Nigerian Journal of Fisheries and Aquaculture 4(1): 1 – 8, May 2016

Department of Fisheries, Faculty of Agriculture, University of Maiduguri, Nigeria
Available on line: http://www.unimaid.edu.ng
ISSN-2350-1537

Response of Juvenile *Heterobranchus bidorsalis* (Geoffroy - St. Hilaire 1809) to Mega Doses of Supplemental Dietary Vitamin E (Alpha-tocopherol)

*Udo, I. U. and Isangedighi, I. A.
Department of Fisheries and Aquatic Environmental Management, University of Uyo, Uyo Nigeria

Received October 27, 2015 Accepted December 23, 2015

ABSTRACT

Heterobranchus bidorsalis is one of the commercially important species of fish for rapid aquaculture expansion in Nigeria and elsewhere in the developing world. Despite the aquaculture potentials of this fish species, not much research has been conducted especially in fixing the requirements and studying its response to different doses of vitamin E. This experiment therefore examined the response of juvenile *H. bidorsalis* to mega doses of supplemental dietary vitamin E. Sixty *Heterobranchus bidorsalis* juveniles (20 per replicate) of about 2.63 ± 0.70 g were fed on 3 diets containing 0,250 and 500 mg/kg^{-1} of vitamin E in triplicate for 113 days. Final weight, average daily gain and specific growth rate decreased with increasing dietary vitamin E supplementation. No significant difference was noticed between the 250 and 500 mg kg^{-1} diet. Protein efficiency ratio and feed conversion ratio also decreased with the increasing dietary vitamin E supplementation while mortality increased with increasing supplementation. Fish fed on the control diet had significantly better growth performance and feed utilization than others. These results indicate that the optimal dietary vitamin E level for *H. bidorsalis* fingerlings is below 250 mg kg^{-1} diets. Feed formulators should therefore use vitamin E levels below 250 mg kg^{-1} in the diet of *H. bidorsalis* and other Clariids.

Key words: Vitamin E, mega doses, *Heterobranchus bidorsalis*, response

INTRODUCTION

Vitamin E is a lipid–soluble vitamins that comprise eight naturally occurring tocopherols. Among them, d-α-tocopherol has the highest bio-potency (Bueno *et al.*, 2010). Other than its vitamin E activity, d-α-tocopherol is a potent biological antioxidant that can protect biological membranes and lipid components containing unsaturated fatty acids against attack from free radicals (Lee and Shiau, 2004; Mehrad *et al*., 2012). The antioxidative functions of vitamin E include scavenging of free radicals to terminate damage to unstable intercellular components including membranes, nucleic acids and enzymes (Hung *et al*., 1980; Kiron *et. al*., 2004). Most deficiency signs observed in fish which include: nutritional muscular dystrophy, fatty liver degeneration, anaemia, exudative diathesis; erythrocyte haemolysis, haemorrhages, depigmentation and reduction of fertility relate to the peroxidative damage of cellular membranes (Huang *et al*., 2004).

A dietary requirement of vitamin E has been demonstrated in a number of fish, which includes 120 mg kg^{-1} diet (Hamre and Lie, 1995) for Atlantic salmon, 30 to 50 mg kg^{-1} diet for channel catfish (Murai and Andrews, 1974; Wilson *et al*., 1984), 200 to 300 mg kg^{-1} diet for common carp (Watanabe *et al*., 1977), 26.6-29.6 mg kg^{-1} dry diet for young-of-the-year beluga (Amlashi *et al.*, 2012**),** 131.91 mg kg^{-1} dry diet for rohu (*Labeo rohita*) fry (Sau *et al*., 2004). According to Mehrad and Sudagar (2010), 1000 mg kg^{-1} of vitamin E is required by guppy (*Poecilia reticulata*) for optimum growth and reproductive performance. Shiau and Shiau (2001) reported the re-evaluated optimum dietary vitamin E requirement of juvenile hybrid tilapia to be 40 – 44 and 60–66 mg in 50 and 120 g lipid per kg diets, respectively.

*Corresponding Author: email: dorime_2004@yahoo.com, +234(0) 8182260328

Heterobranchus bidorsalis is one of the commercially important species of fish for rapid aquaculture expansion in Nigeria and elsewhere in the developing world, due to its fast growth rate, high feed conversion ratio and utilization, high resistance to stress and disease, desirable as food, readily accepts artificial diets, and feeds over a wide range of diets. It is omnivorous and possesses accessory breathing organs which enable them to tolerate adverse aquatic conditions where other cultivable fish species cannot survive (Fagbenro *et al*., 1991; Fagbenro, 1992; Agbebi *et al*., 2009; Owodeinde and Ndimele, 2011). Despite the aquaculture potentials of this fish species (Fagbenro *et al*., 1993) not much research has been conducted especially in determining the nutrient requirements and studying its response to different doses of vitamin E. This experiment therefore investigates the response of juvenile *H. bidorsalis* to mega dose of supplemental dietary vitamin E.

MATERIALS AND METHODS

Location and climate of the experimental site

This study was carried out at the University of Uyo fish farm located in the Southern part of Nigeria (Latitude 05°03′04’’N; Longitude 07°56′00’’E). The water source was a borehole. The area has an altitude of around 45 m above sea level. The climate in the area can be divided into two main seasons: wet and dry. The wet season lasts from May to October and the dry season from November to April. Annual rainfall averages about 570 mm and the peak rainfall occurs in the period between August and September. The average minimum and maximum temperatures are about 16 and 32°C, respectively. The mean ambient temperatures recorded were 28-32°C in the middle of the day during the experimental period (July to December, 2014). Data were obtained from the Nigerian Meteorological Agency.

Collection and processing of feed ingredients

Feed ingredients used in this experiment include soybeans, fish meal (*Ethmalosa fimbriata*), palm kernel cake and white maize. All the ingredients were procured locally from the Uyo main market. The ingredients were brought to the processing unit where they were finally processed as follows: Soybean was toasted until it became brown in colour and the chaff was blown out before grinding it into powders. Fishmeal, white maize and palm kernel cake were also ground into powder.

Proximate composition of feeds ingredient

Ingredients used include fishmeal; soybean meal; white maize meal and palm kernel cake while nutrient considered were dry matter, crude protein, crude fibre, ether extract, phosphorus, calcium and nitrogen free extract. Others are lysine, methionine and digestible energy (Table 1). These were adapted from standard nutritional tables (ADCP, 1993; NRC, 1993).

Table 1: Proximate composition and digestible energy of the feed ingredients used in this experiment

Feedstuff	**Content (%) (Kcal kg^{-1} DM)**										
	DM	**CP**	**CF**	**EE**	**Ash**	**P**	**Ca**	**NFE**	**LS**	**MT**	**DE**
FM	91.12	52.89	3.11	5.78	21.90	2.89	5.14	16.32	4.85	2.62	2861.00
SBM	88.50	88.50	6.50	3.50	5.67	0.20	0.20	31.33	2.80	0.60	2230.00
WMM	88.51	7.31	2.00	3.20	0.51	0.09	0.01	76.59	0.30	0.18	3432.00
PKC	91.6	20.40	9.0	8.90	5.70	0.60	0.30	56.60	0.75	0.94	3137.00

FM=fishmeal; SBM=soybean meal; WMM=white maize meal; PKC=palm kernel cake DM=dry matter CP=crude protein; CF=crude fibre; EE=ether extract; P=phosphorus; Ca=calcium; NFE=nitrogen free extract; LS=lysine; MT=methionine; DE=digestible energy. Source: ADCP (1993).

Diet formulation

Diet for this experiment was formulated using feed formulation software for windows (Winfeed 2.8) which formulates feed by linear programming technique. All diets were formulated on dry matter basis using the proximate compositions of the feed ingredients (Table 2).

Table 2: Ingredient and nutrient compositions of the experimental diet

Ingredient (%)	**0Mg Kg^{-1}ATO (NoATO)**	**250Mg Kg^{-1} ATO (250ATO)**	**500Mg Kg^{-1} ATO (500ATO)**
Palm kernel cake	2.22	2.21	2.21
Soyabean meal	23.38	23.38	23.38
Fishmeal	10.00	10.00	10.00
White maize meal	64.40	64.40	64.40
Total	100.00	100.00	100.00
Proximate composition (%)			
Crude protein	40.00	40.00	40.00
Ether extract	4.09	4.09	4.09
Crude fibre	3.73	3.73	3.73
Ash	4.41	4.41	4.41
Nitrogen free extract	67.17	67.17	67.17
Methionine	1.51	1.51	1.51
Phosphorus	0.45	0.45	0.45
Calcium	0.63	0.63	0.63
Digestible energy	3476.23	3476.23	3476.23
Cost kg^{-1} (₦)	**219.00**	**219.00**	**219.00**

Feed milling and drying

Diets formulated in percentage were then converted to weight basis. The ingredients were measured using Camry kitchen weighing balance into a mixer where they were mixed thoroughly. Two per cent of cassava starch was used as a binder. Hot water was then added into the mixture and mixing continued. After 10 minutes, they were then pelleted using manual pelletizer of 2.0 mm die ring and dried accordingly. Vitamin E (Korea, United Pharm. Inc. 404-10, Nojang RI, Jeondong-Myeon, Yeongi-Kun, Chongnam Korea) was dissolved in liquid hexane at 100 mg/0.25 litre of liquid hexane. This was sprayed on the pellets according to different treatments and then air-dried for 15 minutes.

Experimental fish

Two hundred and ten (210) juvenile *H. bidorsalis* were procured from a fish farm located in Ikot Abasi Akwa Ibom State, Nigeria**.** The experimental fish were transported in an opened-top, 20 litre plastic container half-filled with water to the experimental site. Allowance was made for 2 % mortality during transportation. The fish were acclimated for one week in the hatchery unit of the fish farm. During this period the fish were fed *ad libitum* with 45 per cent crude protein diet.

Experimental procedures

Nine tarpaulin tanks (3000 litres each) were used for the experiment. The tanks were washed, dried and filled with water supplied from the bore-hole; water level used in stocking the fish was 20 cm from the bottom. The setup took the form of completely randomized design (CRD). One hundred and eighty fingerlings were randomly distributed into nine experimental tanks (20 per tank). These were assigned to three treatments and replicated thrice. Treatment 1 (control) was diet prepared with all the ingredients without alpha-tocopherol (ATO) supplementation, designated NoATO. Treatment 2 was the basal diet with 250 mg kg^{-1} ATO supplementation, designated 250ATO while treatment 3 was the basal diet with 500 mg kg^{-1} ATO supplementation, designated 500ATO.

Stocking, feeding and water management

One hundred and eighty juvenile of *H. bidorsalis* having a mean body weight of 2.63 ± 0.70 g were randomly stocked in the nine tanks with the same water levels. They were fed thrice a day at 5% body weight (BWD) at the hours of 8.00, 12.00 and 18.00 for 113 days. The feeding rate was adjusted accordingly during each sampling date. Water was changed according to the result of the routine water analysis.

Data collection

Data on fish growth were recorded weekly. The weight of individual fish was measured with an electronic top loading, weighing balances (Mettle Toledo, model PB 602). The experimental tanks were inspected daily to remove dead fish. Final weight (g), feed consumed (g) and mortality daily. The following growth parameters were determined:

i) Daily Weight Gain (g) (DWG) = (FW – IW)/N, Where: DWG=Daily weight gain, FW=Final weight of fish, IW=Initial weight of fish, N=number of days of the experiment.

ii) Specific Growth Rate (SGR): = (In FBW – In IBW)/N × 100, Where: FBW= Final body weight at each harvest, IBW= Initial body weight, In= Natural logarithm N=Number of days

iii) Feed Conversion Ratio (FCR) = Dry weight of feed fed (g)/Weight gain (g)

iv) Survival Rate (SR) in % = (Total fish number harvested/ Total fish number stocked) 100

v) Protein Efficiency Ratio (PER) = Weight gain/protein intake

Physico-chemical parameters

Physico-chemical parameters such as pH, temperature, ammonia and dissolved oxygen were checked thrice a week to ensure optimum water quality. Every week water quality was monitored according to APHA, (1995), throughout the experimental period.

Statistical analysis

Data obtained were subjected to one-way analysis of variance (ANOVA). The means from the various treatments were compared for significant differences ($P<0.05$), using Duncan's multiple range test with the aid of SPSS V.19 for windows.

RESULTS

Growth response

Growth response and nutrient utilization of *H. bidorsalis* fed diets supplemented with varying levels of ATO for 113 days is presented in table 4. The highest daily weight gain was recorded in control diet while the lowest was observed in fish fed Diet 500 mg kg^{-1} ATO. The specific growth rate also followed the same trend. Fish fed diets 250 mg kg^{-1}ATO and 500 mg kg^{-1}ATO exhibited a poor appetite attested by feed intake and decreasing body weight (Fig. 1). The growth trend reveals a similar growth pattern until the third week when fish fed the control diet started growing faster than fish fed both diet 250 mg kg^{-1} ATO and 500 mg kg^{-1}ATO. The mean weight of fish fed the control diet was significantly higher ($p<0.05$) than both diets 250 mg kg^{-1}ATO and 500 mg kg^{-1}ATO. However, mean weight of the fish fed diets 250 mg kg^{-1}ATO and 500 mg kg^{-1} ATO were not ($P>0.05$) significantly different. Specific growth rate was also significantly higher in the control diet.

Feed utilization and survival

The control diet was also better utilized than other diets. This is attested by the values of feed conversion ratio and protein efficiency ratio, which were significantly better ($P<0.05$) in the control than the other diets. The rate of survival was higher in the control, but lower in other groups. The survival rate of the group fed diet 500 mg kg^{-1}ATO was not significantly higher than that of 250 mg kg^{-1}ATO.

Table 4: Mean (±SD) growth performance and feed utilization of *H. bidorsalis* fed different concentrations of Alpha-tocopherol for 113 days

Parameter	Alpha-tocoperol inclusion level (mg kg^{-1})		
	NoATO	**250ATO**	**500ATO**
Initial weight (g)	52.25±0.29^{a}	52.37±0.17^{a}	52.70±0.08^{a}
Final weight (g)	146.89±8.07^{a}	52.63±20.80^{b}	46.00±8.29^{b}
Feed intake (g)	176.98±3.85^{a}	89.44±2.05^{b}	86.22±1.65^{b}
Daily Weight Gain (g fish^{-1})	0.84±0.02^{a}	-0.01±0.10^{b}	-0.056±0.01^{b}
Specific Growth Rate	0.93±0.01^{a}	0.42±0.02^{ab}	-0.14±0.01^{b}
Feed conversion ratio	1.87±0.10^{a}	1.98±0.12^{b}	2.31±0.20^{a}
Protein efficiency ratio	1.65±0.01^{a}	0.43±0.02^{ab}	0.03±0.01^{b}
Survival rate	61.67^{a}	30.00^{b}	36.67^{b}

Means with the same superscript letter are not significantly different (P>0.05)

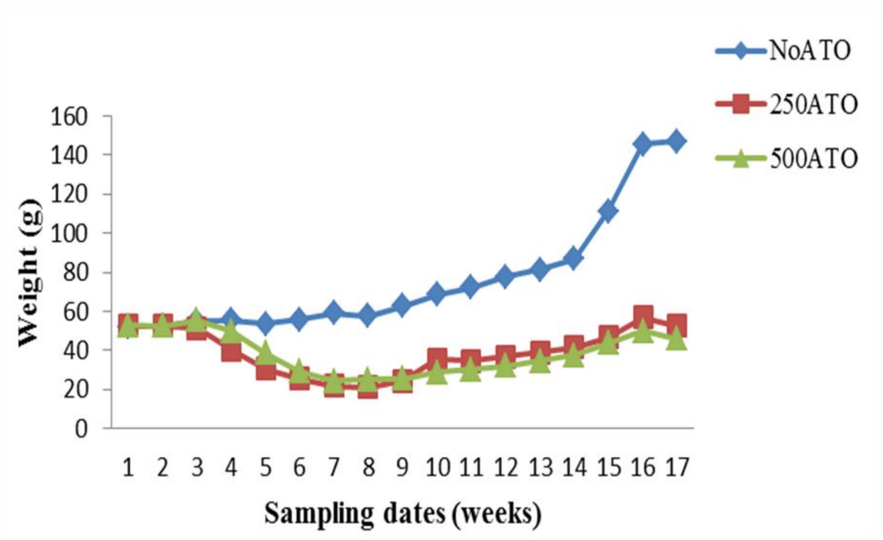

Fig. 1: Weekly growth trend of *Heterobranchus bidorsalis* feed different concentration of Alpha-tocopherol for 113 days

Water quality parameter

Physico-chemical parameters of the cultured water are shown in table 3. The water quality parameters were within the optimum range recommended for the culture of freshwater fishes in the tropical region (Boyd and Lichtkoppler, 1979). No significant variation (P>0.05) was observed in the physical-chemical parameters among the treatments during the experiment.

Table 3. Mean (±SD) physico-chemical parameters during the 113 days culture period

Parameter	**NoATO**	**250ATO**	**500ATO**
Dissolved oxygen (mg l^{-1})	5.46±0.85^{a}	5.78±0.71^{a}	6.04±0.82^{a}
Morning temperature (^{o}C)	27.51±1.29^{a}	27.48±1.95^{a}	26.61±1.85^{a}
pH	7.10±0.61^{a}	6.97±0.49^{a}	6.97±0.48^{a}
Ammonia (mg l^{-1})	0.02±0.01^{a}	0.01±0.02^{a}	0.03±0.02^{a}

Means with the same superscript letter are not significantly different (P>0.05), NoATO=No alpha-tocopherol; 250ATO=250 mg kg^{-1} alpha-tocopherol; 500ATO=500 mg kg^{-1} alpha-tocopherol

DISCUSSION

Hypervitaminosis is a condition in which adverse effects are caused by taking in too much of one or more vitamins. Hypervitaminosis E results in poor growth, toxic liver reaction, and death, but the question is what dose can be said to be too much for a particular fish species since different species respond differently. Supplementation of dietary vitamin E at 250 to 500 mg kg^{-1} resulted in poor growth and death of *H. bidorsalis* fingerlings in this experiment. This is in agreement with the findings of Bai and Lee (1998) who reported that the highest level of DL-α-tocopheryl acetate supplementation (diet E 500) did not result in greater growth performance of the juvenile Korean rockfish, *Sebastes schlegeli* than the other diets. Similarly, Bueno *et al*. (2010) also reported that growth performance and feed utilization of juvenile parrot fish (*Oplegnathus fasciatus*) fish fed the 25 mg kg^{-1} were significantly higher compared to that of fish fed the other diets (50, 75, 100 and 500 mg kg^{-1} diet). In the juvenile grass carp (*Ctenopharyngodon idellus*), Li *et al*. (2014) also reported that the maximum weight gain (WG), specific growth ratio (SGR) and feed intake (FI) were achieved in fish fed on a diet supplemented with 100 mg kg^{-1} vitamin E. Mohammad and Hosein (2014) also had similar findings on juvenile rainbow trout (*Oncorhynchus mykiss*). However, when the concentration of dietary vitamin E was between 0 to 100 mg kg^{-1} diet, Baker and Davies (1996) found that dietary regime had no significant effect on growth ($P > 0{\cdot}05$) of juvenile African catfish (*Clarias gariepinus* Burchell), although haematocrit values increased with increasing tocopherol dose above 15 mg kg^{-1}. By broken-line analysis these authors found that the requirement for this species to suppress lipid peroxidation was estimated as 30 to 40 mg all-rac-a-tocopheryl acetate per kg dry diet.

The toxic effects of supplementation of massive dose of dietary alpha – tocopherol acetate with respect to growth performance have been reported in brook trout fry (Poston and Livingstone 1969) and rainbow trout (Kiron *et al*., 2004). Discrepancies observed among research reports may be due to species-specific response, the level of polyunsaturated fatty acids in the diet and their degree of unsaturation (Harris and Embree 1963; Lin and Shiau, 2005), the presence and amount of other nutrients such as selenium (Poston *et al*., 1976), and vitamin C (Shiau and Hus, 2002), the oxidative quality of the oil (Scott, 1978), the stability of the vitamin and the condition under which it is stored (Berasategi *et al*., 2012).

Commercial feed producers maintained that there is 100-200 mg kg^{-1} vitamin E in their feed, but vitamins must be added in the diet freshly. So, the vitamin dosage in the diet diminished slowly depending on stock conditions. The dietary requirement of vitamin E has been demonstrated in *H. longifilis* (Ibiyo Lenient *et al*., 2008). The authors used a dosage of 0-150 mg kg^{-1} diet and fixed the requirement at 112 mg kg^{-1}. They finally concluded that for optimum performance of the liver and health of mudfish fingerlings vitamin E supplementation is necessary whenever poultry premix is used in fish feed formulation. Our results are in agreement with these findings. More research should be conducted to fix the vitamin requirement for *H. bidorsalis*

Conclusion

The results of this study clearly show that vitamin E supplementation above 250 mg kg^{-1} in the diet of juvenile *H. bidorsalis* will lead to retarded growth, decreased feed utilization and death. Feed formulators should therefore use vitamin E at the dose of 112 mg kg^{-1} diet recommended for *H. longifilis* when formulating feed for *H bidorsalis* pending when the actual dose is determined.

REFERENCES

ADCP (Agriculture Development and Coordination Program) (1993). *Fish feeds and feeding in Developing Countries*. Rome, FAO ADCP/REP/83/18-97. FAO, Rome Italy.

Agbebi, O. T., Olufeagba, S. O., Mbagwu, I. G., Ozoje, M. O. and Aremu, A. (2009). Morphological characteristics and body indices of *Heterobranchus bidorsalis* from three geographical locations in Nigeria. *Journal of Fisheries International*, 4(4):68-72.

Amlashi, A. S., Falahatkar, B and Sharifi, S. D. (2012). Dietary vitamin E requirements and growth performance of the young-of-year beluga, *Huso huso* (L.) (Chondrostei: Acipenseridae). *Arch. Pol. Fish*., 20:299-306.

APHA, (1995). *Standard Methods for the Examination of water and wastewater*. 19th Ed. American Public Health Association, Washington, D. C., USA, 124-450.

Bai, S. C. and Lee, K. J. (1998). Different levels of dietary DL- a-tocopherylacetate affect the vitamin E status of juvenile Korean rockfish, *Sebastes schlegeli*. *Aquaculture*, 161: 405-414.

Baker, R. T. M., and Davies, S. J. (1996). Oxidative nutritional stress associated with feeding ranci oils to African catfish, *Clarias gariepinus* (Burchell) and the protective role of a-tocopherol. *Aquac Res*., 27:795–803.

Berasategi, I., Barriuso, B., Ansorena, D. and Astiasaran, I. (2012). Stability *of* avocado oil during heating*:* Comparative study to olive oil. *Food Chem*., 132*:*439-446.

Boyd, C. E. and Lichtkoppler, F. (1979). Water Quality Management in Fish Ponds. *Research and Development Series* No. 22, International Centre for Aquaculture (J.C.A.A) Experimental Station Auburn University, Alabama, 45-47.

Bueno, G., Sung-sam K. and Kyeong-jun, L. (2010). Effect of different dietary levels of vitamin E on growth performance, non-specific immune response, and disease resistance against *Vibrio anguillarum* in parrot fish (*Oplegnathus fasciatus*). *Asian-Aust J. Anim. Sci.*, 23(7):916-923.

Fagbenro, A. O. (1992). The dietary habits of the Clariid catfish, *Heterobranchus bidorsalis* (Goeffroy St. Hilaire 1809) in Owena Reservior, Southern Nigeria. *Trop. Zool*., 5:11-17.

Fagbenro, O. A., Adedire, C. O., Owoseni, E. A. and Ayotunde, E. O. (1993). Studies on the biology and aquaculture potential of feral catfish, *Heterobranchus bidorsalis* (Geoffroy St. Hilaire, 1809) (Clariidae). *Trop. Zool*., 6: 67-79.

Fagbenro, O. A., Olaniran, T. S. and Esan, A. O. (1991). Some aspects of the biology of the catfish, *Heterobranchus bidorsalis*, Goeffroy Saint Hilaire, 1809 (Clariidae) in river Ogbesse, Nigeria. *J. Afr. Zool*., 105:363-372.

Hamre, K. and Lie, O. (1995). Minimum requirement of vitamin E for Atlantic salmon (*Salmo salar* L.) at first feeding. *Aquac. Res*., 26:175-184.

Harris, P.L. and Embree, N. (1963). Quantitative consideration of the effects of polyunsaturated fatty acids contents of the diets upon the requirement for vitamin E. *Am. J. Clin. Ntr.* 13:385-392.

Huang C. H., Higgs, D. A., Balfry, S. K. and Devlin, R. H. (2004). Effect of dietary vitamin E level on growth, tissue lipid peroxidation and erythrocyte fragility of transgenic Coho salmon, *Oncorhynchus kisutch. Comp. Biochem. Physiol. Part A*, 139:199-204.

Hung, S. S., Cho, C. Y., and Slinger, S. J. (1980). Measurement of oxidation in fish oil and its effect on vitamin E nutrition on rainbow trout. *Can. J. Fish. Aquat. Sci*., 37:1248-1253.

Ibiyo-Lenient, M. O., Atteh, O. J., Omotosho, S. J. and Madu, T. C. (2008). Response of *Heterobranchus longifilis* to supplemental dietary vitamin E. *Journal of Fisheries and Aquatic Science*, 3:22-30.

Kiron, V., Puangkaew, J., Ishizaka, K., Satoh, S. and Watanabe, T. (2004). Antioxidant status and nonspecific immune responses in rainbow trout (*Oncorhynchus mykiss*) fed two levels of vitamin E along with, three lipid sources. *Aquaculture*, 234: 361-379.

Lee, M. H., and Shiau, S. Y. (2004). Vitamin E requirement of juvenile grass shrimp, *Penaeus monodon*, and effect on non-immune response – *Fish Shellfish Immunol*., 16: 475-485.

Li, J., Liang, X. Tan, Q. Yuan, X., Liu, L., Zhou, Y. and Li, B. (2014). Effects of vitamin E on growth performance and antioxidant status in juvenile grass carp, *Ctenopharyngodon idellus. Aquaculture*, 430(20): 21-27.

Lin, Y. H. and Shiau, S. Y. (2005). Dietary vitamin E requirement of grouper, *Epinephelus malabaricus*, at two lipid levels, and their effects on immune responses. *Aquaculture*, 248:235-244.

Mehrad, B. and Sudagar, M. (2010). Dietary vitamin E requirement, fish performance and reproduction of guppy (*Peocilia reticulata*). *AACL Bioflux*, 3(3):239-246.

Mehrad, B., Jafaryan, H. and Taati, M. (2012). Assessment of the effect of dietary vitamin E on

growth performance and reproduction of Zebra fish, (*Danio rerio*) (Pisces, Cyprinidae) – *Journal of Oceanography and Marine Science*, 3(1):1-7.
Mohammad, R. and Hosein, O. (2014). Effects of vitamin E on growth performance, survival rate, hematological parameters response to heat stress in rainbow trout (*Oncorhynchus mykiss)* at two stocking densities. *Journal of Aquaculture Feed Science and Nutrition*, 6:39 - 46.
Murai, T. and Andrews, J.W. (1974) – Interactions of dietary α-tocopherol, oxidized menhaden oil and ethoxyquin on channel catfish (*Ictalurus punctatus*) *J. Nutr*., 104:1416-1431.
NRC (National Research Council) (1993). *Nutrient requirements of Fish*. National Academy Press, Washington, DC, 114 Pp.
Owodeinde, F. G. and Ndimele, P. E. (2011) Survival, growth and feed utilization of two Clariid catfish (*Clarias gariepinus*, Burchell 1822 and *Heterobranchus bidorsalis*, Geoffroy, 1809) and their reciprocal hybrids. *J. Applied Ichthyol*., 27:1249-1253.
Poston, H. A., Combs, G. F. and Leibovitz, L. (1976). Vitamin E and selenium interrelations in the diet of Atlantic salmon (*Salmo salar*): Gross, histological and biochemical deficiency signs. *J. Nutr*., 106:892-904.
Poston, H. G. and Livingston, D. L. (1969). Effect of massive dose of dietary vitamin E on fingerling broook trout. *Fish. Res. Bull*. N.Y. State Conserv. Dep., 33:9–12.
Sau, S. K., Paul, B. A., Mohanta, K. N. and Mahanty, S. N. (2004) Dietary vitamin E requirement, fish performance and carcass composition of rohu (*Labeo rohita*) fry. *Aquaculture*, 240: 359–368.
Scott, M. L. (1978). Vitamin. 133–210 In: *Handbook of Lipid Research. 2*. The Fat-Soluble Vitamins, H. F. DeLuca, editor, ed. New York: Plenum Press.
Shiau, S. Y. and Shiau L. F. (2001) Reevaluation of the vitamin E requirements of juvenile hybrid tilapia, *Oreochromis niloticus* × *O. aureus- Anim*. *Sci*., 72: 529-534.
Shiau, S. Y., and Hsu, C. Y. (2002). Vitamin E sparing effect by dietary vitamin C in juvenile hybrid tilapia, *Oreochromis niloticus*×*O. aureus*. *Aquaculture*, 210: 335–342.
Watanabe, T. and Takeuchi, T., Matsui, M., Ogino, C., and Kawabata, T. (1977). Effect of α-tocopherol deficiency on carp. VII. The relationship between dietary levels of linoleate and α-tocopherol requirement. *B. Jpn. Soc. Sci. Fish*, 43: 935-946.
Wilson, R. P., Bowser, P. R. and Poe, W. E. (1984) Dietary vitamin E requirement of fingerling channel catfish. *J. Nutr*. 114(11):2053-2058.