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

Amber Iqbal1, Mahroze Fatima2, Syed Z. H. Shah1,∗, Muhammad Afzal1, Moazama Batool1, Muhammad Bilal1, Naheed Bano6, Ayesha Khizar2 and Sumbal Iqbal7

1Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan
2Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore, Pakistan.
3Department of Zoology, University of Gujrat, Gujrat, Pakistan
4Department of Zoology, Govt. College Women University, Sialkot, Pakistan
5School of Life Science and Food Engineering, Huaibin Institute of Technology, Huaian, China
6Department of Veterinary and Animal Science, MNS-University of Agriculture, Multan, Pakistan
7Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad Sub Campus Toba Tek Singh, Pakistan

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
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 Zakipur et al. (2012). 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 (SOD, CAT, GPX) activities and fatty acid profile at day 0, 15 and 30 of storage. TBARS were found in the fish containing higher values of α-tocopherol acetate in feed at the level of 0, 25, 50, 75, 100 and 125 mg/kg, to formulate six experimental diets namely D1, D2, D3, D4, D5 and D6. The α-tocopherol acetate was mixed in feed ingredients were ground and sieved to required particle size and mixed with α-tocopherol acetate as vitamin E supplement in Atlantic halibut and turbot (Ruff et al., 2002). 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 -20°C. The composition of experimental diets has already been described in a previous article (Zulfiqar et al., 2022), while Table 1 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\textsubscript{2}SO\textsubscript{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.

| Fatty acid  | D\textsubscript{1} | D\textsubscript{2} | D\textsubscript{3} | D\textsubscript{4} | D\textsubscript{5} | D\textsubscript{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           |
| Monounsatu-| 37.61           | 37.77           | 37.98           | 37.85           | 36.58           | 36.18           |
| rated      |                 |                 |                 |                 |                 |                 |
| 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/  | 0.611           | 0.622           | 0.640           | 0.626           | 0.589           | 0.575           |
| polyenes   |                 |                 |                 |                 |                 |                 |

D\textsubscript{6}, 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 1 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\textsubscript{6}. 

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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 (D6) with 30 days of storage time. The minimum values for all enzyme activities were observed in control group (D1).

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  |
|------|----------------|----|-----|-----|-------|
|      |                |    |     |     |       |
| D1  | 2.29           | 3.56 | 5.04 | 3.63 |       |
| D2  | 2.45           | 3.71 | 5.15 | 3.77 |       |
| D3  | 2.61           | 3.85 | 5.23 | 3.90 |       |
| D4  | 2.71           | 3.94 | 5.3  | 3.98 |       |
| D5  | 2.79           | 4.03 | 5.45 | 4.09 |       |
| D6  | 2.83           | 4.17 | 5.53 | 4.18 |       |
| Mean| 2.61           | 3.88 | 5.28 |       |

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 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 |
|      | D2             | 2.45 | 3.71 | 5.15 | 3.77 |
|      | D3             | 2.61 | 3.85 | 5.23 | 3.90 |
|      | D4             | 2.71 | 3.94 | 5.3  | 3.98 |
|      | D5             | 2.79 | 4.03 | 5.45 | 4.09 |
|      | D6             | 2.83 | 4.17 | 5.53 | 4.18 |
| Mean |                | 2.61 | 3.88 | 5.28 |       |

CAT  

| Diet | Storage (days) | 0 | 15 | 30 | Mean |
|------|----------------|---|----|----|------|
|      |                |   |    |    |      |
| D1   | 22.99          | 35.29 | 41.18 | 33.15 |       |
| D2   | 25.63          | 36.54 | 42.16 | 34.78 |       |
| D3   | 29.03          | 37.59 | 42.71 | 36.44 |       |
| D4   | 30.57          | 38.13 | 42.78 | 37.16 |       |
| D5   | 32.31          | 38.53 | 43.37 | 38.07 |       |
| D6   | 33.43          | 38.86 | 43.45 | 38.58 |       |
| Mean |                | 28.99 | 37.49 | 42.61 |       |

GPX  

| Diet | Storage (days) | 0  | 15  | 30  | Mean  |
|------|----------------|----|-----|-----|-------|
|      |                |    |     |     |       |
| D1   | 66.75          | 73.85 | 91.84 | 87.55 |       |
| D2   | 67.46          | 75.55 | 92.56 | 87.25 |       |
| D3   | 68.02          | 77.80 | 92.80 | 85.49 |       |
| D4   | 68.14          | 80.33 | 92.42 | 83.06 |       |
| D5   | 68.60          | 82.52 | 93.47 | 81.53 |       |
| D6   | 69.15          | 84.44 | 93.53 | 82.37 |       |
| Mean |                | 68.02 | 79.08 | 92.77 |       |

5SOD= Superoxide dismutase (U/mg protein); 6CAT= Catalase (U/mg protein); 7GPX= 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 |
|-------|-------|---------|---------|
| 14:0 n-0 |      |         |         |
| C3.20 | a     | b       | c       |
| C3.28 | a     | b       | c       |
| C3.24 | a     | b       | c       |
| C3.35 | a     | b       | c       |
| C3.33 | a     | b       | c       |
| 16:0 n-0 |      |         |         |
| B3.87 | a     | b       | c       |
| B3.83 | a     | b       | c       |
| B3.79 | a     | b       | c       |
| B3.75 | a     | b       | c       |
| B9.67 | a     | b       | c       |
| 18:0 n-0 |      |         |         |
| A5.95 | a     | b       | c       |
| A5.65 | a     | b       | c       |
| A5.63 | a     | b       | c       |
| A5.59 | a     | b       | c       |
| A5.62 | a     | b       | c       |
| 16:1 n-7 |      |         |         |
| A12.77| a     | b       | c       |
| A12.21| a     | b       | c       |
| A12.09| a     | b       | c       |
| A12.01| a     | b       | c       |
| A11.93| a     | b       | c       |
| 18:1 n-7 |      |         |         |
| A19.82| a     | b       | c       |
| A19.67| a     | b       | c       |
| A19.61| a     | b       | c       |
| A19.58| a     | b       | c       |
| A19.65| a     | b       | c       |
| 18:1 n-9 |      |         |         |
| A20.21| a     | b       | c       |
| A20.17| a     | b       | c       |
| A20.20| a     | b       | c       |
| A20.17| a     | b       | c       |
| A20.30| a     | b       | c       |
| 18:2 n-6 |      |         |         |
| B4.13 | a     | b       | c       |
| B4.16 | a     | b       | c       |
| B4.23 | a     | b       | c       |
| B4.22 | a     | b       | c       |
| B4.35 | a     | b       | c       |
| 20:4 n-6 |      |         |         |
| B3.12 | a     | b       | c       |
| B3.20 | a     | b       | c       |
| B3.21 | a     | b       | c       |
| B3.16 | a     | b       | c       |
| B3.15 | a     | b       | c       |
| 18:3 n-3 |      |         |         |
| A6.97 | a     | b       | c       |
| A7.34 | a     | b       | c       |
| A7.27 | a     | b       | c       |
| A7.25 | a     | b       | c       |
| A7.33 | a     | b       | c       |
| 20:5 n-3 |      |         |         |
| B17.74| a     | b       | c       |
| B17.76| a     | b       | c       |
| B17.77| a     | b       | c       |
| B17.77| a     | b       | c       |
| B17.66| a     | b       | c       |
| 22:5 n-3 |      |         |         |
| B17.90| a     | b       | c       |
| B17.79| a     | b       | c       |
| B17.84| a     | b       | c       |
| B17.87| a     | b       | c       |
| B17.78| a     | b       | c       |
| 22:6 n-3 |      |         |         |
| B7.00 | a     | b       | c       |
| B6.97 | a     | b       | c       |
| B6.99 | a     | b       | c       |
| B7.01 | a     | b       | c       |
| B6.97 | a     | b       | c       |
| 17.19 | a     | b       | c       |
| 46.51 | a     | b       | c       |
| 53.19 | a     | b       | c       |
| 53.07 | a     | b       | c       |
| 53.04 | a     | b       | c       |
| 53.01 | a     | b       | c       |
| n-3 FA |      |         |         |
| A14.51| a     | b       | c       |
| A15.33| a     | b       | c       |
| A15.34| a     | b       | c       |
| A15.39| a     | b       | c       |
| A15.37| a     | b       | c       |
| n-6 FA |      |         |         |
| A6.91 | a     | b       | c       |
| A7.13 | a     | b       | c       |
| A7.20 | a     | b       | c       |
| A7.32 | a     | b       | c       |
| A7.47 | a     | b       | c       |
| n-9 FA |      |         |         |
| A18.34| a     | b       | c       |
| A17.47| a     | b       | c       |
| A17.42| a     | b       | c       |
| A17.34| a     | b       | c       |
| A17.41| a     | b       | c       |
| ARA/EPA |      |         |         |
| A0.18 | a     | b       | c       |
| A0.18 | a     | b       | c       |
| A0.18 | a     | b       | c       |
| A0.18 | a     | b       | c       |
| A0.18 | a     | b       | c       |
| EPA/DHA |      |         |         |
| A1.32 | a     | b       | c       |
| A1.32 | a     | b       | c       |
| A1.32 | a     | b       | c       |
| A1.32 | a     | b       | c       |
| A1.32 | a     | b       | c       |
| n-3/n-6 |      |         |         |
| A3.76 | a     | b       | c       |
| A3.78 | a     | b       | c       |
| A3.81 | a     | b       | c       |
| A3.83 | a     | b       | c       |
| A3.83 | a     | b       | c       |

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.

Data are mean of three replicates.
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 (Scalf 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 kis 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 (Scalf et al., 2000). Similarly, TBARS values in Oncorhynchus kis 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 kis W. fillets (Yildiz et al., 2000) and Nawaz et al. (2020) determined the induced TABRS values in sea bass (Dicentrarchus labrax) and mori (Cirrhinus miragala) 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/polynenes 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 showed no effect against increasing storage time in all the treatments. The EPA/DHA ratio supplementation and diminished with increase in the amounts of these fatty acids were increased. The n-6 fatty acids, n-9, and the ratios of monoenes/polyenes were revealed that by enhancing the supplemental level 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.

CONCLUSION

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.

REFERENCES

AOAC (Association of Official Analytical Chemists), 1995. Official methods of analysis. 15th Ed., Association of Official Analytical Chemist, Washington, D.C. USA., pp. 1094.

Baker, R.T.M., 2001. The effect of certain micronutrients on fish flesh quality. In: Farmed fish quality (eds. S.C. Kestin and P.D. Warriss). Blackwell Science Ltd., Oxford, UK. pp. 180-191.

Bell, J.G., 1998. Current aspects of lipid nutrition in fish farming. In: Biology of farmed fish (eds. K.D. Black and A.D. Pickering). Sheffield, UK: Sheffield Academic Press. pp. 114-145.

Bonnel, A.D., 1989. Quality assurance in seafood processing. Chapman and Hall, New York.

Chance, B. and Maehly, A.C., 1955. Assay of catalase and peroxidases. Methods Enzymol., 2: 764-775. https://doi.org/10.1016/S0076-6879(55)02300-8

Chen, Y.C., Nguyen, J., Semmens, K., Beamer, S. and Jaczynski, J., 2008. Chemical changes in omega-3-enhanced farmed rainbow trout (Oncorhynchus mykiss) fillets during abusive-temperature storage. Fd. Chem., 19: 599-608. https://doi.org/10.1016/j.fdc.2007.06.011

Civello, P.M., Marting, G.A., Chaves, A.R. and Anan, M.C., 1995. Peroxidase from strawberry fruit by partial purification and determination of some properties. J. Agric. Fd. Chem., 43: 2596-2601. https://doi.org/10.1021/jf000058a008

Fatima, M., Afzal, M. and Shah, S.Z.H., 2019. Effect of dietary oxidized oil and vitamin E on growth performance, lipid peroxidation and fatty acid profile of Labeo rohita fingerlings. Aquac. Nutr., 25: 281-291. https://doi.org/10.1111/anu.12851
2009. Dietary α-tocopheryl acetate on fillet quality of tilapia. *Acta Sci. Anim. Sci.*, 31: 439-445. https://doi.org/10.4025/actascianimsci.v31i4.6929

Frigg, M., Prabucki, A.L. and Ruhdel, E.U., 1990. Effect of dietary vitamin E levels on oxidative stability of trout fillets. *Aquaculture.*, 84: 145-158. https://doi.org/10.1016/0044-8486(90)90344-M

Gatta, P.P., Pirini, M., Testi, S., Vignola, G. and Monetti, P.G., 2000. The influence of different levels of dietary vitamin E on sea bass *Dicentrarchus labrax* flesh quality. *Aquac. Nutr.*, 6: 47-52. https://doi.org/10.1046/j.1365-2095.2000.00127.x

Giannopolitis, C.N. and Ries, S.K., 1997. Superoxide dismutase I occurrence in higher plants. *Pl. Physiol.*, 59: 309-314. https://doi.org/10.1104/pp.59.2.309

Hosseini, S.V., Abedian-Kenari, A., Rezaei, M., Nazari, R.M., Feas, X. and Rabbani, M., 2010. Influence of the in vivo addition of alpha-tocopheryl acetate with three lipid sources on the lipid oxidation and fatty acid composition of Beluga sturgeon, *Hucho huso*, during frozen storage. *Fd. Chem.*, 118: 341-348. https://doi.org/10.1016/j.foodchem.2009.04.131

Huang, S.L., Weng Y.M. and Huang, C.H., 2004. Lipid peroxidation in sarcoplasmic reticulum and muscle of tilapia is inhibited by dietary vitamin E supplementation. *J. Fd. Biochem.*, 28: 101-111. https://doi.org/10.1111/j.1745-4514.2004.tb00058.x

International Union of Pure and Applied Chemistry, (IUPAC), 1987. *Standard methods for the analysis of oils, fats and derivatives*, 7th Rev. enlarged (eds. C. Paquot and A. Hautfenne) Blackwell Scientific, London.

Jasour, M.S., Rahimabadi, E.Z., Elhsani, A., Rahnama M. and Arshadi, A., 2011. Effects of refrigerated storage on fillet lipid quality of rainbow trout (*Oncorhynchus mykiss*) supplemented by α-Tocopheryl acetate through diet and direct addition after slaughtering. *J. Fd. Process. Technol.*, 2: 2-5. https://doi.org/10.4172/2157-7110.1000124

Jittinandana, S., Kenney, P.B., Slider, S.D., Kamireddy, N. and Hankins, J.S., 2006. High dietary vitamin E affects storage stability of frozen-refrigerated trout fillets. *J. Fd. Sci.*, 71: 91-96. https://doi.org/10.1111/j.1365-2621.2006.tb08888.x

Jittrepotch, N., Ushio, H. and Ohshima, T., 2006. Oxidative stabilities of triacylglycerol and phospholipids fractions of cooked Japanese sardine meat during low temperature storage. *Fd. Sci.*, 99: 360-367. https://doi.org/10.1016/j.foodchem.2005.08.002

Kamireddy, N., Jittinandana, S., Kenney, P.B., Slider, S.D., Kiser, R.A., Mazik, P.M. and Hankins, J.A., 2011. Effect of dietary vitamin E supplementation and refrigerated storage on quality of rainbow trout fillets. *J. Fd. Sci.*, 76: 233-241. https://doi.org/10.1111/j.1750-3841.2011.02121.x

Lee, B.L., New A.L. and Ong, C.N., 2003. Simultaneous determination of tocotrienols, tocopherol, retinol and major carotenoids in human plasma. *Clin. Chem.*, 49: 2056-2066. https://doi.org/10.1373/clinchem.2003.022681

Lygren, B., Hamre, K. and Waagbo, R., 2000. Effect of induced hyperoxia on the antioxidant status of Atlantic salmon *Salmo salar* L. fed three different levels of dietary vitamin E. *Aquac. Res.*, 31: 401-407. https://doi.org/10.1046/j.1365-2109.2000.00459.x

McDonald, S., Prenzler, P.D., Antolovich, M. and Robard, K., 2001. Phenolic content and antioxidant activity of olive extracts. *Fd. Chem.*, 73: 73-84. https://doi.org/10.1016/S0044-8486(02)00064-9

Mourente, G., Diaz-Salvago, E., Bell, J.B. and Tocher, D.R., 2002. Increased activities of hepatic antioxidant defense enzymes in juvenile gilthead sea bream (Sparus aurata L.) fed dietary oxidized oil: attenuation by dietary vitamin E. *Aquaculture*, 214: 343-361. https://doi.org/10.1016/S0044-8486(02)00064-9

Nawaz, T., Fatima, M., Shah, S.Z.H. and Afzal, M., 2020. Coating effect of rosemary extract combined with chitosan on storage quality of mori (*Cirrhinus mrigala*). *J. Fd. Process. Preserv.*, 44: e14833. https://doi.org/10.1111/jfpp.14833

Nogala-Kalucka, M., Korsczak, J., Dratwia, M., Lampart-Szcza, E., Siger, A. and Buchowsk, M., 2005. Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests. *Fd. Chem.*, 93: 227-235. https://doi.org/10.1016/j.foodchem.2004.09.021

Pascual, P., Pedrajas, J.R., Toribio, F., Lopez-Barea, J. and Peinado, J., 2003. Effect of food deprivation on oxidative stress biomarkers in fish (*Sparus aurata*). *Chem. Biol. Interact.*, 145: 191-199. https://doi.org/10.1016/S0009-2797(03)00002-4

Quinn, P.K., Coffman, D.J., Bates, T.S., Welton, E.J., Covert, D.S., Miller, T.L., Johnson, J.E., Maria, S., Russell, L., Arimoto, R. and Carrico, C.M., 2004. Aerosol optical properties measured on board the Ronald H. Brown during ACE-Asia as a function of aerosol chemical composition and source region. *J. Geophysic. Res.*, 109: 1-28. https://doi.org/10.1029/2004JD004732
Vitamin E Affects Quality of Refrigerated Rohu

Rahimabadi, Z.E., Jasour, M.S., Ehsani, A., Rahnama, M. and Arshadi, A., 2012. Dietary supplementation versus direct postmortem addition of α-tocopherol acetate on fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) fillets during refrigerated storage. *Int. Fd. Res. J.*, 19: 1145-1151.

Rowland, S.J. and Ingram, B.A., 1991. Diseases of Australian native fishes. *Fish. Bull.* 4. NSW Fisheries, Sydney, NSW, Australia.

Ruff, N., Fitz-Gerald, R.D., Cross T.F. and Kerry, J.B., 2003. The effect of dietary vitamin E and C level on market-size turbot (*Scophthalmus maximus*) fillet quality. *Aquac. Nutr.*, 9: 91-103. https://doi.org/10.1046/j.1365-2095.2003.00230.x

Sau, S.K., Paul, B.N., Mohanta, K.N. and Mohanty, S.N., 2004. Dietary vitamin E requirement, fish performance and carcass composition of rohu (*Labeo rohita*) fry. *Aquaculture*, 240: 359-368. https://doi.org/10.1016/j.aquaculture.2004.02.008

Scaife, J.R., Onibi, G.E., Murray, I., Fletcher, T.C. and Houlihan, D.F., 2000. Influence of α-tocopherol acetate on the short-and long-term storage properties of fillets from Atlantic salmon (*Salmo salar*) fed a high lipid diet. *Aquac. Nutr.*, 6: 65-71. https://doi.org/10.1046/j.1365-2095.2000.00128.x

Shiau, S.K. and Hzu, C.Y., 2002. Vitamin E sparing effect by dietary vitamin C in juvenile hybrid Tilapia, *Oreochromis niloticus* x *O. aureus*. *Aquaculture*, 210: 335-342. https://doi.org/10.1016/S0044-8486(01)00853-5

Sies, H., 1986. Strategies of antioxidant defense. *Eur. J. Biochem.*, 215: 213-219. https://doi.org/10.1111/j.1432-1033.1993.tb18025.x

Steel, R.G.D., Torrie, J.H. and Dickey, D.A., 1996. *Principles and procedures of statistics*, 3rd Ed. McGraw Hill International Book Co.Inc., New York, USA, pp. 336-352.

Wang, Y., Yuen, K.H. and Wing-Keong, N.G., 2006. Deposition of tocotrienols and tocopherols in the tissues of red hybrid tilapia, *Oreochromis* sp., fed a tocotrienol-rich fraction extracted from crude palm oil and its effect on lipid peroxidation. *Aquaculture*, 253: 583-591. https://doi.org/10.1016/j.aquaculture.2005.08.013

Yamamoto, Y., Fujisawa, A., Hara, A. and Dunlap, W.C., 2001. An unusual vitamin E constituent provides enhanced antioxidant protection in marine organisms adapted to cold-water environments. *Proc. natl. Acad. Sci.*, 98: 13144-13148. https://doi.org/10.1073/pnas.24102498

Zakipour, R.E., Jasour, M.S., Ehsani, A., Rahnama, M. and Arshadi, A., 2012. Dietary supplementation versus direct postmortem addition of α-tocopherol acetate on fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) fillets during refrigerated storage. *Int. Fd. Res. J.*, 19: 1145-1151.

Zhang, X.D., Wu, T. W., Cai L.S. and Zhu, Y.F., 2007. Effects of α-tocopherol acetate supplementation in preslaughter diet on antioxidant enzyme activities and fillet quality of commercial-size *Sparus macroscephalus*. *J. Zhejiang Univ. Sci.*, 8: 680-685. https://doi.org/10.1631/jzus.2007.B0680

Zhou, Q.C., Wang, L.G., Wang, H.L., Wang, T., Elmada, C.Z. and Xie, F.J., 2013. Dietary vitamin E could improve growth performance, lipid peroxidation and non-specific responses for juvenile cobia (*Rachycentron canadum*). *Aquacult. Nutr.*, 19: 421-429. https://doi.org/10.1111/j.1365-2095.2012.00977.x