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Effect of Refrigerated Storage on Fillet Quality of Vitamin E Fed Rohu, *Labeo rohita*

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AI conducted the experiment and perfomed the analysis. MF performed post-harvest and statistical analysis. SZHS conceived the experiment and wrote the manuscript. MA supervised the experiment. MB helped in analysis. M.Bilal reviewed the manuscript. NB and SI helped in the conduction of the experiment. AK helped in analysis and manuscript write up.

Key words
α-tocopherol, TBARS, Fatty acid profile, Antioxidant enzyme activities, *Labeo rohita*, Storage

ABSTRACT

The dietary effect of vitamin E supplementation on fingerlings of *Labeo rohita* during refrigerated storage was investigated. Six graded levels of vitamin E, between 0-125 mg/kg vitamin E, were fed to fish. After 60-day feeding trial, fish were sacrificed, fillets were stored in refrigerator at -20°C and analyzed on 0, 15 and 30th day of storage. Upon refrigeration the vitamin E fed fish showed reduced level of Thiobarbituric acid reactive substances (TBARS) at all storage days. The enzyme activities were improved by elevating the levels of vitamin E in diet as well as by storage period ($P < 0.05$) for all treatments. Fatty acid profile indicated that n-3, n-6 fatty acids and the ratios of ARA/EPA and n-3/n-6 were increased by increasing vitamin E supplementation and decreased with an increase in the storage period. Conclusively, the dietary vitamin E reduced lipid peroxidation and improved the fillet quality of stored fish.

INTRODUCTION

The biological materials containing unsaturated fatty acids invariably face severe lipid peroxidation. In case of fish, it is more serious problem as fish body contains highly unsaturated fatty acids in prodigious quantities as compared to other species of animals (Huang *et al*., 2004). The peroxidation of lipid results in change of color and development of rotten taste in fish which affects the consumer acceptability (Nogala-Kalucka *et al*., 2005).

Use of antioxidants and refrigeration is an efficacious method to reduce the oxidation and toxic oxidative product formation that deteriorate the food quality (Sau *et al*., 2004). Freezing method is used for the preservation of fish by impeding the growth of microorganisms and biochemical reactions. However, deterioration takes place in fish quality during freezing such as changes in texture, color and taste. Dietary supplementation of antioxidants such as vitamin E is an excellent approach to enhance the tissue lipids stability of fish and to increase the durability of products (Yildiz *et al*., 2006; Fatima *et al*., 2019).

Vitamin E (α-tocopherol) as an antioxidant has gained much importance in the farmed fish nutrition (Baker, 2001). α-tocopherol is the most powerful biological antioxidant that safeguards biological membranes (Wang *et al*., 2006). It protects the lipid integrant having unsaturated fatty acids from free radicals of reactive oxygen species (Yamamoto *et al*., 2001). It was found that the dietary supplementation of α-tocopherol in fish diet increases its level in the fish muscles. The enhanced levels of α-tocopherol in muscles

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restrain the lipid peroxidation of frozen storage fish products in numerous species of fish like Atlantic halibut and turbot (Ruff *et al*., 2002).

The supplementation of supra nutritional quantities of α-tocopheryl acetate to fish diet, can enhance the quality of whole-body fish (Ruff *et al*., 2002). Alpha tocopherol in tissues captures the free radicals that initiate and aggravate the process of lipid peroxidation, and hence protect against its worse effects (Yildiz *et al*., 2006). Shiau and Hzu (2002) also reported the reduced postmortem lipid peroxidation in *Oreochromis niloticus* x *O. aureus* (hybrid tilapia) fed with α-tocopherol. Furthermore, supplementation of α tocopherol in the feed of rohu significantly enhanced the level of polyunsaturated fatty acids while decreased the saturated fatty acids (SFA) and monounsaturated fatty acids in the study of Fatima *et al*. (2019). However, Zakipur *et al*. (2012) contemplated in his study that variant concentrations of vitamin E supplementation in *Oncorhynchus mykiss* does not affect the muscle fatty acid profile during refrigerated storage. They also observed that the fatty acid profile is only affected by dietary fatty acid level.

Fogaca *et al*. (2009) in their studies on tilapia determined that thiobarbituric acid reactive substances (TBARS) values have a linear relationship with α-tocopherol level in fish muscles upon refrigerated storage. By increasing the α-tocopherol levels in the diet decreases the TBARS value. The treatment containing higher degree of vitamin E (200 mg/kg diet) has conspicuously lower levels of TBARS value. Zhang *et al*. (2007) found in their study on *Sparus macrocephalus* during refrigerated storage that TBARS value are significantly reduce in response to increased dietary α-tocopherol level. The lower values of TBARS were found in the fish containing higher values of α tocopherol in the diet (i.e. 1069 mg/kg).

This research was undertaken to inspect the antioxidant impacts of vitamin E by supplementing it through diet on TBARS, antioxidant enzyme activity, proximate and fatty acid composition, of fish fillet during refrigeration.

MATERIALS AND METHODS

This experiment was conducted in Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan, after the ethical approval by the review committee of the university.

Fish and experimental conditions

After acquiring the *Labeo rohita* from Government Fish Hatchery, Faisalabad, they were permitted to be acclimatize for a period of two weeks to experimental conditions. Before the start of the experimental trial, the fish fingerlings were given prophylactic dip containing NaCl solution (5g/L) (Rowland and Ingram, 1991). For experiment trial, 15 fingerlings were stocked in each tank and triplicate tanks were allotted for each experimental diet. Fish were given respective diet up to apparent satiation, twice a day. Dissolved oxygen in water media was monitored by using digital meter (HANNA, model HI 9147). Similarly, water temperature and pH were estimated by using AMPROBE pH meter (model WT-80). During experimental period aeration was rendered to tanks through capillary system. The feeding experiment lasted for 60 days.

Formulation of experimental diet

The feed ingredients were procured from nearby market and examined chemically (AOAC, 1995). The feed ingredients were ground and sieved to required particle size and mixed with α-tocopherol acetate as vitamin E at the level of 0, 25, 50, 75, 100 and 125 mg/kg diet, to formulate six experimental diets namely D_1, D_2, D_3, D_4, D_5 and D_6. The α-tocopherol acetate was mixed in feed after mixing in fish oil. The experimental ingredients were mixed electrically, water was added to make dough, which was extruded further to make pellets. The finished pellets were dried and stored at -20ºC. The composition of experimental diets has already been described in a previous article (Zulfiqar *et al*., 2022), while Table I shows its fatty acid profile.

Fish harvesting and storage for analyses

At the termination of feeding trial, fish were harvested from each replicate, euthanized by MS 222 and sacrificed. Fish were dissected and fillets were collected and stored at -20°C for further analysis. Fillets were analyzed for TBARS, antioxidant enzyme (SOD, CAT, GPX) activities and fatty acid profile at day 0, 15 and 30 of storage.

Analysis of fatty acid profile and α-tocopherol contents

Using soxtec system, fat from the samples was extracted by petroleum ether. By adopting the method reported by Lee *et al*. (2003), the α-tocopherol contents of experimental diets were examined on HPLC. The fatty acid profile was examined from extracted fat from fish muscles and diet samples. For the examination of fatty acid profile in diet and muscles International Union of Pure and Applied Chemistry (IUPAC, 1987) standard method was followed. Fatty acid methyl esters (FAME) were used for the determination of fatty acid profile. Methanol was used to prepare FAMEs. A sample of 200 to 300 mg of fat was taken in flask and refluxed with 0.5 N

KOH solution for 3-5 min. When the solution was hot, 15 ml of ammonium-chloride-methanol-H_2SO_4 mixture was added and reflux for 15 min. Swirled to mix and refluxed for 3 min. Cooled and added light petroleum ether and shake. Then the ether layer was separated and evaporated the ether under vacuum or nitrogen. The residues were diluted in petroleum ether (3-10 ml) to evaluate the fatty acid profile by gas chromatography. Gas chromatography (GC) (SHIMADZU, model GC-17A FID) was used to analyze FAMEs.

Table I. Fatty acid (percent of total fatty acid detected) profile of experimental diets.

Fatty acid	D_1	D_2	D_3	D_4	D_5	D_6
14:0 n-0	4.53	4.75	4.41	4.37	4.68	4.20
16:0 n-0	11.06	11.34	11.28	10.98	11.21	11.13
18:0 n-0	2.59	2.64	2.81	2.69	2.44	2.40
16:1 n-7	10.80	11.60	10.96	11.67	10.22	12.09
18:1 n-7	9.43	8.95	11.24	9.86	9.42	7.26
18:1 n-9	17.38	17.22	15.78	16.32	16.94	16.83
18:2 n-6	2.89	3.11	2.94	3.07	3.15	3.40
20:4 n-6	1.17	1.20	1.27	1.23	1.19	1.31
18:3 n-3	5.01	4.99	5.19	4.95	5.00	4.94
20:5 n-3	13.14	13.78	14.09	13.44	13.87	14.33
22:5 n-3	15.89	14.78	13.69	15.04	15.36	15.34
22:6 n-3	6.11	5.64	6.34	6.38	6.52	6.77
Total	100	100	100	100	100	100
Saturated	18.18	18.73	18.50	18.04	18.33	17.73
Monounsaturated	37.61	37.77	37.98	37.85	36.58	36.18
n-3	40.15	39.19	39.31	39.81	40.75	41.38
n-6	4.06	4.31	4.21	4.30	4.34	4.71
n-9	17.38	17.22	15.78	16.32	16.94	16.83
ARA/EPA	0.089	0.087	0.090	0.091	0.086	0.091
EPA/DHA	2.15	2.44	2.22	2.11	2.13	2.12
n-3/n-6	2.31	2.28	2.49	2.44	2.41	2.46
Monoenes/polyenes	0.611	0.622	0.640	0.626	0.589	0.575

D, experimental diet.

TBARS assay

The quantification of thiobarbituric acid reactive substances (TBARS) in fish fillets was analyzed by following Gatta *et al*. (2000). A solution containing 3 ml of 80 mM tris-maleate and KCl (11.5 g/L) pH 7.4, was homogenized with each sample (1 g) in a homogenizer. To induce lipid peroxidation, 1 ml ascorbic acid (2mM) was included in the samples and incubated for 30 min at a temperature of 37°C. By including 5 ml of thiobarbituric acid (TBA) containing 0.05 M and 0.7 M HCl (5ml) and boiling the sample tubes for 25 min colorimetric reaction was obtained. Then, with the inclusion of 5 ml trichloroacetic acid (200 g/L) samples were refrigerated, centrifuged and TBA values were determined and expressed photometrically at 530 nm as l g malondialdehyde (MDA) equivalents mg muscle tissue. For calculating the amount of MDA in fish muscles, MDA standard solution was utilized to acquire a standard curve which was correlated with absorbance values.

Enzyme extraction

After weighing the muscle samples, phosphate buffer (pH 7.4) was added 3 times more than the samples weight i.e. 1:3. After homogenization, samples were passed through muslin cloth to remove the biomass. The resultant liquid was filtered using Whatman filter paper no. 1 before being centrifuged for 15 min in a refrigerated centrifugal machine at 10,000 rotations per minute. For further analysis, the supernatants were separated. All the enzyme isolation steps were carried out at 4°C.

By following the Giannopolitis and Ries (1997) method, the activity of superoxide dismutase (SOD) in muscles of fish was measured by assessing its propensity to restrain the nitroblue tetrazole (NBT) reduction by superoxide. By adopting Chance and Maehly (1955) method, the activity of catalase (CAT) was evaluated by assessing its ability to decompose hydrogen peroxide concentration at wavelength of 240 nm. The activity of peroxidase was determined by measuring its capability to lower the hydrogen peroxide concentration at 470 nm wavelength (Civello *et al*., 1995).

Statistical analysis

To statistically analyze data, two-way analysis of variance was executed using CoStat computer package (Version 6.303, PMB320 Monterey, CA, 93940 USA). Tuckey's significant difference test was used for means comparison by considering the significant level of $p<0.05$ (Steel *et al*., 1996).

RESULTS

The results revealed that the values of TBARS were decreased with the increasing α-tocopherol level in diet. By expanding the storage time from 0 to 30 days, the TBARS values were increased in a striking pattern. The interaction of α-tocopherol and storage time was found significant for TBARS values in all the treatments. The lowest value of TBARS in the present study was determined in the D_6

treatment which had maximum level of vitamin E (125mg/kg diet) at 0 day of storage than all the other treatments (Table II).

The results for antioxidant enzyme activities evinced that by increasing the vitamin E level and the storage duration, the activities of CAT, peroxidase and SOD were found to be increased significantly in all the treatments accordingly (Table III). The interaction of α-tocopherol and storage time was found significant for all enzyme's activities in all the treatments. The maximum activity of all the enzymes under present study was observed in treatment containing 125 mg/kg vitamin E in diet (D_6) with 30 days of storage time. The minimum values for all enzyme activities were observed in control group (D_1).

Table II. Effect of refrigerated storage on fillets thiobarbituric acid reactive substances (TBARS; mg/g) of vitamin E fed *L. rohita.*

Diet	Storage (days)			
	0	15	30	Mean
D_1	2.29	3.56	5.04	3.63^{a}
D_2	2.45	3.71	5.15	3.77^{b}
D_3	2.61	3.85	5.23	3.90^{c}
D_4	2.71	3.94	5.3	3.98^{d}
D_5	2.79	4.03	5.45	4.09^{e}
D_6	2.83	4.17	5.53	4.18^{f}
Mean	2.61^{C}	3.88^{B}	5.28^{A}	

Data are mean of three replicates. Means within columns having different superscripts (a, b) are significantly different at $p<0.05$. Means within rows having different superscripts (A, B) are significantly different at $p<0.05$

Effect of α-tocopherol supplementation to fish through diet on fatty acid profile of muscle is shown in Table IV. The fish fillets fatty acid composition comprised of myristic acid (14:0 n-0), palmitic acid (16:0 n-0), stearic acid (18:0 n-0), palmitoleic acid (16:1 n-7), vaccenic acid (18:1 n-7), oleic acid (18:1 n-9), linoleic acid (18:2 n-6), arachidonic acid (20:4 n-6), linolenic acid (18:3 n-3), eicosa pentaenoic acid (20:5 n-3), decosa pentanoic acid (22:5 n-3), decosa hexanoic acid (22:6 n-3), saturated fatty acids, monounsaturated fatty acid, n-3, n-6, n-9 fatty acids and the ratios of monoenes/polyenes, ARA/EPA, EPA/DHA, n-3/n-6 fatty acids. The fish fillets fatty acids composition was not affected due to the supplementation of vitamin E. The results of present study manifested some variance in the different fatty acids proportions during refrigerated storage against different dietary α-tocopherol levels. By increasing supplemental level of α-tocopherol, the levels of 14:0 n-0, 16:0 n-0, 18:0 n-0, saturated fatty acids, 16:1 n-7, 18:1 n-7, 18:1 n-9, monounsaturated fatty acids, n-9, and the ratios of monoenes/ polyenes were decreased while with increase in the storage time from 0 to 30 days, the levels of these fatty acids were increased. The 18:2 n-6, 20:4 n-6, 18:3 n-3, 20:5 n-3, 22:5 n-3, 22:6 n-3, n-3, n-6 fatty acids and the ratios of ARA/EPA and n-3/n-6 were observed to be increased by incrementing the supplementation of α-tocopherol and decreased by expanding the storage time in all the treatments. The EPA/DHA ratios showed no effect against increasing storage time in all the treatments, but the level declined by increasing dietary α-tocopherol. The interaction of vitamin E and storage time was found to be significant in all the groups of fatty acids in all the treatments.

Table III. Effect of refrigerated storage on fillets antioxidant enzyme activities of vitamin E fed *L. rohita.*

	Diet	Storage (days)			
		0	15	30	Mean
SOD†	D1	2.29	3.56	5.04	3.63^{f}
	D2	2.45	3.71	5.15	3.77^{e}
	D3	2.61	3.85	5.23	3.90^{d}
	D4	2.71	3.94	5.3	3.98^{c}
	D5	2.79	4.03	5.45	4.09^{b}
	D6	2.83	4.17	5.53	4.18^{a}
	Mean	2.61^{C}	3.88^{B}	5.28^{A}	
CAT‡	D1	22.99	35.29	41.18	33.15^{f}
	D2	25.63	36.54	42.16	34.78^{e}
	D3	29.03	37.59	42.71	36.44^{d}
	D4	30.57	38.13	42.78	37.16^{c}
	D5	32.31	38.53	43.37	38.07^{b}
	D6	33.43	38.86	43.45	38.58^{a}
	Mean	28.99^{C}	37.49^{B}	42.61^{A}	
GPX§	D1	$66.75^{f\,C}$	$73.85^{f\,B}$	$91.84^{f\,A}$	77.48^{f}
	D2	$67.46^{e\,C}$	$75.55^{e\,B}$	$92.56^{e\,A}$	78.52^{e}
	D3	$68.02^{d\,C}$	$77.80^{d\,B}$	$92.80^{d\,A}$	79.54^{d}
	D4	$68.14^{c\,C}$	$80.33^{c\,B}$	$92.42^{c\,A}$	80.30^{c}
	D5	$68.60^{b\,C}$	$82.52^{b\,B}$	$93.47^{b\,A}$	81.53^{b}
	D6	$69.15^{a\,C}$	$84.44^{a\,B}$	$93.53^{a\,A}$	82.37^{a}
		68.02^{C}	79.08B	92.77^{A}	

†SOD= Superoxide dismutase (U/mg protein); ‡CAT= Catalase (U/mg protein); §GPX= Glutathione peroxidase (mU/mg protein); Data are mean of three replicates; Means within columns having different superscripts (a, b) are significantly different at $p<0.05$; Means within rows having different superscripts (A, B) are significantly different at $p<0.05$

Table IV. Effect of refrigerated storage on fillets fatty acid profile of vitamin E fed *L. rohita.*

	0 day						15 days						30 days					
	D_1	D_2	D_3	D_4	D_5	D_6	D_1	D_2	D_3	D_4	D_5	D_6	D_1	D_2	D_3	D_4	D_5	D_6
14:0 n-0	5.51^{aC}	3.20^{bC}	3.28^{cC}	3.24^{cC}	3.35^{cC}	3.33^{cC}	6.71^{aB}	3.87^{bB}	3.83^{cB}	3.79^{cB}	3.75^{cB}	3.80^{cB}	9.67^{aA}	5.95^{bA}	5.65^{cA}	5.63^{cA}	5.59^{cA}	5.62^{cA}
16:0 n-0	7.61^{aC}	5.56^{bC}	5.41^{cC}	5.37^{cC}	5.36^{cC}	5.43^{cC}	8.11^{aB}	5.95^{bB}	5.61^{cB}	5.60^{cB}	5.65^{cB}	5.55^{cB}	11.42^{aA}	7.63^{bA}	7.07^{cA}	7.10^{cA}	7.10^{cA}	7.11^{cA}
18:0 n-0	4.08^{aC}	1.78^{bC}	1.77^{bC}	1.83^{bC}	1.81^{bC}	1.73^{bC}	6.71^{aB}	2.08^{bB}	2.13^{bB}	2.11^{bB}	2.20^{bB}	2.18^{bB}	9.17^{aA}	4.76^{bA}	4.76^{bA}	4.69^{bA}	4.65^{bA}	4.69^{bA}
16:1 n-7	14.42^{aC}	7.72^{bC}	7.70^{cC}	7.74^{dC}	7.70^{dC}	7.75^{dC}	16.26^{aB}	8.05^{bB}	8.21^{cB}	8.05^{dB}	8.08^{dB}	8.04^{dB}	18.30^{aA}	12.77^{bA}	12.21^{cA}	12.09^{dA}	12.01^{dA}	11.93^{dA}
18:1 n-7	12.25^{aC}	8.81^{bC}	8.75^{bC}	8.74^{bC}	8.77^{bC}	8.69^{bC}	12.80^{aB}	8.90^{bB}	8.92^{bB}	8.94^{bB}	8.85^{bB}	8.91^{bB}	13.56^{aA}	9.15^{bA}	9.15^{bA}	9.13^{bA}	9.16^{bA}	9.15^{bA}
18:1 n-9	19.84^{aC}	12.45^{bC}	12.67^{bC}	12.60^{bcC}	12.58^{bcC}	12.53^{cC}	20.21^{aB}	13.43^{bB}	13.38^{bB}	13.34^{bcB}	13.42^{bcB}	13.38^{cB}	22.37^{aA}	15.59^{bA}	15.38^{bA}	15.39^{bcA}	15.36^{bcA}	15.33^{cA}
18:2 n-6	1.70^{eA}	4.31^{dA}	4.43^{cA}	4.40^{bA}	4.37^{bA}	4.33^{aA}	1.46^{eB}	4.13^{dB}	4.16^{cB}	4.23^{bB}	4.22^{bB}	4.35^{aB}	1.46^{eB}	4.10^{dB}	4.16^{cB}	4.28^{bB}	4.32^{bB}	4.35^{aB}
20:4 n-6	1.16^{cA}	3.50^{bA}	3.46^{aA}	3.52^{aA}	3.50^{aA}	3.55^{aA}	0.99^{cB}	3.12_{bB}	3.20^{aB}	3.21^{aB}	3.16^{aB}	3.15^{aB}	0.73^{cC}	0.80^{bC}	1.90^{aC}	1.95^{aC}	2.01^{aC}	1.92^{aC}
18:3 n-3	4.09^{cA}	8.26^{bA}	8.43^{aA}	8.41^{aA}	8.43^{aA}	8.37^{aA}	3.80^{cB}	8.03^{bB}	8.10^{aB}	8.20^{aB}	8.17^{aB}	8.17^{aB}	1.21^{cC}	6.97^{bC}	7.25^{aC}	7.27^{aC}	7.25^{aC}	7.33^{aC}
20:5 n-3	13.80^{cA}	18.94^{bA}	18.79^{bA}	18.81^{abA}	18.86^{abA}	18.91^{aA}	11.30^{cB}	17.57^{bB}	17.74^{bB}	17.76^{abB}	17.75^{abB}	17.76^{aB}	5.98^{cC}	12.37^{bC}	12.40^{bC}	12.45^{abC}	12.42^{abC}	12.46^{aC}
22:5 n-3	11.98^{bA}	18.35^{aA}	18.22^{aA}	18.24^{aA}	18.21^{aA}	18.25^{aA}	9.19^{bB}	17.90^{aB}	17.78^{aB}	17.82^{aB}	17.77^{aB}	17.78^{aB}	5.14^{bC}	14.23^{aC}	14.34^{aC}	14.32^{aC}	14.40^{aC}	14.44^{aC}
22:6 n-3	3.59^{bA}	7.15^{aA}	7.12^{aA}	7.14^{aA}	7.10^{aA}	7.15^{aA}	2.49^{bB}	7.00^{aB}	6.97^{aB}	6.99^{aB}	7.01^{aB}	6.97^{aB}	1.02^{bC}	5.70^{aC}	5.76^{aC}	5.72^{aC}	5.74^{aC}	5.70^{aC}
SFA	17.19^{aC}	10.54^{bC}	10.46^{cC}	10.43^{cC}	10.52^{cC}	10.48^{cC}	21.52^{aB}	11.89^{bB}	11.56^{cB}	11.49^{cB}	11.60^{cB}	11.53^{cB}	30.26^{aA}	18.34^{bA}	17.47^{cA}	17.42^{cA}	17.34^{cA}	17.41^{cA}
MUFA	46.51^{aC}	28.98^{bC}	29.12^{cC}	29.07^{dC}	29.04^{dC}	28.97^{eC}	49.26^{aB}	30.37^{bB}	30.50^{cB}	30.32^{dB}	30.34^{dB}	30.32^{eB}	54.22^{aA}	37.51^{bA}	36.74^{cA}	36.61^{dA}	36.53^{dA}	36.41^{eA}
n-3 FA	33.45^{dA}	52.69^{cA}	52.55^{bA}	52.60^{abA}	52.59^{abA}	52.68^{aA}	26.77^{dB}	50.50^{cB}	50.58^{bB}	50.76^{abB}	50.69^{abB}	50.67^{aB}	13.34^{dC}	39.25^{cC}	39.74^{bC}	39.75^{abC}	39.81^{abC}	39.92^{aC}
n-6 FA	2.86^{dA}	7.81^{cA}	7.89^{bA}	7.92^{aA}	7.86^{aA}	7.88^{aA}	2.45^{dB}	7.25^{cB}	7.36^{bB}	7.44^{aB}	7.38^{aB}	7.50^{aB}	2.19^{dC}	4.90^{cC}	6.06^{bC}	6.23^{aC}	6.33^{aC}	6.27^{aC}
n-9 FA	19.84^{aC}	12.45^{bC}	12.67^{bC}	12.60^{bcC}	12.58^{bcC}	12.53^{cC}	20.21^{aB}	13.43^{bB}	13.38^{bB}	13.34^{bcB}	13.42^{bcB}	13.38^{cB}	22.37^{aA}	15.59^{bA}	15.38^{bA}	15.39^{bcA}	15.36^{bcA}	15.33^{cA}
ARA/EPA	0.084^{cA}	0.185^{bA}	0.184^{aA}	0.187^{aA}	0.185^{aA}	0.188^{aA}	0.088^{cB}	0.178^{bB}	0.180^{aB}	0.181^{aB}	0.178^{aB}	0.177^{aB}	0.122^{cC}	0.065^{bC}	0.153^{aC}	0.157^{aC}	0.162^{aC}	0.154^{aC}
EPA/DHA	3.85^{aA}	2.65^{bA}	2.64^{bA}	2.63^{bA}	2.66^{bA}	2.65^{bA}	4.55^{aA}	2.51^{bA}	2.55^{bA}	2.54^{bA}	2.53^{bA}	2.55^{bA}	5.89^{aA}	2.17^{bA}	2.15^{bA}	2.18^{bA}	2.17^{bA}	2.19^{bA}
n-3/n-6	1.69^{dA}	4.23^{cA}	4.15^{cA}	4.18^{bA}	4.18^{bA}	4.20^{aA}	1.32^{dB}	3.76^{cB}	3.78^{cB}	3.81^{bB}	3.78^{bB}	3.79^{aB}	0.596^{dC}	2.52^{cC}	2.58^{cC}	2.58^{bC}	2.59^{bC}	2.60^{aC}
Monoenes/ polyenes	0.828^{aC}	0.397^{bC}	0.398^{cC}	0.398^{dC}	0.398^{dC}	0.396^{eC}	0.997^{aB}	0.427^{bB}	0.428^{cB}	0.424^{dB}	0.424^{dB}	0.424^{eB}	1.431^{aA}	0.628^{bA}	0.600^{cA}	0.596^{dA}	0.594^{dA}	0.592^{eA}

Data are mean of three replicates. Means within columns having different superscripts (a, b) are significantly different at $p<0.05$; Means within rows having different superscripts (A, B) are significantly different at $p<0.05$

DISCUSSION

α-tocopherol is an antioxidant which plays its important role in preventing the lipid peroxidation. The level of TBARS can be utilized as a key factor for the assessment of fish meat standard (Frigg *et al*., 1990). The percentage inhibition of lipid oxidation by an antioxidant system can be measured by TBARS (McDonald *et al*., 2001). The motive of the present research was to assess the fatty acid profile and lipid peroxidation in fillets of *Labeo rohita* fingerlings fed with α-tocopherol supplemented diet during storage.

The outcomes of present study revealed that by enhancing the α-tocopherol supplementation in fish diet TBARS contents also increased in all the treatments. During storage, the TBARS value indicated a significant increment with increase in storage time. The interaction of α-tocopherol and storage time was found significant for TBARS values in all the treatments. α-tocopherol has a conspicuous effect on lipid peroxidation (Scaife *et al*., 2000; Gatta *et al*., 2000; Ruff *et al*., 2003; Huang *et al*., 2004; Yildiz *et al*., 2006; Zhang *et al*., 2007; Jasour *et al*., 2011). Yildiz *et al*. (2006) discern that by elevating the level of α-tocopherol in diet, the value for TBARS decreases during the refrigerated storage of *Oncorhynchus mykiss W*. fillets (Yildiz *et al*., 2006). Use of α-tocopherol in fish diet deposited in the muscles of fish and curtails the lipid peroxidation which improves the products shelf life (Zhang *et al*., 2007). The outcomes of present research are in accordance with many other related studies in which significant results for TBARS in response to cold storage and vitamin E supplementation were recorded. The supplementation of α-tocopherol through diet to Atlantic salmon (*Salmo salar*) showed a pronounced drop ($p < 0.001$) in lipid peroxidation over one-year storage period in fillets from the fish fed with high doses of α-tocopherol acetate compared to the fish which were provided with lower levels of α-tocopherol in their diet (Scaife *et al*., 2000). Similarly, TBARS values in *Oncorhynchus mykiss W.* fillets were found to be enhanced significantly ($p < 0.05$) through 9 days of storage period. Moreover, higher value was obtained in those fish which were supplemented with minimum level (100 mg/kg diet) of α-tocopherol and vice versa (Yildiz *et al*., 2006). Huang *et al*. (2004) reported increased TBARS values with increasing storage time in all groups of hybrid tilapia regardless of different vitamin E levels used in their diet. During refrigeration of *Sparus macrocephalus* tissues, TBARS level was also significantly increased ($p < 0.05$). Furthermore, the treatment containing more quantities of α-tocopherol in diet showed low values of TBARS ($p < 0.05$) while a higher value was observed for the treatment containing low level of vitamin E (Zhang *et al*., 2007). Similar results were observed in turbot (*Scophthalmus maximus*) for a storage duration of 9 days (Ruff *et al*., 2003). The TABRS values were found to increase continuously ($p < 0.05$) in *Oncorhynchus mykiss* nourished with distinct concentrations of dietary α-tocopherol in all the group treatments through 12 days of storage period (Jasour *et al*., 2011). Gatta *et al*. (2000) and Nawaz *et al*. (2020) determined the induced TABRS values in sea bass *(Dicentrarchus labrax)* and mori *(Cirrhinus mrigala)* fillets, respectively, after different storage periods. Fish supplemented with diet containing high α-tocopherol levels showed low TBARS value compared to low level of α-tocopherol.

The relationship between antioxidant enzyme activities and dietary α-tocopherol has been described in many research works on fish. However, the impacts of α-tocopherol reported for the antioxidant enzymes in fish is highly varied (Lygren *et al*., 2000; Mourente *et al*., 2002). The results of present research manifested that the activities of CAT, SOD and POX enzymes enhanced by incrementing the supplemental level of vitamin E through diets. Moreover, these enzymatic activities also increased among all the treatments due to increase in the storage period from 0 to 30 days. The interaction of α-tocopherol and storage time was found significant for all enzyme activities in all the treatments. Living organisms possess many defense mechanism systems in response to reactive oxygen species that include antioxidant enzymes like CAT, GPX, SOD and many more. The antioxidants having low molecular weight, for example vitamin E and C work in synergy with these enzymatic systems (Pascual *et al*., 2003). When pro-oxidant forces overcome the defense mechanism of antioxidant system and the reactive oxygen species are not sufficiently evacuated then the oxidative stress occurs (Sies, 1986). The results of many studies on antioxidant enzymes activities are in line with the present research. Zhang *et al*. (2007) evaluated the serum antioxidant enzyme activities in *Sparus macrocephalus.* The fish supplemented with diet containing high concentration of vitamin E exhibited significantly ($p < 0.05$) higher activities of SOD enzyme, in contrast to fish supplemented with diet containing lower dose of vitamin E. The GPX activities did not show any remarkable change among the groups. Zhou *et al*. (2013) determined the activity of SOD in plasma of juvenile cobia (*Rachycentron canadum*). The SOD activity of fish increased by enhancing the concentration of vitamin E up to a certain limit in fish nourished with α-tocopherol containing diet as compared to the fish provided with basal diet.

Fish lipids are consisted of some long chain fatty acids which contain a number of double bonds and are highly unsaturated (Jittrepotch *et al*., 2006). The fatty acid

composition and lipid contents present in fish fillet usually reflect dietary contents of lipids and fatty acids (Bell, 1998; Chen *et al*., 2008). The supplementation of α-tocopherol in diet has no influence on fish fillet fatty acid composition (Yildiz *et al*., 2006). The results of present investigation revealed that by enhancing the supplemental level of vitamin E, the amounts of monounsaturated and saturated fatty acids, n-9, and the ratios of monoenes/polyenes were decreased and by increasing storage time from 0 to 30 days, the amounts of these fatty acids were increased. The n-6 and n-3 fatty acids and the ratios of ARA/EPA and n-3/n-6 were found to be enhanced by enhancing the α-tocopherol supplementation and diminished with increase in the storage time in all the treatments. The EPA/DHA ratio showed no effect against increasing storage time in all the treatments, but the level dropped against increasing dietary α-tocopherol. The interaction of vitamin E and storage time was found to be significant in all the groups of fatty acids in all treatments. During storage a gradual change is known to occur in the fatty acids composition in which a variation in the proportion of unsaturated and saturated fatty acids takes place (Yildiz *et al*., 2006; Jittinandana *et al*., 2006; Hosseini *et al*., 2010; Rahimabadi *et al*., 2012). It is a well-known fact that during refrigerated storage the hydrolysis of lipids and poly unsaturated fatty acids decreases with an increase in peroxides content. These two are the major factors responsible for the quality degradation of fish meat (Bonnel, 1989). The interior hydrophobic part of biological membranes contains α-tocopherol with its phytol chain where it protects the polyunsaturated fatty acids by giving its hydrogen atom to the radicals of lipid peroxyl and by chain reactions shattering which are responsible for the peroxidation of lipid (Quinn *et al*., 2004). Several other studies affirm the outcomes of present research. Variations in the percentage of different fatty acids in rainbow trout (*Oncorhynchus mykiss*) fillets were found associated with the increased storage time of fillets and prolonged duration of fatty acids feeding, instead of vitamin E supplementation (Kamireddy *et al*., 2011). During period of storage the fatty acid composition of *Oncorhynchus mykiss W.* fillets got significantly ($p< 0.05$) affected by concentration of α-tocopherol (Yildiz *et al*., 2006). In comparison to our research, the supplementation of α-tocopherol to *Oncorhynchus mykiss* does not affect the composition of fatty acid in fillets significantly ($p > 0.05$). While, during refrigeration, the composition of fatty acid was affected significantly ($p < 0.05$) in all treatments (Rahimabadi *et al*., 2012). Likewise, Jittinandana *et al*. (2006) studied that the levels of α-tocopherol supplementation do not affect the fatty acid composition of *Oncorhynchus mykiss* frozen fillets. Feeding α-tocopherol for a longer period improves the frozen fish meat quality by improving its fatty acid profile.

CONCULSION

The supplementation of dietary vitamin E ameliorated the fillet quality of *L. rohita* during refrigerated storage by restraining the lipid peroxidation and improving the enzyme activities and ratios of polyunsaturated fatty acids.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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