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Invited Review Article

Functions, metabolism, interactions, growth and requirements of vitamin E in fish: A Review

P. Bera[1], S. K. Sau[1*], B. N. Paul[2], M. H. Ali[1] and T. K. Ghosh[1]

[1]Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata- 700 094, West Bengal, India; [2]Regional Research Centre, ICAR-Central Institute of Freshwater Aquaculture, Rahara, Kolkata- 700 118, West Bengal, India

Abstract

Vitamin E is a necessary lipid soluble micronutrient which prevents unsaturated fatty acid from oxidation and protects cell structure. Optimal amount of vitamin E increases the cell wall strength, erythrocytes and haemoglobin concentration, lysozyme activity, Na^+, K^+, ATPase activity in fish enterocytes. Better muscle superoxide dismutase, glutathione peroxidase, catalase activity and inferior malondialdehyde content in fish, and better non-specific immune responses in shrimps have been seen when diet was supplied with vitamin E supplements. Vitamin E fed fish have shown improved phagocytic activity, less histological damages in internal organs during occurrence of diseases. When the inclusion rate of vitamin E in fish diet increases, it shows positive effects on growth performance. Requirement of vitamin E in fish varies among their life stages based on the growth rate. Vitamin E reduces the deficiency signs of vitamin C in fish body. It shows synergistic effects with vitamin C and selenium. Vitamin E improves acceptance of fishery products by improving its storage quality. It shows a negative correlation with TBARS value during sensory study of fish flesh. It also positively helps in increasing ovulation rate, spawn survival rate, larval growth rate etc. during breeding season.

Key words: Cell function, Dietary requirement, Fish growth, Flesh quality, Vitamin E

Highlights

- Anti-oxidative, disease resistance and cell maintenance functions of vitamin E in fish.
- Effects of vitamin E on fish growth, carcass composition, flesh quality and immunity.
- Interaction of vitamin E with selenium and vitamin C.
- Requirement of vitamin E for different fish species.

INTRODUCTION

Vitamins are one of the most precious ingredients for preparation of nutritionally balanced feed. Based on solubility, vitamins are divided in two groups- one is soluble in water (vitamin C, vitamin B complex) and another in lipid (vitamin A, vitamin D, vitamin E and vitamin K). Among them, vitamin E is an indispensable micronutrient playing an essential role in various physiological and biochemical processes in fish (Hamre, 2010; Le *et al.*, 2014; Lu *et al.*, 2016). Vitamin E (VE) cannot be produced in the fish body, so they depend on dietary intake to fulfill their requirement (He *et al.*, 1992). It occurs in several natural forms and comprises four tocotrienols and four tocopherols (α, β, γ and δ for both). Among them, the highest VE activity is shown by α-tocopherol. Among different types of α-tocopherols, stable DL-α-tocopherol acetate (DL-α-TOA) is the most commonly used animal feed ingredients (NRC, 1993). Upon ester hydrolysis, intestine absorbed α-tocopherol along with dietary fats.

VE supplemented diets improve growth (Roem *et al.*, 1990; Huang and Huang, 2004), restore impaired immunity, enhance immunity, shelf-life and oxidative stability of fish (Lygren *et al.*, 2001; Wu *et al.*, 2017). On another side, it helps to maintain resistance power of RBC

*Corresponding Author, E mail: suryaksauaqua2020@gmail.com

against lysis, flesh quality and the penetrability of capillaries (Abdel-Hameid *et al.*, 2012).

It also has a sparing mechanism with vitamin C (ascorbic acid). It protects oxidation of low-density lipoproteins by reduction of α-tocopherol (α-TOH) with the help of ascorbate by regenerating α-TOA. Vitamin C-E sparing mechanism had increased growth, immunity and composition of tissue in some fish species (Frischknecht *et al.*, 1994). Selenium (Se) also protects oxidative damage in cell membranes by the glutathione peroxidase (GPx) activity as an antioxidant, which catalyzes fatty acid hydroperoxides and hydrogen peroxide to convert into fatty acid, water and alcohol (Dawood *et al.*, 2019).

Deficiency of VE may lead to liver degeneration, lipid oxidation, oedema, haemorrhages, erythrocyte haemolysis and peroxidative damage in cell membranes (NRC, 1993). Therefore, requirement of VE in diet at optimum level is necessary to estimate. Additionally, VE requirement is elevated when there is an increase in polyunsaturated fatty acids (PUFA) content in fish diet (Lin and Shiau, 2005). The content of selenium and vitamin C could impact the VE requirement in fish (Naderi *et al.*, 2019). The metabolism, interactions with other nutrients, requirement, growth, flesh quality, immunity, stress, requirements and functions of VE are reviewed in this article.

Functions of vitamin E in fish

Vitamin E as antioxidant: Malondialdehyde (MDA) is the most important indicator of lipid oxidation and the direct evidence that free radicals can cause injury to cell membrane (Huang and Huang, 2004). VE prevents oxidation of polyunsaturated lipids in fish tissues. It mainly protects the cellular structures from the reactive products and oxygen free radicals of lipid peroxidation as a scavenger by giving their hydrogen which was attached to phenol to oxygen free radicals (Li *et al.*, 2014). Artificial antioxidants, such as butylated hydroxyl anisole, butylated hydroxyl toluene and ethoxyquin which may spare dietary VE in fish by reducing rancidity (NRC, 1993).

Haematological functions of vitamin E: Optimal VE in diet proved to be best in increasing the cell wall strength of erythrocytes (Sau *et al.*, 2004). Addition of VE increased carbohydrate and haematocrit value in plasma of the fish groups receiving oxidized oil like largemouth bass (Chen *et al.*, 2013). The haemoglobin concentration and haematocrit increased significantly when dietary VE concentrations increased up to 140 mg/kg in *Channa punctatus*. The osmotic fragility of erythrocyte decreased with increasing dietary VE concentration @140 mg/kg diet and in hypotonic salt solutions it became more resistant to haemolysis (Abdel-Hameid *et al.*, 2012). Deficiency of VE increased haemoglobin contents and haematocrit value of blood, decreased catalase activities, glutathione peroxidase, and superoxide dismutase activity in genetically improved farmed tilapia (Jun *et al.*, 2019). It was found in Atlantic salmon that when concentration of VE decreased in diet, the concentration of blood haemoglobin also decreased. Less than 5000 mg of dl-α tocopherol (dl-α-toh)/kg in diet was found successful to increase erythrocyte concentration in fingerling brook trout (Jittinandana *et al.*, 2006). The superoxide dismutase (SOD) activity and intracellular O_2 production rate were highest in shrimp @100 mg VE kg/day

Stress functions of vitamin E: ATPase, sodium and potassium ions in fish intestinal cell were enhanced with increasing levels of VE in diet. Glutathione maintains oxidation state intracellularly (Pflugmacher *et al.*, 1998). VE must be reduced by Glutathione-SH to restore its antioxidant function (Jiang *et al.*,2009). Vitamin C together with VE supplementation may be useful to protect against the effects of stress (Sandor *et al.*, 2017). The decreased cortisol and glucose levels demonstrated that overall metabolic stress could be reduced by VE @500 mg/kg in feed (Farsani *et al.*, 2017). Cortisol played this role by reducing number of antibodies producing cells and circulating lymphocytes (Nardocci *et al.*, 2014). This study

also showed enhanced lysozyme activity in fish when feed was supplied with VE. The increase in ATPase, Na^+, and K^+ activity was observed in shrimp when feed was given with VE @100 and 600 mg/kg when exposed to 5% and 50% of salinity.

Disease resistance functions of vitamin E: Waagbo *et al.* (1993) found superior effects of dietary VE in Atlantic salmon when challenged with bacteria (*Vibrio salmonicida)*, depending upon temperature of water and composition of fatty acid in feed. Best survival was found in salmon when fed with an increased level of n-3 PUFA (sardine oil) supplemented with 300 mg VE/kg and reared at low water temperatures (Lygren *et al.*, 2001). The phagocyte oxidative burst activity was higher in unvaccinated Atlantic salmon post smolt fed with 400 mg VE/kg after 23 weeks of feeding. Wahli *et al.* (1998) observed increased respiratory burst activity with VE (800 mg/kg) and C (2000 mg/kg) in combination for 8 months in rainbow trout. VE was able to prevent histological damage in the liver by maintaining the size of the nuclear diameter of hepatocytes by dilation of the proximal convoluted tubules and the disorganization of the distal convoluted tubules in fish (Guzman-Guillen *et al.*, 2015). The challenge test by Li *et al.* (2019) showed that river prawn fed deficient VE diet showed higher cumulative mortality than those fed with VE at 160 mg/kg diet after injection of bacteria. Gill necrotic tissues and intestinal health (villi surface area, villi length and goblet cells count) showed improvement when VE supplementation was provided with *Quillaja Saponaria* (soapbark tree) extract in Nile tilapia (Elkaradawy *et al.*, 2021). The innate immunity against *Aeromonas hydrophila* of *L. rohita* had also been improved with dietary supplementation of VE (Priyadarsini *et al.*, 2021).

Cell maintenance of vitamin E: VE promotes antioxidant capacity of fish enterocyte by removing free radicals, inhibiting lipid peroxidation and increasing non-enzymatic antioxidants (Musalmah *et al.*, 2002). The cell wall credibility was increased with increased VE content in diet for all groups of rohu. When VE was added to the feed @160 mg/kg, then cells were filled with mitochondria and fat droplets without any surface depression. The number of Golgi bodies and mitochondria increased, but microvilli and nuclei were normal. However, excess VE @640 mg/kg in diet decreased R cells count and increased B cells count (Li *et al.*, 2019).

Effects on growth

With increasing addition of VE, the weight gain (WG) and specific growth rate (SGR) of fishes first elevated and then deteriorated, but feed conversion ratio (FCR) elevated after deterioration. Growth depression may happen due to gathering of VE particles by inducing oxidative stress. The excess level of α-tocopherol may lead to growth arrest by reducing mobility of protein kinase C (PKC) in involuntary muscle. Sau *et al.* (2004) observed increasing protein efficiency ratio (PER), WG and SGR but lowest FCR in rohu with increasing VE up to 115.70 mg/kg. Grass carp supplied with VE @100 mg/kg had significantly better SGR and WG, but it had no significant influence on PER. Dietary lipid oxidation changed the taste of the diet by altering its odour consequently (Gao *et al.*, 2012). VE improved the edibility of feed to promote ingestion by reducing fatty acid oxidation (Li *et al.*, 2014). The increase in growth of *Cirrhinus mrigala* was observed with addition of VE @ 99 mg/kg diet (Paul *et al.*, 2004) and VE @98 mg/kg diet in catla (Paul *et al.*, 2005). An evenly improving effect was observed in proximate composition (Saheli *et al.*, 2021). GIFT tilapia supplied with 40 mg VE/kg feed had maximum growth. Feeding efficiency was improved in fish when the diet was supplied with 60 mg VE/kg (Jiang *et al.*, 2019). But rancid oil in feed reduced the VE necessity of hybrid tilapia apparently (Huang and Huang, 2004). *Channa punctatus* fingerlings fed with VE at 140 mg/kg diet had significantly higher WG and the lowest FCR.

However, further inclusion of dietary VE at 220 and 260 mg/kg in diets significantly depressed growth, FCR and nutrient retention efficiency (Abdel-Hameid *et al.*, 2012). The feed with 60 and 120 mg VE/kg were enough to increase survival and growth rate of Atlantic salmon. The best feeding parameters of juvenile eel were obtained when fed with VE supplementation @16.5 mg/kg diet (Bae *et al.*, 2012). Diet containing 100 mg VE/kg was enough to support optimal growth of *Ompok pabda* brood fish (Sarowar and Mollah, 2009). The largemouth bass fed with 79.53 mg VE /kg diet got superior protein efficiency ratio, protein retention rate and apparent digestibility coefficient. But the lipid retention rate possibly was unaffected by VE supplementation (Li *et al.*, 2018). Growth performances (SGR and WG) and feed utilization rate (FER and PER) parameters were remarkably affected by rancid oil inclusion diet in largemouth bass (Chen *et al.*, 2013). 300 mg VE/kg feed increased life span of hybrid striped bass significantly (Sealey and Gatlin III, 2002).

VE supplementation of 100 mg/kg diet caused an increase in SGR of shrimp (Liu *et al.*, 2007). The WG and survival of shrimp were highest @75 mg VE/kg diet (Lee and Shiau, 2004). The gonadosomatic index of male shrimp was significantly higher with increasing VE from 80 to 640 mg/kg in diet. But muscle VE contents slightly increased as these organs were lipid rich tissues (Li *et al.*, 2019).

Carcass composition with supplementation of vitamin E

Increased supply of VE leads to increased catabolism rate. The content of crude protein in *Labeo rohita* fry was higher when fed with 66.50 mg VE/kg diet. The deposition of VE in *Labeo rohita* was maximum when fed with 217.80 mg/kg diet (Sau *et al.*, 2004). Dietary VE had elevated hepatic VE content and it increased supremely in Nile tilapia supplied with VE @240 mg /kg feed (Jiang *et al.*, 2019). In *Channa punctatas* fingerlings, VE had a positive association with moisture content but a negative correlation with ash and fat content (Mourente *et al.*, 2007). Body protein of *Channa punctatus* was intended to increase significantly when VE was provided up to 140 mg/kg diet (Abdel-Hameid *et al.*, 2012). VE concentration was increased in liver, muscle, kidney and gonad significantly with elevated inclusion rate in Japanese eel (Shahkar *et al.*, 2017). The liver VE content in largemouth bass increased significantly when level of VE increased from 14.24 to 118.70 mg/kg (Li *et al.*, 2018). Deposition of α-tocopherol in the whole body of *Anguilla japonica* linearly increased, and highest level found @120 mg/kg diet (Bae *et al.*, 2012). Hepatic and muscular VE contents was reasonably high in rainbow trout given with VE @500 mg/kg for 60 days (Naderi *et al.*, 2019). Young Atlantic salmon has reduced ability to digest and absorb VE, so they require a high dose of VE for production of free radicals and maintenance of body stores.

The n-6 polyunsaturated fatty acid (Σn-6), linoleic acid (LOA, mC18:2n-6) and docosapentaenoic acid (DPA, C22:5n-3) content except eicosapentaenoic acid (EPA, C20:5n-3) showed a decreasing trend in the hepatopancreas with increasing VE levels of male shrimp diet. VE elevated the total content of saturated fatty acid, but PUFA increased a little (Li *et al.*, 2019). The supply of histidine in fish diet with 50 mg VE/kg might be due to the accretion of peroxides (Li *et al.*, 2014). Hepatopancreatic VE quantity was uppermost in grass shrimp when provided with VE @200 mg/kg feed. TBA values of muscle and hepatopancreas also decreased in shrimp generally (Lee and Shiau, 2004).

But in some studies, incorporating VE in feed had untouched effect on the imminent nutrient composition of *Oreochromis niloticus* (Jiang *et al.*, 2019), largemouth bass (Li *et al.*, 2018), *Anguilla japonica* (Bae *et al.*, 2012) and body lipid and ash content in rohu (Sau *et al.*, 2004).

Effects of vitamin E on immunity

A proper quantity of VE in feed may promote

innate immunity, while excessive VE levels may inhibit its activity. The manufacture of humoral and cellular components in immune system will be increased in fish with VE supplementation (Biller-Takahashi *et al*., 2015). Lowest muscle MDA content and the improved muscle catalase (CAT), superoxide dismutase (SOD) GPx activities were found in juvenile grass carp (Li *et al.*, 2014), tilapia (Jiang *et al.*, 2019) and in rainbow trout group fed with both VE and selenium (Naderi *et al.*, 2019). Lysozyme activity was highest with 160 mg VE/kg diet. The increase in VE to 118.70 mg/kg in diet improved lysozyme and SOD action both in liver and serum of largemouth bass significantly (Li *et al.*, 2018). Decrease of antioxidant capacity in rainbow trout was observed under high density rearing without any VE supplementation in diet (Yarahmadi *et al.*, 2016). The interdependent activity of selenium and VE to elevate GPx action was observed in liver of Channel catfishes (Wise *et al.*, 1993). The pro-oxidant effect of excess α-tocopherol in feed might counteract with the raised activities of hepatic enzymes (Li *et al.*, 2014). In sea bass, reduced survival, growth of larvae and increased incidence of muscular lesions were observed (Betancor *et al.*, 2012).

In the study of male shrimp, when level of VE increased in feed, activity of immune enzymes (LSZ, ACP and AKP) initially rose and then fell, but opposite trend was observed in the activity of enzymes with antioxidant properties (CAT, SOD and GSH Px). Deficiency of VE caused lowest survival (85%) and darkening of the hepatopancreas in shrimp (He and Lawrence, 1993). O_2 production rate was superior within cell when VE was supplied with 100 mg/kg feed in shrimp (Lee and Shiau, 2004).

Vitamin E in broodstock diets

According to Nascimento *et al.* (2014), female Nile tilapia showed better fertilization rate, spawning rate, and fecundity when fed with 400 mg VE/kg diet during the reproductive period. Heavy gonad, higher fecundity, good hatching rate, a greater number of eggs were resulted with VE @300 mg/kg diet of goldfish (James *et al.*, 2008). In bloch, gonadosomatic index, ovarian development and fecundity were higher with 100 mg VE/kg diet, but better breeding performance, hatching rate and larval survival rate were observed with 200 mg VE/kg diet (Mollah *et al.*, 2003). There was a favorable effect of VE incorporation on the breeding performance of female *O. pabda*. Highest fertilization rate, ovulation rate of broodfish, hatching percentage, growth rate, and survival rate of the larvae were obtained with 100 mg VE/kg diet (Sarowar and Mollah, 2009).

Flesh quality on supplementation of vitamin E

VE has improved the storage quality of fishery products giving better fillet texture. The dietary VE levels should be more than the nutritional requirements of fish based on growth performance (Wu *et al.*, 2017). VE is also effective to introduce natural antioxidants into fish muscle to reduce adverse effects of several engineered nanomaterials (Naderi *et al.*, 2019).

Chewiness increased with increasing dietary VE levels. Similarity was found in flexibility and springiness within the VE enriched groups. Fillets from fish supplied with high VE content were darker, and less yellow while diet did not affect fillet redness. The maximum hardness, chewiness and cohesiveness rate of the GIFT tilapia fillet were at 582.3 mg, 573.8 mg and 1000 mg VE/kg groups, respectively (Wu *et al.*, 2017). After frozen storage rancid products of tail muscle were remarkably affected by VE levels in shrimp. It had the highest TBA value when fed the VE-free diet (He *et al.*, 1992). Diet did not affect trout fillet fat, muscle pH and moisture content in the study of Jittinandana *et al.* (2006).

TBARS (Thiobarbituric acid-reactive substances) value decreased significantly with increase in VE levels in rohu, which notified that high α-tocopherol content and lipid oxidation rate were disproportional to each other (Sau *et al.*, 2004) and similar trend was found in other fish (Lozano *et al.*, 2016). Tissue TBARS concentrations showed a negative

interrelation with VE up to140 mg/kg feed for *Channa punctatus* (Abdel-Hameid *et al.*, 2012).

Interactions of vitamin E

Interactions with vitamin C: In the study of Frischknecht *et al.* (1994), fish fed only with VE, without vitamin C (C/E- 0/800) suffered from scoliosis and lordosis, and signs of paralysis with cartilage degeneration and haemorrhages in the peridental mucosa, distortions of the secondary gill lamellae, reduction in sensitivity and atrophy in caudal muscle of the body, haemorrhages in front lobe of the eye and on the pectoral and pelvic fin bases were observed. Vitamin C also causes loss of VE in the diet by auto-oxidation (Roberts, 1989). There might be not only reduction of the antioxidant property of vitamin C in fish to recycle α-TOH or capture peroxyl particles from the fluidity phase but also construction of good antioxidant molecules (Betancor *et al.*, 2012). Negative impacts of vitamin C deficiency in blood components were prevented by VE correlation between these two vitamins in rainbow trout. This combination increased feed intake efficiency, growth, survival rate, vitamin and oxidation levels in tissue of hybrid striped bass as observed by Sealey and Gatlin III (2002).

Interactions with vitamin E and selenium: The incorporation of nano Se with VE might improve respiratory burst activity by increasing the oxidation rate during phagocytes, which is an important process of innate immunity in fish. Dawood *et al.* (2019) observed fishes fed feeds incorporated with both nano Se and VE were more fore-bearing of oxidative stress which was the indication of a higher health status. Nile tilapia improved its WG, SGR, and FER as VE was added with Seat nano scale in diet. Both VE and Se inclusion could protect oxidative damage by dietary oil rancidity in largemouth bass, although they could not significantly improve growth parameters (Chen *et al.*, 2013). The supplementing VE and Se mixture in diets of yellow tail kingfish increased the WBC counts and haemoglobin levels (Li *et al.*, 2014). However, dietary VE and nano Se interact synergistically to deposit muscle protein in rainbow trout. Cortisol acts in this process by reducing lymphocytes and antibody producing cells in blood (Nardocci *et al.*, 2014). Furthermore, titer of agglutinated antibody was remarkably influenced by the correlation of VE and nano Se as the titer was elevated by the VE and Se combined diet. The proportion of albumin and globulin was remarkably lower than control group when both VE and nano Se were supplied with feed (Naderi *et al.*, 2019). The results in *Penaeus vannamei* suggested that Se could not prevent lipid oxidation as VE by replacing it (He and Lawrence, 1993).

Requirements of vitamin E in fish

The optimum demand for VE was 131.91 mg/kg in rohu (Sau *et al.*, 2004), 99 mg/kg in mrigal (Paul *et al.*, 2004), 98 mg/kg in catla (Paul *et al.*, 2005)) and 100.36 mg/kg in grass carp (Li *et al.*, 2014). Dietary VE level of GIFT was established to be 66.0-76.1 mg/kg (Jiang *et al.*, 2019). The dietary VE should be maintained at 73-108 mg/kg for largemouth bass (Li *et al.*, 2018). The optimum VE required between 140 and 169 mg/kg diet for *C. punctatus* (Abdel-Hameid *et al.*, 2012). The requirement of vitamin E for different species is shown in Table I. The minimum requirement of VE for Atlantic salmon was between 30 and 60 mg/kg feed. Juvenile eel required <21.2 mg VE/kg diet (Bae *et al.*, 2012). Combined incorporation of VE (500 mg/kg) and nano Se (1 mg/kg) in feed showed improvement in health status and growth parameters in intensive culture of rainbow trout (Naderi *et al.*, 2019). 99 mg VE/kg diet was mandatory for better growth of *P. vannamei* (He and Lawrence, 1993). The adequate dietary VE requirement was approximately 85-89 mg/kg feed for better growth and immunological parameters of *P. monodon* (Lee and Shiau, 2004). For optimal growth, fatty acid composition and immunity, male *M. nipponense* shrimp required 80-160 mg/kg VE in diet (Li *et al.*, 2019).

Table 1. Requirement of vitamin E as studied in different species

Species	Requirement mg kg^{-1} dry diet	References
Rohu	131.91	Sau *et al.*, 2004
Catla	98	Paul *et al.*, 2005
Mrigal	99	Paul *et al.*, 2004
Grass carp	100.36	Li *et al.*, 2014
GIFT	66-76	Jiang *et al.*, 2019
Largemouth bass	73-108	Li *et al.*, 2018
Atlantic Salmon	30-60	Hamre, 2010
P. vennami	99	He and Lawrence, 1993
P. monodon	85-89	Le and Shiau, 2004
M. nipponense	80-160	Li *et al.*, 2019

Conclusion and future perspectives

The summary of the above discussion is that lipid soluble VE, which may be supplemented through meals, primarily shields lipids from oxidation, i.e. it acts as an antioxidant *in vivo* as well as during frozen storage of fish fillets, but shortage was observed in the majority of fishes. VE aids in the conservation of cellular structures, cell wall strength, and cellular oxidative damage reduction. It also boosts the body's VE concentration, protein level, and moisture content. VE reduces oxidative stress in fish by boosting serum and liver levels of SOD, CAT, and GPx, which aids in scavenging free radicals produced by lipid oxidation and lowering MDA and ALT levels. Boosted dietary VE levels increased overall growth rate, gonadosomatic index, immunity, and survival rate of both finfish and shellfish by lowering respiratory burst activity, erythrocyte osmotic fragility, and haemolysis etc. The supply of both vitamins C and vitamin E with diets showed sparing and synergistic effects when VE was given along with nano Se in fish diets, making VE a dietary essential nutrient for fish.

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