



Assessment of Dietary Requirement of Vitamin E for Rohu, *Labeo rohita* Fingerlings Fed Practical Diet

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ABSTRACT

An 8-weeks feeding experiment was performed to study the requirement of vitamin E for rohu fingerlings. Six practical diets were fed to fingerlings including one control diet (E0) and five test diets (E25, E50, E75, E100 and E125) supplemented with graded vitamin E levels including 0, 25, 50, 75, 100 and 125 mg/kg. After the completion of trial, fish growth, vitamin E, antioxidant enzymes activities and fatty acids profile was determined. From the results it was concluded that the graded supplemental vitamin E levels improve the utilization of feed and fish growth. The antioxidant enzymes activities also showed significant results by increasing vitamin E level in the diet. However, an inverse relation was recorded between the contents of liver thiobarbituric acid reactive substances (TBARS) and vitamin E level in the diet. Moreover, analysis of fatty acids profile showed decrease in saturated and monounsaturated fatty acids (MUFAs). While, polyunsaturated fatty acids (PUFAs) were increased in all vitamin E supplemented diets compared to control group. Furthermore, vitamin E level of 80.62 mg/kg was found optimum for maximum growth performance in *L. rohita* fingerlings, beyond which no increase in growth was observed.

Article Information

Received 11 November 2021

Revised 08 December 2021

Accepted 24 December 2021

Available online 12 March 2022
(early access)

Authors' Contribution

UZ conducted the experiment and performed the analysis. MF performed post-harvest and statistical analysis. SZHS conceived the experiment and wrote the manuscript. MA supervised the experiment. SS helped in the conduction of the experiment. SR helped in analysis. MB helped in analysis and manuscript write up and MB reviewed edited the manuscript.

Key words

Fatty acids profile, *Labeo rohita*, α -tocopherol, Antioxidant enzymes activity, Growth performance, TBARS

INTRODUCTION

Vitamins are among the costly nutrients which are used in the preparation of nutritionally balanced diet for fish. Vitamin E has great importance in fish nutrition because of its role in many physiological processes and biochemical such as the prevention of unsaturated fats oxidation in fish tissues (Zhong *et al.*, 2007). It is a lipid soluble vitamin and found in the form of four tocotrienol and four tocopherol molecules. Among these different forms, α -tocopherol has highest activity of vitamin E

(Mehrad *et al.*, 2014). Supplementation of α -tocopherol in feed improved the growth performance in various species of fish (Li *et al.*, 2014; Mehrad *et al.*, 2014; Saleh *et al.*, 2014; Bae *et al.*, 2013; Li *et al.*, 2013).

The requirement of vitamin E in the diet varies from fish species to species depending upon age, type of species, culture conditions and the polyunsaturated fatty acids (PUFAs) level (Waagbo *et al.*, 1991; Puangkaew *et al.*, 2005). The quantitative dietary α -tocopherol requirements of fish have been studied for various cultured species (Peng and Gatlin, 2009; Paul *et al.*, 2004; Kocabas and Gatlin, 1999). Vitamin E deficiency in fish creates anemia, muscular dystrophy, skin discoloration and erythrocyte fragility (Mourente *et al.*, 2007).

The peroxidation process of lipid involves the formation, propagation of lipid radicals, Unsaturated lipids undergo double bond rearrangement, leading to membrane lipid degradation and the manufacturing of a variety of breakdown products such as alkanes, ethers, aldehydes, ketones and alcohols (Dianzani and Barrera, 2008). These free radicals initiate the destruction of the

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0030-9923/2022/0001-0001 \$ 9.00/0



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intracellular components including nucleic acid, enzymes and biological membranes which lead to the pathological conditions (Paul *et al.*, 2004). Vitamin E's antioxidant effect is based on its reaction with free radicals, particularly the radicals of peroxy and oxygen singlet molecule ($1O_2$) (Sies *et al.*, 1992). The most important indicator for the determination of lipid peroxidation is the analysis of biochemical profiles like the thiobarbituric acid reactive substances (TBARS) level (Bahmani *et al.*, 2001). Several studies have determined the vitamin E requirement on level of thiobarbituric acid (TBA) in different organs of fish species and it was observed that TBA values decrease when the level of vitamin E increases in various species of fish (Jiang *et al.*, 2009; Galaz *et al.*, 2010; Li *et al.*, 2013, 2014; Zhang *et al.*, 2016).

Organisms have developed different types of antioxidant defenses to protect themselves from free radicals including the antioxidant enzymes activity e.g., catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Among these enzymes, SOD and CAT which reacts on superoxide (O_2^-) and hydrogen peroxide (H_2O_2) respectively, are active oxygen species scavengers (Miller *et al.*, 1993). Tocopherol supplementation showed significant variations in activities of these enzymes (Glucin *et al.*, 2005).

The popular freshwater fish species in Asia is *Labeo rohita*, particularly in the subcontinent of India (Khan *et al.*, 2004). Normally, it is cultured under semi-intensive poly-cultured system (Meshe, 1985). Due to its fast growth rate, increased market demand and capacity to live in a range of agro-climatic conditions, it accounts for 35% of total output of Indian major carps (Mir *et al.*, 2017). Production of *L. rohita* grew globally from 1.1 million tons in 2010 to about 2 million tons in 2018. As a result, it has been recognized as a significant aquaculture species, accounting for around 3.7% of world aquaculture production in 2018 (FAO, 2018).

Vitamin E requirement of *L. rohita* fingerlings in purified diets had already been reported (Sau *et al.*, 2004) however, there has been no work done on requirement of vitamin E for the fingerlings of *L. rohita* when practical diet being provided. The current study aimed to find an optimum vitamin E level in fish diet as well as its antioxidant role in practical diet of rohu fingerlings.

MATERIALS AND METHODS

The current experiment was performed in the Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan. The experimental trial was ethically permitted from the same university's ethical review committee.

The *L. rohita* fingerlings were procured from

Government Fish Seed Hatchery, Faisalabad and were given basal diet for two weeks to acclimatize to laboratory conditions. On the basal diet, the fingerlings were fed once in a day until the satiation appeared throughout the acclimation period. The fingerlings of *L. rohita* were dip in 5g/L solution of NaCl as an antiseptic to avoid infections and diseases before start of the experiment (Rowland and Ingram, 1991). The fish was stocked in triplicates containing 15 fish in each aquarium having equal initial weight (3.02 g) and fed with experimental diets. During the feeding trial, the continuous aeration was given in all aquaria through capillary system. The physio-chemical parameters were checked throughout the feeding trial. The dissolved oxygen (DO) of water was checked by digital meter (HANNA, model HI 9147) and kept constant at 5.8-7.3 mg/L. Likewise, the temperature (24.9-28.7°C) of water and pH (7.4-8.6) were assessed by pH meter of AMPROBE (model WT-80) following Fatima *et al.* (2019) method.

Experimental diets preparation

The dry ingredients of feed were bought from the market and examined chemically by AOAC (1995) method. Before the formulation of vitamin E supplemented diets, the dry feed ingredients were finally ground in powdered form. The vitamin E was added in the diet in the form of α -tocopherol acetate (Sigma-Aldrich) after mixing in the fish oil. The dough was formed with the addition of 15% water. After making the dough from the mixture of ingredients, pellets were made by using hand pelleting machine. Pellets were air dried for approximately 48 hours. The chemical analysis of test diets was assessed by AOAC (1995). The moisture was measured by oven drying method by placing the test diets in oven at 105°C for 12h, the protein content ($N \times 6.25$) was measured through Kjeldahl's apparatus, the fat (%) of diet was analyzed by the method of ether extraction through Soxhlet apparatus (Soxtec HT2, 1045 system), the crude fiber content was analyzed from the samples by ignition loss process of residues of dried lipid-free after digestion with 1.25% H_2SO_4 and 1.25% NaOH; ash by ignition at 650°C for 12 h in electric furnace (Eyela-TMF, 3100) to a constant weight. The feed ingredients and chemical analysis of test diets are given in Table I.

Chemical analysis

Growth performance was evaluated fortnightly by weighing fish of each tank. Growth performance and utilization of feed was measured with regard to weight gain percentage, FCR, specific growth rate (SGR) and rate of survival (%).

The fish from each replicate was euthanized with an overdose (3000 mg/L) of clove oil for 40-60s (Khajepour *et al.*, 2012) then scarified and livers of five fish were

Table I. Ingredients composition (g/kg) and Proximate composition of experimental diet.

Diet composition	Diets					
	E0	E25	E50	E75	E100	E125
Ingredients						
Sunflower	400	400	400	400	400	400
Fishmeal	200	200	200	200	200	200
Rice polish	180	180	180	180	180	180
Wheat flour	100	100	100	100	100	100
Fish oil	60	60	60	60	60	60
Mineral mixture [†]	30	30	30	30	30	30
Vitamin premix without vitamin E [‡]	30	30	30	30	30	30
Total	1000	1000	1000	1000	1000	1000
Vitamin E (mg/kg)	0	25	50	75	100	125
Proximate composition						
Moisture (g/kg)	909.6	910.6	913.2	906.5	911.2	909.0
Crude protein (g/kg)	329.0	330.6	328.3	330.5	329.5	328.0
Crude fat (g/kg)	100.3	100.8	99.8	100.8	96.4	98.9
Gross energy (Kcal/kg)	4073.1	4104.5	4079.1	4092	4088.7	4079.3
α -tocopherol (vitamin E) (mg/kg)	14.62	39.51	57.14	86.2	104.02	122.9

[†]Each kg of mineral mixture contains; Ca (Calcium) 155 g, P (Phosphorous) 135g, Mg (Magnesium) 55g, Na (Sodium) 45g, Zn (Zinc) 3000 mg, Mn (Manganese) 2000 mg, Fe (Iron) 1000 mg, Cu (Copper) 600 mg, Co (Cobalt) 40 mg, I (Iodine) 40mg, Se (Selenium) 3mg. [‡]Each Kg of Vitamin premix contains; Vitamin A (Retinoic acid) 5.0 mg, Vitamin B1 (Thiamine) 0.5 mg, Vitamin B2 (Riboflavin) 3.0 mg, Vitamin B3 (Niacin) 5.0 mg, Vitamin B6 (Pyridoxine) 1.0 mg, Vitamin B7 (Biotin) 0.05 mg, Vitamin B9 (Folic acid) 0.18 mg, Vitamin B12 (Cobalamin) 0.002 mg, Vitamin C (Ascorbic acid) 5.0 mg, Vitamin D3 (Cholecalciferol) 0.002 mg, Cellulose 815.26 mg, Choline 100 mg.

separated to analyze the antioxidant enzymes activity, total lipid content, fatty acids profile, vitamin E content and TBARS level.

SOD enzymatic activity in the fish liver was analyzed by [Giannopolitis and Ries \(1997\)](#) in which the superoxide inhibits the nitro blue tetrazole reduction. One unit activity of SOD is described as enzyme concentration affecting half the reduction of NBT through maximum inhibition. CAT was assessed by determining its capacity to breakdown the hydrogen peroxide concentration at wavelength of 240 nm according to [Chance and Maehly \(1955\)](#). The CAT unit is defined as the quantity of catalyzing 1 μ mol of H₂O₂ in one second. The ability of peroxidase to lower the absorption of hydrogen peroxide at 470 nm wavelength was used to assess activity of peroxidase ([Civello et al., 1995](#)).

The fish liver fatty acid profile was measured by [IUPAC \(1987\)](#) standard method. The lipid of trans esterified containing the derivatives of fatty acid methyl esters (FAME) were used to evaluate the profile of fatty acid in the liver. FAMES were prepared by using methanol.

TBARS in the liver of fish was measured by the method of [Gatta et al. \(2000\)](#). The liver samples (1g) from each treatment were homogenized. After the addition of acid to the sample, 30 min incubation was done at 37 °C for the stimulation of peroxidation of lipids. For calorimetric reaction, the sample tubes were boiled for

approximately 25 min after adding HCl and TBA. After cooling, trichloroacetate (TCA) was intermixed and sample centrifugation was done at 495 x g for approximately 5 min. Thiobarbituric acid values were expressed as μ g MDA equivalents mg⁻¹ tissue. The spectrophotometer (UV-VIS 2001, Spectrophotometer) was used to check the absorbance of sample and recorded as compared to the blank sample at 530 nm. The α -tocopherol analysis was assessed by high performance liquid chromatography (HPLC) following [Anwar et al. \(2006\)](#).

Statistical analysis

The means and pooled standard errors (PSE) are used to present the data. The statistical analysis was done using one-way analysis of variance (ANOVA) when the pre-ANOVA assumptions were fulfilled ([Steel et al., 1996](#)). Tukey's honestly significant difference test was applied to compare the differences among means, and p<0.05 was considered significant. The vitamin E optimum requirement in the diet was measured by applying broken line regression on weight gain % data ([Snedecor and Cochran, 1991](#)).

RESULTS

The study was undertaken to find the requirement of vitamin E for *L. rohita* fingerlings.

Growth performance

Vitamin E supplementation affects the growth of *L. rohita* and given below in Table II. Up to 75 mg/kg level, the SGR indicated a linear increase, after which it started to level off with a decreasing tendency. Moreover, minimum values for FCR were also recorded in 75 mg/kg vitamin E supplemented diets. However, survival rate remained unaffected from all treatments. Vitamin E optimum requirement for *L. rohita* is 80.62 mg/kg which was obtained after broken line regression analysis (Fig. 1).

Antioxidant enzymes activities

Activities of GPx, CAT and SOD are given in Table III. These enzymatic activities were measured at their lowest levels in the diet containing 0 mg/kg (Control). While, the increase in 125 mg/kg vitamin E level showed a linear increase in their activities.

Level of TBARS

Effect of graded vitamin E levels on TBARS analysis from liver sample is given below in Table III. In contrast to antioxidant enzyme activities, a linear decrease in TBARS level was recorded with increase in the supplementation of dietary vitamin E. Maximum TBARS value were recorded in control group while minimum results were observed in diet containing the vitamin E at 125 mg/kg.

Fatty acid profile

The dietary vitamin E containing graded levels effect the profile of fatty acids which is given below in Table IV. The fatty acid profile showed significant results in the fingerlings when vitamin E in their diet was added. The

decrease in monounsaturated and saturated fatty acids while the significantly improved results observed in PUFAs when supplemented with vitamin E. The n-3 and n-9 percentages was observed significantly increased by raising the level of vitamin E from 0 to 75 mg/kg while with the further increase in level up to 125 mg/kg caused a significant decrease in the percentages of these fatty acids. However, n-6 fatty acids responded differently to different doses of vitamin E supplementation. Furthermore, the total ratio of n-3/n-6 increased significantly from 0 mg/kg to 50 mg/kg vitamin E while it started to decrease significantly with higher supplementation of vitamin E (75-125 mg/kg).

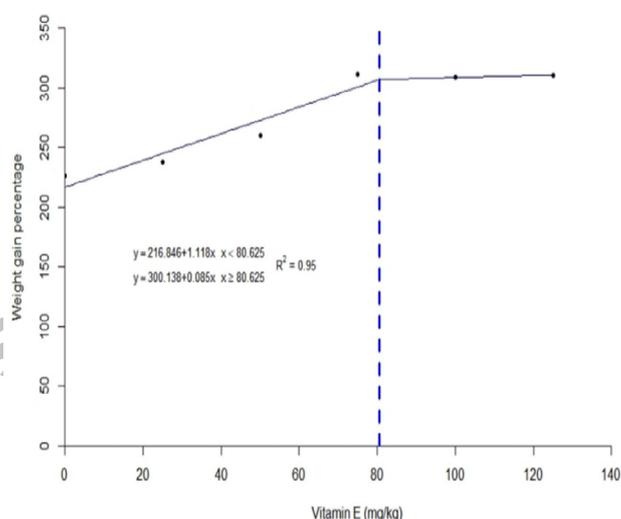


Fig. 1. Broken line regression on weight gain% showing optimum vitamin E requirement of *L. rohita*.

Table II. Dietary vitamin E response on growth performance of *L. rohita* fingerlings.

Parameters	Experimental diets						PSE	p value
	E0	E25	E50	E75	E100	E125		
Initial weight (g)	3.02	3.03	3.03	3.03	3.03	3.03		
Final weight (g)	9.85 ^d	10.21 ^c	10.89 ^b	12.48 ^a	12.38 ^a	12.43 ^a	0.06	$p < 0.05$
AWG (g) [†]	6.83 ^d	7.19 ^c	7.86 ^b	9.45 ^a	9.35 ^a	9.40 ^a	0.06	$p < 0.05$
Weight gain% [‡]	225.99 ^d	237.52 ^c	259.84 ^b	311.72 ^a	308.58 ^a	310.75 ^a	2.16	$p < 0.05$
SGR (%/day) [§]	1.97 ^d	2.03 ^c	2.13 ^b	2.36 ^a	2.35 ^a	2.35 ^a	0.01	$p < 0.05$
Feed intake(g)	13.80 ^a	13.10 ^b	11.98 ^c	10.92 ^d	11.44 ^d	10.94 ^d	0.15	$p < 0.05$
FCR [¶]	2.02 ^a	1.82 ^b	1.52 ^c	1.16 ^d	1.22 ^d	1.16 ^d	0.02	$p < 0.05$
Survival rate (%)	100.00	96.67	100.00	100.00	96.67	100.00	1.92	$p > 0.05$

The data represents mean of three replicates; Mean values containing different superscript letters within a row are significantly different ($p < 0.05$) while others show non-significant results; PSE, Pooled standard error = $\sqrt{\text{MSE}/n}$ (where MSE=mean-squared error); [†]AWG= Absolute weight gain=final weight (g) – initial weight. [‡]Weight gain%= (final weight - initial weight)/initial weight $\times 100$. [§]SGR= Specific growth rate = (ln final weight – ln initial weight)/experimental duration $\times 100$. [¶]FCR, Feed conversion ratio = feed intake (g)/AWG (g).

Table III. Effect of Dietary vitamin E response on TBARS and antioxidant activities in liver of *L. rohita* fingerlings.

	Level of vitamin E (mg/kg)						PSE	p value
	E0	E25	E50	E75	E100	E125		
TBARS[†]								
Liver	3.31 ^c	3.13 ^d	2.86 ^c	2.67 ^b	2.56 ^{ab}	2.44 ^a	0.04	<i>p</i> <0.05
Antioxidant enzyme activities (Liver)								
SOD [‡]	5.78 ^f	5.97 ^c	6.24 ^d	6.36 ^c	6.60 ^b	6.86 ^a	0.03	<i>p</i> <0.05
CAT [§]	65.49 ^f	69.83 ^c	79.40 ^d	86.72 ^c	95.06 ^b	99.45 ^a	1.21	<i>p</i> <0.05
GPX [¶]	95.21 ^f	97.23 ^c	103.23 ^d	104.95 ^c	109.29 ^b	114.51 ^a	0.42	<i>p</i> <0.05

The data represents mean of three replicates; Different superscripts letters on the mean value shows significantly different results (*p* < 0.05); PSE, Pooled standard error= $\sqrt{\text{MSE}/n}$ (where MSE, mean-squared error); [†]TBARS, Thiobarbituric acid reactive substances (mg/g protein); [‡]SOD, Superoxide dismutase (Units/min/mg protein); [§]CAT, Catalase= (Units/min/mg protein); [¶]GPX, Glutathione peroxidase= (mUnits/min/mg protein).

Table IV. Dietary vitamin E response on fatty acid profile in liver of *L. rohita* fingerlings.

Fatty acids [†]	Levels of vitamin E (mg/kg)						PSE	p value
	E0	E25	E50	E75	E100	E125		
14:0 n-0	4.17 ^a	2.42 ^b	2.40 ^b	2.40 ^b	2.40 ^b	2.38 ^b	0.03	<i>p</i> <0.05
16:0 n-0	15.37 ^a	9.00 ^b	9.00 ^b	8.98 ^b	9.01 ^b	9.03 ^b	0.04	<i>p</i> <0.05
18:0 n-0	5.21 ^a	3.25 ^b	3.38 ^b	3.43 ^b	3.39 ^b	3.37 ^b	0.07	<i>p</i> <0.05
16:1 n-7	12.70 ^a	7.84 ^b	7.74 ^b	7.69 ^b	7.80 ^b	7.80 ^b	0.04	<i>p</i> <0.05
18:1 n-7	13.84 ^a	8.93 ^b	8.87 ^b	8.85 ^b	8.88 ^b	8.90 ^b	0.03	<i>p</i> <0.05
18:1 n-9	17.35 ^a	9.05 ^b	9.01 ^{bc}	8.82 ^c	8.97 ^{bc}	8.94 ^{bc}	0.04	<i>p</i> <0.05
18:2 n-6	2.09 ^b	5.86 ^a	5.81 ^a	5.74 ^a	5.78 ^a	5.80 ^a	0.04	<i>p</i> <0.05
20:4 n-6	1.21 ^b	3.74 ^a	3.75 ^a	3.68 ^a	3.70 ^a	3.68 ^a	0.03	<i>p</i> <0.05
18:3 n-3	3.83 ^b	7.60 ^a	7.61 ^a	7.68 ^a	7.60 ^a	7.60 ^a	0.04	<i>p</i> <0.05
20:5 n-3	9.86 ^b	16.59 ^a	16.61 ^a	16.69 ^a	16.61 ^a	16.58 ^a	0.03	<i>p</i> <0.05
22:5 n-3	10.05 ^b	17.47 ^a	17.48 ^a	17.60 ^a	17.62 ^a	17.57 ^a	0.08	<i>p</i> <0.05
22:6 n-3	4.34 ^b	8.27 ^a	8.37 ^a	8.47 ^a	8.28 ^a	8.37 ^a	0.05	<i>p</i> <0.05
Others[‡]								
Saturated	24.74 ^a	14.67 ^b	14.78 ^b	14.81 ^b	14.79 ^b	14.78 ^b	0.06	<i>p</i> <0.05
Monounsaturated	43.89 ^a	25.82 ^b	25.61 ^b	25.36 ^c	25.64 ^b	25.64 ^b	0.04	<i>p</i> <0.05
n-3	28.08 ^c	49.92 ^b	50.06 ^b	50.43 ^a	50.10 ^b	50.11 ^b	0.04	<i>p</i> <0.05
n-6	3.30 ^b	9.60 ^a	9.56 ^a	9.41 ^a	9.48 ^a	9.48 ^a	0.06	<i>p</i> <0.05
n-9	17.35 ^a	9.05 ^b	9.01 ^{bc}	8.82 ^c	8.97 ^{bc}	8.94 ^{bc}	0.04	<i>p</i> <0.05
ARA/EPA	0.12 ^b	0.23 ^a	0.23 ^a	0.22 ^a	0.22 ^a	0.22 ^a	0.00	<i>p</i> <0.05
EPA/DHA	2.27 ^a	2.01 ^b	1.98 ^b	1.97 ^b	2.01 ^b	1.98 ^b	0.02	<i>p</i> <0.05
n-3/n-6	1.62 ^c	5.52 ^b	5.56 ^b	5.72 ^a	5.59 ^b	5.61 ^b	0.03	<i>p</i> <0.05
Monoenes/ Polyenes	0.90 ^a	0.38 ^b	0.37 ^b	0.36 ^c	0.37 ^b	0.37 ^b	0.00	<i>p</i> <0.05

The data represents mean of three replicates; Mean values containing different superscript letters within a row are significantly different (*p* < 0.05); PSE, Pooled standard error= $\sqrt{\text{MSE}/n}$ (where MSE, mean-squared error); [†]Fatty acid = % total fatty acid detected; [‡]Others= Sum of 15:0, 15:1, 16:1 n-9, 16:2 n-7, 17:0, 17:1 n-7, 18:2 n-3, 20:1 n-9, 21:5 n-3, 22:1 n-9, 22:2 n-6, 22:4 n-6.

DISCUSSION

Present study showed an increased growth

performance when fed with graded vitamin E levels to *L. rohita*. Specifically, the significant (*p*<0.05) increase in the final weight of fingerlings, SGR, AWG, weight gain%

while decrease in FI and FCR was observed by enhancing the vitamin E supplementation. The fingerlings did not show any effect on rate of survival rate when vitamin E was supplemented in fish diet. Same results were found in *Sebastes schlegeli*, (Bai and Lee, 1998), gilthead sea bream, turbot, halibut (Tocher *et al.*, 2002), mori (Paul *et al.*, 2004), grouper (Lin and Shiau, 2005), parrot fish (Galaz *et al.*, 2010), guppy (Mehrad and Sudagar, 2010), *Herichthys cyanoguttatus* (Montajami *et al.*, 2012), *Huso huso* (Amlashi *et al.*, 2012), *Channa punctatus* (Hameid *et al.*, 2012), angel fish (Nekoubin *et al.*, 2012), hybrid catfish (Pitaksong *et al.*, 2013), gilthead seabream (Atalah *et al.*, 2012), Eel (Bae *et al.*, 2013), darkbarbel catfish (Li *et al.*, 2013), pacu (Sado *et al.*, 2013), Sea bream (Saleh *et al.*, 2014), grass carp (Li *et al.*, 2014), zebra fish (Mehrad *et al.*, 2014) and Persian sturgeon (Kenari and Naderi, 2015).

In the current study addition of vitamin E at 80.62 mg/kg was found optimum for the supplementation of dietary vitamin E in *L. rohita* fingerlings diet at which maximum growth performance was observed. Sau *et al.* (2004) performed an experiment on dietary vitamin E requirement of *L. rohita* fed semi purified diet for optimum growth and found best growth performance at 131.91 mg/kg level. Vitamin E requirement for various species have been recorded such as for rainbow trout the 30 mg kg⁻¹ supplementation of vitamin E was optimum (Woodall *et al.*, 1964), for common carp the required level was 200 to 300 mgkg⁻¹ (Watanabe *et al.*, 1977), Channel catfish requires 30 to 50 mgkg⁻¹ vitamin E in the diet (Wilson *et al.*, 1984), *Salmo salar* needs 35 mgkg⁻¹ dietary vitamin E (Lall *et al.*, 1988), blue tilapia requires 2 mg/kg vitamin E (Roem *et al.*, 1990), the yellowtail fish needs 119 mg/kg vitamin E (Shimeno, 1991), Atlantic salmon requires 120 mgkg⁻¹ vitamin E (Hamre and Lie, 1995), while, the Korean rock fish and hybrid striped bass require 45 mgkg⁻¹ and 28 mgkg⁻¹ vitamin E (Bai and Lee, 1998; Kocabas and Gatlin, 1999), 99 mg/kg vitamin E for mori (Paul *et al.*, 2004), 30 to 60 mgkg⁻¹ for Salmonid (Wen, 2006) and for red drum (31 mgkg⁻¹) (Peng and Gatlin, 2009). The vitamin E requirement in fish species varies on the factors like PUFAs level in diet (Lin and Shiau, 2005) and amount of oxidized lipid in the diet (Huang *et al.*, 2004).

In the current experimental trial, a linear decrease in the level of TBARS from liver sample of *L. rohita* fingerlings was observed when vitamin E in diet increases from 0 to 125 mg/kg. The change in the lipid oxidation is due the presence of TBARS which is an important indicator (Shahidi and Hong, 1991). Many studies confirm this inverse relation as was observed in grouper, rainbow trout, jian carp, parrot fish, darkbarbel catfish, juvenile cobia, Atlantic salmon, sea bream, grass carp and dojo loach, (Lin and Shiau, 2005; Glucin *et al.*, 2005; Jiang

et al., 2009; Galaz *et al.*, 2010; Li *et al.*, 2013; Zhou *et al.*, 2013; Faizan *et al.*, 2013; Saleh *et al.*, 2014; Li *et al.*, 2014; Zhang *et al.*, 2016).

Organisms developed many antioxidant defense systems like antioxidant enzymes including CAT and SOD. Both these antioxidants are scavengers of active oxygen species (Miller *et al.*, 1993). Level of the antioxidants also effect the vitamin E requirement in diet of fish (Bae *et al.*, 2013). The current experiment showed increased results significantly ($p < 0.05$) in the values of antioxidant enzyme (SOD, CAT, GPX) activities in liver of fingerlings by increasing the dietary vitamin E level. Hence, this study may support in the hypothesis that enhanced activity of enzymes in fish liver can counterbalance the effect of pro-oxidant which is caused by vitamin E consumption (Li *et al.*, 2014). Glucin *et al.* (2005) claimed that these enzymes are activated by vitamin E. Similar findings were observed in rainbow trout, cobia, dark barbel catfish, and sea cucumber (Puangkaew *et al.*, 2005; Zhou *et al.*, 2013; Li *et al.*, 2013; Wang *et al.*, 2015). Related results were also found in several marine species (Ortuno *et al.*, 2000; Puangkaew *et al.*, 2005; Lin and Shiau, 2005; Li *et al.*, 2008). The variations in activities of SOD and CAT revealed that an optimum vitamin E level in the fish diet could provide enough protection in contradiction to oxidative stress, and that the vitamin E level in high amount may not be important.

In the current study, the profile of liver fatty acids of fingerlings showed variations according to the change of vitamin E level. Saturated and monounsaturated fatty acids were decreased while PUFAs were increased significantly in all the diets of vitamin E supplementation than that of non-supplemented diet. Further investigations that the percentage of n-3 and n-9 was improved significantly when fed with increased vitamin E in diet of fish from 0 mg/kg to 75 mg/kg, however, with the increase in the concentration of up to 125 mg/kg vitamin E showed decrease in the percentages of n-3 and n-9. These findings highlight the vitamin E importance in preventing polyunsaturated fatty acids peroxidation. The PUFAs peroxidation inhibition by vitamin E supplementation was prominent. Similar results were observed in the studies of Bai and Lee (1998) on Korean rockfish by feeding dietary vitamin E. Similar type of results were reported by Kenari and Naderi (2015) on Persian sturgeon larvae by feeding Artemia enriched diet and vitamin E supplemented soybean meal.

Moreover, the deficiency of supplementation of vitamin E might change the fatty acids composition, however, addition of vitamin E above the required amount showed slight variations in the fish fatty acids (Watanabe *et al.*, 1977). Percentages of eicosapentaenoic acid and docosahexaenoic acid were increased in *H. hippoglossus*

in diets containing vitamin E levels (Tocher *et al.*, 2002). No change was observed in fatty acid profile of liver by vitamin E supplementation (Peng *et al.*, 2009). Contents of fatty acids in red sea bream were improved when vitamin E in fish diet was increased (Gao *et al.*, 2012). The percentage of neutral lipids enhanced with increase in vitamin E level while decreased the polar lipids percentage in loach fingerlings (Zhang *et al.*, 2016).

CONCLUSION

In conclusion, *L. rohita* fingerlings showed significantly increased results in growth performance when different supplemental levels of vitamin E was added and maximum growth performance was observed at level of 80.62 mg/kg, after which no further increase in growth performance was observed with further addition of vitamin. Moreover, activities of antioxidant enzymes were increased while, TBARS level was significantly decreased when vitamin E level in fish diet increases. This study will assist the fish feed industry and farmers in the formulation of nutritionally balanced practical feed with an optimum dose of vitamin E to reduce the oxidative load of *L. rohita*.

ACKNOWLEDGEMENT

This study received no specific support from public, private, or non-profit funding agencies.

Conflict of interest Statement

The equal contribution of all authors in this study.

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