilis* Fingerlings to Supplemental Dietary Vitamin E

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Abstract: A study was conducted to determine the dietary vitamin E supplementation into a basal diet containing the common poultry premix as diet of *Heterobranchus longifilis* fingerlings. 0, 25, 50, 75, 100, 125, or 150 mg vitamin E kg^{-1} diet as DL-α-tocopheryl acetate (Teva Pharmaceutical Industries, Petach Tikva) were fed to *H. longifilis* fingerlings initially averagely weighing 1.83±0.06 for 12 week. Fish fed the basal diet were visually lighter in colour. Specific growth rate showed significant difference which was not significantly manifested in weight gain, feed consumed, feed efficiency and survival at the end of the trial as there was no significant difference in all levels with respect to these parameters. The Thiobarbituric Acid Reactive Substance (TBARS) of the liver, stored carcass and plasma however showed significant difference. Fish receiving the basal diet had significantly highest TBARS in both plasma and tissues. The haematological parameters also showed significant difference, while the Hb and PCV reflected the supplementation levels, the RBC and WBC showed no definite trend. Regression analysis of the liver TBARS data using the broken line model indicated a 112 mg vitamin E kg^{-1} diet supplementation. It was concluded that for optimum performance of the liver and health of mud-fish fingerlings vitamin E supplementation is necessary whenever poultry premix is used in fish feed formulation.

Key words: Vitamin E, *Heterobranchus longifilis*, growth response, haematology, TBARS

INTRODUCTION

Heterobranchus longifilis have gained widespread as a promising species in aqua cultural production in Nigeria. A number of studies have been carried out during the last decade to investigate various nutritional requirements of *Heterobranchus longifilis* to improve and refine practical diet formulations for use in aqua cultural production. Research to date has focused primarily on determining dietary protein (Fagbenro *et al*., 1992; Eyo, 1995) and relative use of lipid (Babalola and Apata, 2006). The mineral and vitamin requirements have received limited attention (Eyo, 1999) except the need for vitamin C and its requirement (Ibiyo *et al*., 2006, 2007).

One vitamin that has been shown to be of considerable importance in fish nutrition is vitamin E. Dietary requirements for vitamin E which functions primarily in protecting cell membranes from oxidation, have been established for a number of fish species (NRC, 1993). Most fish feed formulation in Nigeria involves the use of the common poultry premix as a source of vitamins and trace minerals

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in fish diets. This present study was undertaking to determine whether the poultry premix meets the needs of *H. longifilis* in terms of vitamin E or there is need for dietary supplementation and also to determine the effect of vitamin E on stored *H. longifilis* products.

MATERIALS AND METHODS

Experimental Design and Diets

A feeding trial was conducted using a completely randomised design with three replicates to determine the supplemental dietary vitamin E requirement of *H. longifilis* (Valenciennes, 1840) fingerlings. Hexane treated clupeid meal was used in the formulation with other ingredients to contain 42.5% crude protein to minimally satisfy the protein requirement of *H. longifilis* (Fagbenro *et al*., 1992; Eyo, 1995). Cod liver oil was used as source of lipid in the diet. The common broiler premix was used in the basal diet formulation and most fish farm practices in Nigeria involve the use of the common poultry premix. Seven experimental diets were prepared with incremental levels of supplemental vitamin E (dl-α-tocopherol acetate, Teva pharmaceutical Industries, Petach Tikva) (0, 25, 50, 75, 100, 125 and 150) mg kg^{-1}diet at the expense of silica (Table 1). The ingredients were grinded, milled, weighed, mixed and cold pelleted with meat mincer through a 2 mm die. After pelleting the feed were air dried, put in polyethylene in an air-tight container and kept frozen until used. Sample of the basal diet was taken for proximate analysis (Table 2) and α-tocopherol content.

Fish and Feeding Trial

The feeding trial was conducted at the National Institute for Freshwater Fisheries Research (NIFFR) hatchery complex using 273 six weeks old fingerlings obtained within the hatchery. The fingerlings were conditioned to the basal diet for 2 week. Feed supply was stopped 2 days to the commencement of the feeding trial. After acclimatization, the fingerlings were weighed into 30 L circular plastic tanks in a mini-flow through experimental system. The average initial weight of fish was 1.83±0.06 g. The replicates with 13 fingerlings each were randomly allocated to the treatments. 10 fingerlings were sacrificed and taken for analysis of initial proximate composition.

Each of the dietary treatments was fed for 12 weeks to the randomly assigned replicate tanks. A fixed feeding regime of 5% body weight per day divided into two equal rations and given between

Table 1: Composition of the experimental diets

	Percent inclusion levels of vitamin E (mg kg^{-1})						
Ingredients	0	25	50	75	100	125	150
Vitamin E acetate	0.00	0.01	0.02	0.03	0.04	0.05	0.06
Silica	0.08	0.07	0.06	0.05	0.04	0.03	0.02
Basal*	99.92	99.92	99.92	99.92	99.92	99.92	99.92
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

*: With a premix that 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; Choline chloride 0.1 mg

Table 2: Proximate composition of the basal diet

Parameters	Analysed content
Crude protein (%)	43.39
Crude fat (%)	6.55
Crude fibre (%)	1.10
Ash (%)	6.19
NFE (%)	37.95
Moisture content (%)	4.82
Metabolizable energy (kcal/100 g)	373.00

the hours of 800-900 and 1700-1800 was adopted except on sampling days in which, time of feeding was altered. Fish were fed daily, tanks washed and drained at two days intervals to prevent microbial growth that could possibly alter the experiment. Group weight measurements were made forth-nightly and feed allotments subsequently adjusted for the next two weeks. During each weighing, time of draining and washing of tanks mortality and the condition of fish were observed and recorded. During the feeding trial the water quality parameters were monitored with the assistance of the staffs of Liminology Division of the Institute.

Sample Collection and Analysis

Eight weeks into the experiment, apparent nutrient retention trial was carried out using the inert indicator, chromic oxide method, with faecal samples collected by gentle press on the ventral part close to the anal pore twice daily for three days at three days intervals to allow the fish recover from the stress imposed before another subsequent collection. The samples were oven dried and bulked for crude protein and crude fat analysis. 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.

At the end of the 12 week, after obtaining final weights, blood and tissue samples were collected from representative randomly selected five fish in each of the triplicate tanks per dietary treatment. Blood samples were taken from the five fish by heparinized syringe from the caudal vasculature. Haematocrit was determined by the micro method (Brown, 1980) and other blood parameters. Plasma was then separated by centrifugation (Centricone, Precision Scientific, Chicago, IL, USA), removed and stored at 80°C until analysed for thiobarbituric acid reactive substance. In addition, the five fish were excised, liver samples collected, pooled per group and stored at -80°C before being assayed for thiobarbituric acid reactive substance (Draper *et al*., 1993) and α-tocopherol (Huo *et al*. (1996). The TBARS of the pooled liver and fish carcass were determined using a method adopted from that of Burk *et al*. (1980). Concentration of vitamin E in tissue was based on peak height ratios (analyte vs. the internal standard tocol).

Statistical Analysis

The data obtained from growth performance and liver α-tocopherol concentration were subjected to analysis of variance with SPSS version 10.0 while the data from TBARS over the period of storage were subjected to two-way ANOVA without replication and in all means of vitamin E levels were separated with Duncan New multiple range test using one way ANOVA in SPSS. The data from TBARS of the liver was subjected to least square regression analysis with broken line model in order to estimate the minimum vitamin E supplementation level of requirement in the diet of *H. longifilis*.

RESULTS

Growth and Nutritional Performance

Survival of *H. longifilis* fingerlings range from 90 to 100% after the 12 week feeding trial and was not significantly affected by dietary vitamin E (Table 3) whereas diet analysis reflected the supplementation levels. There were graded levels of vitamin E ranging from 36 μg g^{-1} dry weight in the unsupplemented diet to 59, 83, 106, 130, 149 and 175 μg g^{-1} in the order of the supplementation respectively. Mortality occurrences were during the first few days of the trial and appeared to be related to stress of sorting and weighing the fish to commence the trial. Weight gain feed consumed and PER were not significantly affected by the graded levels of vitamin E. The group fed diet supplemented with 50 mg vitamin E kg^{-1} exhibited significantly higher daily growth rate compared to the group fed zero supplementation. However, it was not significantly different from the other groups irrespective

Table 3: Effects of vitamin E on the growth and haematology of *H. longifilis* fingerlings 0-12 week

	Supplemental vitamin E levels (mg kg^{-1}) diet							
Parameters	0	25	50	75	100	125	150	SEM
Initial wt. (g)	1.80	1.80	1.80	1.80	1.80	1.90	1.80	0.126
Wt. gain (g $fish^{-1}$)	40.47	39.77	38.60	37.60	40.30	36.37	37.17	1.630
PER	2.44	2.36	2.46	2.44	2.45	2.38	2.38	0.034
FC (g $fish^{-1}$)	38.90	39.56	36.91	34.25	38.69	35.94	36.73	1.830
SGR (% day^{-1})	3.72^{ab}	3.70^{ab}	3.73^{a}	3.65^{abc}	3.62^{abc}	3.60^{bc}	3.50^{cb}	0.031
Survival (%)	100.00	94.86	100.00	100.00	100.00	94.86	97.47	1.730
CP retained (%)	57.09^{d}	62.21^{b}	63.03^{a}	60.99^{bc}	62.37^{ab}	60.20^{c}	61.99^{ba}	0.486
CF retained (%)	59.53^{c}	63.06^{a}	63.00^{a}	63.20^{a}	61.96^{ab}	60.83^{bc}	60.73^{bc}	0.441
Haematocrit (%)	44.00^{c}	48.00^{b}	50.00^{a}	45.00^{c}	44.00^{c}	42.00^{d}	40.00^{e}	0.462
Hb conc. (mg dL^{-1})	11.03^{c}	11.70^{b}	12.60^{a}	11.60^{b}	11.60^{b}	10.43^{d}	10.90^{dc}	0.049
RBC ($\times 16^{6}$ μL^{-1})	2.61^{e}	3.08^{a}	2.77^{d}	2.67^{e}	2.84^{c}	2.74^{d}	2.96^{b}	0.020
WBC (μL^{-1})	228.00^{d}	251.30^{c}	267.20^{a}	248.60^{c}	247.10^{c}	252.60^{c}	260.00^{b}	1.901

a-e: Means within rows with different superscript significantly different

of the supplementation level (Table 3). The crude protein and crude fat retained showed significant differences. The water quality parameters as monitored showed that average value for temperature, dissolved oxygen, hydrogen ion concentration (pH) and conductivity were 30.5°C, 6.6 mg L^{-1}, 7.2 units and 230 μmhos cm^{-3}, respectively.

Clinical Symptoms of Vitamin E, TBARS of Fillets, liver α-Tocopherol Concentration and Blood Parameters

No critical gross deficiency sign manifested itself during the study. However, fish fed the basal diet had a much lighter colour in appearance than the supplemented groups. Apart from the visual colour differential the only observed clinical symptom of vitamin E deficiency was haemorrhage of the fin exhibited by the control group at the latter end of the experiment. Histological examination of the muscle and liver tissues also failed to detect any abnormalities that could have been attributable to differences in dietary vitamin E.

The blood parameters showed significant differences. The Haemoglobin (Hb) content and the haematocrit (PCV) showed definite trend in relation to the vitamin E levels, while the red blood cell count and white blood cell count parameters showed no definite trend though ANOVA indicated significant differences in these cases (Table 3). The liver α-tocopherol concentration increased significantly while TBARS of liver, carcass and plasma significantly decreased with increased level of vitamin E supplementation in the diets of *H. longifilis* fingerlings (Table 4). The same trend was observed in the TBARS of the stored carcass over 12 days. Days of storage indicated significant difference (p-value = 0.045) (Table 4). There was no much difference between the proximate composition of the initial and final fish, so the data are not presented.

Estimation of Supplemental Vitamin E Requirement

Regression analysis using least square error method showed that the extent of oxidation of the liver tissue can be reduced to minimum when the basal diet of *H. longifilis* is supplemented with vitamin E at a level as high as 100 mg kg^{-1} diet to that present in the basal diet. The broken line model with the regression analysis revealed that the best performance of the liver with respect to TBARS will be achieved at 112 mg vitamin E kg^{-1} diet supplementation level. Supplemental level not less than 22 mg kg^{-1} diet was also indicated. Economically, 50 mg vitamin E kg^{-1} diet can however be recommended as the supplementary requirement estimate since no significant difference existed between it and the groups fed 75, 100, 125 and 150 mg vitamin E kg^{-1} diets. Considering SGR for estimation of the requirement level, also supports the same level as TBARS suggested.

Table 4: Effects of vitamin E on thiobarbituric acid reactive substance (TBARS) and α-tocopherol concentration of the plasma, fillets 0-12 week

	Supplemental vitamin E levels (mg kg^{-1}) diet							
Parameters	0	25	50	75	100	125	150	SEM
Liver (nmole g^{-1})	3.31[a]	2.83[b]	2.61[c]	2.60[c]	2.60[c]	2.59[c]	2.57[c]	0.038
Plasma (nmole L^{-1})	2.03[a]	1.96[b]	1.82[c]	1.61[d]	1.47[e]	1.28[f]	1.27[f]	0.024
Carcass (nmole g^{-1})	25.61[e]	23.29[d]	18.69[c]	16.68[b]	16.87[b]	15.01[a]	14.8[a]	0.2
α-TCL (μg g^{-1}) *	2.02[a]	2.52[b]	2.82[c]	4.81[d]	5.05[e]	6.08[f]	7.02[g]	0.042

a-g: Means within rows with different superscript are significantly different; *: α-tocopherol concentration of the liver

DISCUSSION

The average value for water quality parameters recorded was within the range recommended for good performance of freshwater fish although water quality deterioration is unexpected in a flow through system. The general high survival rate observed suggest that the nutrients received from the diets under the prevailing environment met the need of the fingerlings. The failure of weight gain, feed efficiency or survival to be moderated by the levels of vitamin E presence in the diets has been a common observation in many studies evaluating the effect of supplemental vitamin E on fish growth (Cowey *et al*., 1981; Wilson *et al*., 1984; Gatlin *et al*., 1986; Baker and Davies, 1996). These studies were performed in highly controlled environments where the fish's only source of food was formulated diets and yet fish performance was not hindered even at low levels of vitamin E intake. Tocher *et al*. (2002) also showed that Halibot and Turbot were not significantly affected by dietary supplementation of vitamin E when 0, 100 or 1000 mg kg^{-1} diets were fed. The results of this study also suggested that the diets might have supplied an adequate vitamin E needed for the growth of *H. longifilis*. However the colour differential and haemorrhage of the fin that was visible in the group fed unsupplemented basal diet lend support to vitamin E supplementation in this fish species to boost health performance under the use of the most available premix. Channel catfish fed a diet deficient in vitamin E were lighter in colour (Lovell *et al*., 1984) whereas hybrid striped bass fed a basal diet deficient in vitamin E were darker in appearance (Kocabas and Gatlin, 1999). These differential responses in terms of visual colour appearance might be related to species differential in both studies.

Failure to detect severe gross abnormal pathologies was not particularly surprising in this study. Other researchers working with a variety of fish species have had difficulty inducing a vitamin E deficiency severe enough to manifest gross deficiency signs. The work of Baker and Davies (1996) failed to observe any deficiency symptom or reduced growth while feeding African catfish, *Claria gariepinus* a diet with as low as 2.15 mg α-tocopherol kg^{-1} for 10 week. Therefore, in this present study, failure to observe reduced growth or induce severe pathological signs was not inconsistent with previous research studies. The concentration of α-tocopherol in the tissue necessary to prevent any gross abnormal pathologies is not clear from the data presently available. In studies by Murai and Andrews (1974) and Lovell *et al*. (1984), both of which reported signs of gross deficiency in channel catfish, neither α-tocopherol levels in the basal diet, nor tissue levels were determined for further clarification of a minimum level of vitamin E to prevent these signs. The responses of *H. longifilis* tissue to increasing levels of dietary vitamin E in this study were similar to those previously reported in which supplementation of vitamin E increased the concentration of α-tocopherol in various tissues of poultry (Rethwill *et al*., 1981; Lin *et al*., 1989) and fish (Frigg *et al*., 1990; Bai and Gatlin, 1993). Although the significant increase in tissue levels of α-tocopherol was not manifested in weight gain response as the supplementation level increases, Gaylord *et al*. (1998) said the ability of dietary vitamin E supplementation to influence tissue levels of α-tocopherol was not unexpected when the author analyzed for tissue content of vitamin E.

The significant improvement in haematocrit and haemoglobin concentration is an indication that vitamin E's contribution to the general well-being of the fish may be most attainable with 50 mg supplementation level which is also manifested in the TBARS of the liver and carcass (Fig. 1). Kocabas

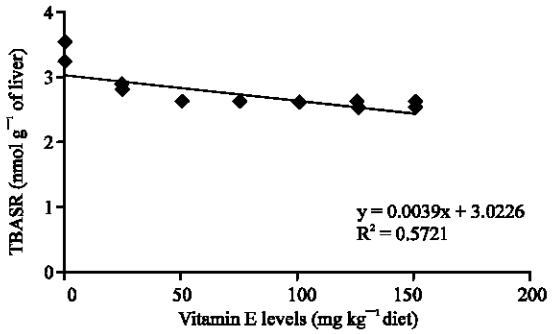

Fig. 1: Effect of vitamin E levels on TBARS of liver 0-12 week

and Gatlin (1999) also observed an improvement in haematocrit with vitamin E supplementation. The lack of definite trend in the statistical difference observed with respect to RBC and WBC parameters might be attributed to some other unexplained factors influencing haematology of the fish.

The ability of a cell to prevent oxidation of its membrane lipids is well documented to be associated with α-tocopherol levels (Gaylord *et al*., 1998). In previous research with fish, when growth has not been responsive to dietary vitamin E levels, a measure of the cell's ability to prevent oxidation of its membrane lipids has been utilized, namely an iron/ascorbate-stimulated lipid peroxidation assay, to assess the vitamin E requirements of those fish. The assay is a sensitive method for measuring the relative α-tocopherol status of the tissue, but a sufficient number of dietary treatments are needed to estimate a level at which lipid peroxidation is sufficiently inhibited to warrant a certain level of vitamin E supplementation. In African catfish, Baker and Davis (1996) found that supplementation of vitamin E acetate from 0 to 500 mg kg^{-1} reduced the susceptibility of muscle and liver tissues to peroxidation. It was determined that a level of 212.2 μg α-tocopherol g^{-1} of liver tissue prevented further iron/ascorbate lipid peroxidation and *in vivo* lipid oxidation was also prevented, as measured by malondialdehyde concentration in the African catfish. Baker and Davis (1996), however, were unable to determine a requirement owing to the lack of intermittent levels between 0 and 80 mg supplemental vitamin E kg^{-1} diet. Requirement estimate have been reported for channel catfish (Wilson *et al*., 1984; Gatlin *et al*., 1986a) and for rainbow trout *Onchorynchus mykiss* (Cowey *et al*., 1981, 1983) based on lipid peroxidation of hepatic macrosomes. However, in the present study it would most likely be inconclusive not to use peroxidation in relation to TBARS as an indicator of dietary vitamin E needed by *H. longifilis* because of the non responsiveness of growth (Gaylord *et al*., 1998). Base on the condition of this experiment, the response of TBARS could be regarded as the index of estimation for supplemental vitamin E need of *H. longifilis*. Weight gain which Woodward (1994) regarded as arguably the best single index of vitamin requirement on which to base diet formulation practice was not influenced in this study. Although, specific growth showed significant difference it was not manifested in the total weight gain, suggesting marginal differences. Increasing levels of dietary vitamin E was seen to progressively decrease the TBARS values of fillet tissues of *H. longifilis*. This was in agreement with the observation that increasing tissue concentrations of α-tocopherol effectively improves oxidation stability of fillet tissues from rainbow trout (Frigg *et al*., 1990) and channel catfish subjected to forced oxidation (Gatlin *et al*., 1992). In the study of Frigg *et al*. (1990) progressive decrease in TBA value of rainbow trout fillets which corresponded to increases in tissue tocopherol levels resulting from dietary supplementation of vitamin E up to 200 mg kg^{-1} was observed. Significant negative correlations between TBA values and tissue tocopherol concentrations were apparent in the study which involved subjection of catfish fillet to forced oxidation (Gatlin *et al*., 1992).

Difficulties occur when trying to extract a set dietary requirement for vitamin E from the studies performed to date. A number of factors have been postulated to influence the requirements for vitamin E. These include vitamin C, selenium, unsaturated lipid as well as other specific response criteria and some other conditions.

The significant difference observed in the crude protein and fat retained is an indication of some influence of vitamin E on the metabolism of *H. longifilis*. Although it did not manifest into differential weight gain, it might have had influence on the general wellbeing of the fish. Vitamin E supplementation of practical diets of *H. longifilis* was evaluated in this study and results indicated that supplementation of the diet containing 36 μg g^{-1} and 160 mg α-tocopherol kg^{-1} analyzed and calculated values respectively did not improve production given the parameters of this experiment. Supplementation may still be necessary to improve health status and product quality of *H. longifilis* fillets, as seen on its effect on haematology and TBARS of liver and carcass in this study just as demonstrated by O'Keefe and Noble (1978) and Gatlin *et al.* (1992) and as well as aided immune function (Wise *et al.*, 1993). Although changing tissue level of TBARS did not appear to affect production performance.

The regression analysis with the broken line model revealed that the best performance of the liver with respect to TBARS will be achievable at 112 mg vitamin E kg^{-1} diet supplementation level. Economically, supplemental level of 50 mg vitamin E kg^{-1} diet could be recommended, as the TBARS of the liver showed similar response from 50 mg vitamin E to the highest supplementation levels. However in terms of storage quality supplementation could be as high as 112 mg vitamin E kg^{-1} diet since the least TBARS occurred at 125 and 150 mg kg^{-1} supplementation levels. Further study will be essential to confirm: Whether longer period (above 12 week) of subjection of *H. longifilis* fingerlings to graded levels of vitamin E could yield similar results; the potential benefit of supplemental vitamin E on these responses of *H. longifilis* under conditions of commercial production and also possible improvement on reproductive performance.

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