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Research Article

Effect of Refrigerated Storage on Fillet Lipid Quality of Rainbow Trout (*Oncorhynchus mykiss* W.) Fed a Diet Containing Different Levels of DL α-Tocopherol Acetate

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Abstract: The effect of refrigerated storage at 1 ± 0.3 ºC on vitamin E level, fatty acid composition and lipid oxidation of fillets was studied in 3 groups of rainbow trout fed diets supplemented with increasing levels (100, 300 and 500 mg kg^{-1} diet, respectively) of α-tocopherol acetate as antioxidant. Fish (initial mean weight 131 ± 1.0 g) were fed experimental diets for 58 days and reached a mean final weight of 299 ± 6.1 g, with growth differences among the 3 experimental groups being insignificant ($P > 0.05$). Fillet samples of fish were analyzed fresh (on day 0) or after storage at 1 ± 0.3 ºC for 9 days. Lipid oxidation was measured using the thiobarbituric acid reactive substances (TBARS) test. Dietary vitamin E levels had a significant effect on fillet α-tocopherol levels and TBARS values of fish by the end of 58 days of feeding. At the end of the feeding trial the fillet α-tocopherol levels of fish fed E_{100}, E_{300} and E_{500} diets were 30.1 ± 0.2, 34.3 ± 0.1 and 40.1 ± 0.1 mg kg^{-1}, respectively, and the fillet α-tocopherol levels were gradually decreased during the 9 days of storage at 1 ± 0.3 ºC ($P < 0.05$). However, at the end of the feeding trial the fillet TBARS values of fish fed diets were 4.4 ± 0.1, 4.2 ± 0.0 and 2.2 ± 0.0 mg malondialdehyde (MDA) kg^{-1}, respectively, and the fillet TBARS values gradually increased during the 9 days of storage at 1 ± 0.3 ºC ($P < 0.05$). Dietary vitamin E levels did not influence the fatty acid composition of the fish fillets ($P > 0.05$). However, during the refrigerated storage period, total saturated fatty acid (SFA) concentration slowly increased and total polyunsaturated fatty acid (PUFA) concentration slowly decreased ($P < 0.05$). Finally, increasing the levels of α-tocopherol acetate in the diets from 130 to 580 mg kg^{-1} can slow down the level of lipid oxidation in fish fillets during refrigerated storage for 9 days at 1 ± 0.3 ºC.

Key Words: Rainbow trout, nutrition, α-tocopherol acetate, fillet, refrigerated storage, lipid oxidation.

Farklı Düzeylerde DL α-Tocopherol Acetate İçeren Diet ile Beslenen Gökkuşağı Alabalığı (*Oncorhynchus mykiss* W.)'nın Fileto Lipit Kalitesine Soğuk Depolamanın Etkisi

Özet: Antioksidant madde olarak artan düzeylerde (sırasıyla 100, 300 ve 500 mg kg^{-1} diyet) α-tocopherol acetate ilave edilen diyetlerle beslenen üç grup gökkuşağı alabalığının filetolarındaki E vitamini düzeyine, yağ asidi kompozisyonuna ve lipit oksidasyonuna 1 ± 0,3 ºC' de yapılan soğuk depolamanın etkisi incelendi. 58 gün süresince deney diyetleri ile beslenen balıklar (ortalama başlangıç ağırlığı, 131 ± 1,0 g) deney sonunda ortalama olarak 299 ± 6,1 g ağırlığa ulaştı ve deney grupları arasındaki istatistiki fark önemsiz bulundu ($P > 0,05$). Balıkların fileto örnekleri taze (0. gün) ve 9 gün süresince 1 ± 0,3 ºC'de yapılan depolamadan sonra analiz edildi. Filetolardaki yağlarda meydana gelen oksidasyonu ölçmek amacıyla tiyobarbutirik asit reaktif (TBARS) testi kullanılmıştır. 58 günlük yemlemenin sonunda diyetlerdeki E vitamini düzeylerinin balıkların filetosundaki α-tocopherol ve TBARS düzeylerini önemli derecede etkilediği görülmüştür. Deney sonunda E_{100}, E_{300} ve E_{500} diyetleri ile beslenen balıkların filetolarındaki α-tocopherol düzeyleri sırasıyla 30,1 ± 0,2, 34,3 ± 0,1 ve 40,1 ± 0,1 mg kg^{-1} olarak bulunmuş ve bu oranlar 9 gün süresince 1 ± 0,3 ºC'de yapılan depolamada giderek azalmıştır ($P < 0,05$). Buna karşın, yemleme deneylerinin sonunda balıkların filetosundaki TBARS değerleri sırasıyla 4,4 ± 0,1, 4,2 ± 0,0 ve 2,2 ± 0,0 mg malondialdehyde (MDA) kg^{-1} olarak bulunmuş ve 9 gün süresince 1 ± 0,3 ºC'de yapılan soğuk depolamada TBARS değerleri giderek azalmıştır ($P < 0,05$). Diyetteki E vitamini düzeyleri balık filetolarındaki yağ asidi kompozisyonunu etkilememiştir ($P > 0,05$). Ancak soğuk depolama sırasında toplam doymuş yağ asitlerinin (SFA) konsantrasyonu giderek artmış ve toplam çok doymamış yağ asitlerinden PUFA'ların konsantrasyonu giderek azalmıştır ($P < 0,05$). Sonuç olarak 130 mg kg^{-1}'dan 580 mg kg^{-1}'a doğru artan düzeylerde α-tocopherol acetate içeren diyetlerle beslenen balıkların filetoları 9 gün süresince 1 ± 0,3 ºC'de depolanmış ve diyetlerde artan α-tocopherol acetate miktarlarına karşın filetolardaki lipit oksidasyonunun azaldığı görülmüştür.

Anahtar Sözcükler: Gökkuşağı alabalığı, besleme, α-tocopherol acetate, fileto, soğuk depolama, lipit oksidasyonu.

Introduction

Fish naturally contain high levels of the highly unsaturated fatty acids (HUFA) eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) and these fatty acids were reported to have beneficial effects on human health (1). Nevertheless, the presence of a high proportion of polyunsaturated fatty acids (PUFA) in fish lipids makes them particularly susceptible to peroxidative damage. The most important signs of deterioration in fish flesh quality after death are related to peroxide increase with HUFA decrease as a result of lipid hydrolysis and formation of unpleasant smells. Numerous strategies are available to minimize the undesirable effects of lipid peroxidation. These include the use of refrigeration and the addition of antioxidants to diets (2,3).

Vitamin E is a lipid-soluble vitamin that comprises 4 tocopherols and 4 tocotrienols in nature. Among them, α-tocopherol has the highest vitamin E activity (4). Vitamin E (α-tocopherol) has been shown to protect muscle lipids against oxidation in meat from chicken thigh and breast, pork, mutton and beef (5). However, fish do not contain high concentrations of vitamin E (6). Increasing dietary levels α-tocopherol has likewise been shown to protect lipids in rainbow trout muscles against oxidation (5,7-9).

The effect of α-tocopherol acetate on tissue lipid quality has been demonstrated for rainbow trout (*Oncorhynchus mykiss* W.) (9-12), channel catfish (*Ictalurus punctatus* R.) (13), turbot (*Scophthalmus maximus* L.) (14-16), halibut (*Hippoglossus hippoglossus* L.) and gilthead sea bream (*Sparus aurata* L.) (14), catfish (*Clarias gariepinus* B.) (17,18), hybrid tilapia (*O. nilotucus* X *O. aureus*) (19) and rockfish (*Sebastes schlegeli* H.) (20). In addition, oxidative changes occurring under refrigeration in tissue lipids of fish fed diets containing vitamin E have been studied in rainbow trout (*Oncorhynchus mykiss* W.) (5), Atlantic salmon (*Salmo salar* L.) (2,8) and European sea bass (*Dicentrarchus labrax* L.) (3,21).

In this study, α-tocopherol concentration in tissues and oxidative changes in tissue lipids during refrigerated storage of rainbow trout fed a commercial diet containing different levels of vitamin E were studied.

Materials and Methods

Fish and experimental diets

The feeding trials were conducted at the Istanbul University, Faculty of Fisheries, Sapanca Inland Waters Research Center, Adapazarı, Turkey. The experimental diets were prepared by adding 3% fish oil (Sürsan Corporation, Samsun, Turkey) and 100 mg kg^{-1} (E_{100}), 300 mg kg^{-1} (E_{300}) or 500 mg kg^{-1} (E_{500}) DL-α-tocopherol acetate (Sigma Chemical Company Ltd., Poole, UK) to a commercial rainbow trout diet produced in Turkey (Çamlı Yem, 4 mm floating pellets). The commercial feed used contained 46.6 ± 0.4% protein, 13.9 ± 0.1% fat, 18.4 ± 0.5% carbohydrate, 2.2 ± 0.2% crude cellulose, 10.9 ± 0.2% ash and 7.9 ± 0.1% moisture. However, analysis revealed that the actual levels of vitamin E level in the experimental diets were 130, 370 and 580 mg kg^{-1}, respectively. Fatty acid composition of the experimental diets is shown in Table 1.

Rainbow trout juveniles with a mean initial weight of 131 ± 1.0 g (± SEM, n = 180) were randomly allocated to six 1200-l fiberglass tanks (30 fish per tank) and they were fed experimental diets twice a day for 58 days. Daily feed portion was adjusted to 2% of the body weight. The tanks received aerated well water with a flow rate of 5-7 l min^{-1}. Temperature, pH, and dissolved oxygen values of the tank water were measured daily and were 12.2 ± 0.1 °C, 6.9 ± 0.0 and 10.2 ± 0.3 mg l^{-1}, respectively.

Sample collection and storage conditions

At the end of the feeding trial, 12 fish collected from each experimental group were immediately killed in a tank containing fresh water and ice, and transferred to the laboratory in a refrigerator. In the laboratory, fish from each trial group were separately packed into a black nylon bag and stored at 1 ± 0.3 °C for 9 days. On days 0 (fresh), 3, 6 and 9, 3 fish from each trial group was filleted and vitamin E, fatty acid composition and TBARS analyzed.

Proximate analysis of feed sample

The methods of the Association of Official Analytical Chemists (22) were used to determine the moisture, crude protein, crude fat, crude cellulose, carbohydrate

and ash content in feed samples. Crude protein was calculated as N X 6.25.

Vitamin E analysis

Vitamin E (α-tocopherol) was determined by high performance liquid chromatography (HPLC) (Shimadzu SCL-10AVP, Kyoto, Japan) with a fluorescence detector, as described in Huo et al. (23). Samples were homogenized in 2 ml of methanol containing 1 mg ml^{-1} BHT (butil hidroxytolune), and tocol (Eisai, Tokyo, Japan) added as an internal standard, using a Potter Elvehjem tube. The samples were then centrifuged at 1500 rpm for 2 min and the supernatant transferred to a polypropylene tube. The solid residue was homogenized in 2 ml methanol/BHT and the extract combined with the first one, and with 1 ml methanol/BHT used to rinse the Potter tube. The combined extracts were centrifuged at 12000 rpm for 10 min and an aliquot of 100 µl was injected to the HPLC. Separation and quantification of α-tocopherol was performed using a 5 µm 250 x 4.6 mm HRC-SIL normal phase column (Shimadzu, Japan). The mobile phase used was HPLC grade hexane: propanol (98:2, v/v), pumped at a rate of 1 ml min^{-1}. Peak areas were integrated and α-tocopherol concentrations calculated from a standard curve derived by chromatographing pure DL- α-tocopherol (Sigma-Aldrich, MO, USA) under similar conditions. Values obtained were expressed as mg kg^{-1} in fish fillet and whole body lipid. Samples were analyzed in duplicate.

Thiobarbituric acid reactive substances (TBARS) test

For the analysis of TBARS in fish fillets, the distillation method was used (24). Frozen fish samples were dissolved at 4±0.5 ºC for 24 h. Then 10 g of fish sample was homogenized for each group and used for TBARS analysis. The color development was measured at 538 nm with a spectrophotometer (Schimadzu UV-160A, UV-visible recording spectrophotometer).

Fatty acid analysis

Crude lipid analysis was carried out by means of ether extraction, and total lipid was extracted after homogenization in chloroform/methanol (2/1 v/v) containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant basically according to the method described by Folch et al. (25). Fatty acid mixtures were prepared by esterification with 1% sulfuric acid in methanol (26) and the fatty acids of the feed and fish samples were analyzed by capillary gas chromatography Perkin Elmer Auto System XL capillary gas chromatography (column 30 x 0.25 mm, FID detector, CP-2330 supelco). Helium was used as the carrier gas. Individual fatty acid methyl esters were identified by reference to known standards (Sigma, 189-19).

Statistical analysis

All data were presented as means ± standard error. The difference between the growth performance and body composition of groups was analyzed with one-way analyses of variance (ANOVA) and Duncan's multiple range test using a statistical software package (SPSS version 10.0) for $P < 0.05$ at the end of the feeding trial. The relation between the fillet α-tocopherol levels and TBARS values or α-tocopherol levels in fillets and diets were also investigated using non-parametric correlation (Spearman rank correlations) analyses. Correlation coefficients were considered significant at $P < 0.01$ (27).

Results

There were no significant differences among body weight gains and specific growth rates and condition factor values of fish fed different levels of vitamin E ($P > 0.05$). The fish reached mean weights of 299 ± 6.1 g after 58 days of feeding (data not shown).

The lipid content and fatty acid composition of fillets revealed no significant differences due to the experimental diets, which were supplemented with different levels of vitamin E and equal levels of (3%) fish oil ($P > 0.05$) (Table 1). The experimental diets had average total lipid concentration of 13.9 ± 0.1%, average total SFA of 34.0 ± 0.9%, average total monounsaturated fatty acid (MUFA) of 27.9 ± 0.1%, average total linoleic polyunsaturated fatty acid (n-6, PUFA) of 13.2 ± 0.0% and average total linolenic polyunsaturated fatty acid (n-3, PUFA) of 20.6 ± 0.1% (Table 1).

α-Tocopherol and TBARS levels in fillets from the fish fed the experimental diets are shown in Table 2. Dietary vitamin E levels had a significant effect on α-tocopherol

Table 1. Total lipid (% dry weight) and fatty acid composition of experimental feeds (% total fatty acids)[1].

Fatty acids	%
14:0	5.9 ± 0.1
16:0	19.3 ± 0.0
18:0	4.7 ± 0.0
16:1n-7	5.4 ± 0.0
18:1n-9	19.4 ± 0.0
20:1n-9	2.4 ± 0.1
22:1n-9	0.1 ± 0.0
24:1n-9	0.3 ± 0.1
18:2n-6	12.2 ± 0.0
18:3n-6	0.1 ± 0.0
20:4n-6	0.9 ± 0.0
18:3n-3	2.0 ± 0.0
20:3n-3	0.1 ± 0.0
20:5n-3	7.4 ± 0.1
22:6n-3	11.0 ± 0.0
Total SFA	34.0 ± 0.1
Total MUFA	27.9 ± 0.1
Total (n-6) PUFA	13.2 ± 0.0
Total (n-3) PUFA	20.6 ± 0.1
Total PUFA	33.8 ± 0.1
Total unsaturated	61.7 ± 1.0
Total lipid	13.9 ± 0.1

[1]: Values are means ± SEM, n = 6
SFA: Saturated fatty acid,
MUFA: Monounsaturated fatty acid,
PUFA: Polyunsaturated fatty acid.

levels and TBARS values of fish fillets. The level of α-tocopherol in the fillet was found to increase significantly with increasing dietary vitamin E levels. At the end of the feeding trial the fillet α-tocopherol levels of fish fed diets E_{100}, E_{300} and E_{500} were 30.1 ± 0.2, 34.3 ± 0.1 and 40.1 ± 0.1 mg kg^{-1} fillet, respectively ($P < 0.05$). The fillet α-tocopherol levels gradually decreased during the 9 days of storage at 1 ± 0.3 ºC in all feeding groups ($P < 0.05$). There was a strong positive correlation ($r = 0.91$) between α-tocopherol levels in fillets and diets. At the end of the feeding trial the TBARS levels from fish fed the experimental diets (E_{100}, E_{300} and E_{500}) were 4.4 ± 0.1, 4.2 ± 0.0 and 2.2 ± 0.0 mg MDA kg^{-1} fillet, respectively ($P < 0.05$). The level of TBARS was significantly higher in fillets from fish fed the lowest level (E_{100}) of vitamin E compared to fish fed the highest level (E_{500}). Lipid oxidation measured as the amount of TBARS increased significantly during the 9 days of storage at 1 ± 0.03 ºC in all feeding groups ($P < 0.05$). During the refrigerated storage period a strong negative relationship was found in the correlation analysis between fillet α-tocopherol levels and TBARS values ($r = - 0.83$).

The fatty acid composition of the fish fillet is shown in Table 3. The fatty acid composition of fish fillet was not influenced by dietary vitamin E levels; for this reason, the data presented in Table 3 are the means of all experimental groups (E_{100}, E_{300} and E_{500}) ($P > 0.05$). The fatty acid composition of fish fillets reflected the fatty acid composition of the diets. The α-tocopherol levels of fish fillets had a significant effect on fillet fatty acid

Table 2. Fillet α-tocopherol (mg/kg fillet) and thiobarbituric acid reactive substances (TBARS) [mg malonaldehyde (MDA)/kg fillet] levels of rainbow trout fed the experimental diets for 58 days[1].

	Groups	Period of storage at 1 ± 0.3 ºC (days)			
		0	3	6	9
Vitamin E	E_{100}	30.1 ± 0.2^{d}	30.0 ± 0.1^{d}	26.6 ± 0.2^{f}	19.1 ± 0.1^{i}
	E_{300}	34.3 ± 0.1^{b}	30.1 ± 0.2^{d}	28.7 ± 0.2^{e}	20.1 ± 0.2^{h}
	E_{500}	40.1 ± 0.1^{a}	31.7 ± 0.1^{c}	31.6 ± 0.1^{c}	24.6 ± 0.3^{g}
TBARS	E_{100}	4.4 ± 0.1^{f}	9.4 ± 0.1^{d}	11.9 ± 0.9^{bc}	16.2 ± 0.1^{a}
	E_{300}	4.2 ± 0.0^{f}	9.3 ± 0.1^{d}	11.7 ± 0.1^{bc}	12.5 ± 0.1^{b}
	E_{500}	2.2 ± 0.0^{g}	8.1 ± 0.1^{e}	11.2 ± 0.3^{c}	11.9 ± 0.2^{bc}

[1]: Values are means ± SEM, n = 2.
Values in each row with different superscript letters are significantly different at $P < 0.05$. Means were tested by ANOVA and ranked by Duncan's multiple range test.

Table 3. Fatty acid composition of fillets of rainbow trout fed the experimental diets during storage (%).

	Period of storage at 1 ± 0.3 °C (days)			
Fatty acids	0	3	6	9
14:0	4.2 ± 0.0^{b}	4.4 ± 0.0^{a}	4.2 ± 0.1^{b}	4.2 ± 0.0^{ab}
16:0	19.2 ± 0.1^{a}	18.2 ± 0.0^{b}	19.3 ± 0.3^{a}	19.0 ± 0.3^{a}
18:0	4.8 ± 0.1^{a}	4.8 ± 0.1^{a}	4.9 ± 0.0^{a}	4.8 ± 0.1^{a}
16:1n-7	5.8 ± 0.1^{a}	5.5 ± 0.1^{a}	5.8 ± 0.1^{a}	5.7 ± 0.2^{a}
18:1n-9	24.5 ± 0.1^{a}	24.5 ± 0.4^{a}	24.8 ± 0.4^{a}	24.6 ± 0.3^{a}
20:1n-9	2.2 ± 0.0^{a}	2.1 ± 0.0^{a}	2.2 ± 0.0^{a}	2.2 ± 0.0^{a}
22:1n-9	0.1 ± 0.0^{a}	0.1 ± 0.1^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
24:1n-9	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}
18:2n-6	13.3 ± 0.1^{a}	13.2 ± 0.3^{a}	12.4 ± 0.2^{a}	12.4 ± 0.3^{a}
18:3n-6	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}
20:4n-6	0.8 ± 0.0^{a}	0.8 ± 0.0^{a}	0.8 ± 0.0^{a}	0.8 ± 0.0^{a}
18:3n-3	1.5 ± 0.0^{ab}	1.6 ± 0.0^{a}	1.5 ± 0.0^{b}	1.5 ± 0.0^{ab}
20:3n-3	0.4 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}
20:5n-3	3.7 ± 0.1^{a}	3.7 ± 0.1^{a}	3.4 ± 0.1^{a}	3.6 ± 0.2^{a}
22:6n-3	12.0 ± 0.1^{a}	11.9 ± 0.1^{ab}	11.6 ± 0.1^{ab}	11.5 ± 0.2^{b}
Total SFA	30.2 ± 0.3^{b}	30.0 ± 0.4^{b}	31.2 ± 0.2^{a}	31.1 ± 0.4^{a}
Total MUFA	33.1 ± 0.1^{a}	33.0 ± 0.5^{a}	33.1 ± 0.6^{a}	33.2 ± 0.4^{a}
Total (n-6) PUFA	14.3 ± 0.1^{a}	14.2 ± 0.3^{ab}	13.4 ± 0.2^{c}	13.3 ± 0.4^{bc}
Total (n-3) PUFA	17.8 ± 0.3^{a}	17.1 ± 0.3^{ab}	16.8 ± 0.1^{b}	17.4 ± 0.3^{ab}
Total PUFA	32.1 ± 0.2^{a}	31.3 ± 0.3^{ab}	30.2 ± 0.2^{b}	30.7 ± 0.4^{b}
Total unsaturated	65.1 ± 0.2^{a}	64.2 ± 0.3^{ab}	63.2 ± 0.4^{b}	63.9 ± 0.4^{ab}

1:Values are means ± SEM, n=6.

Values in each row with different superscripts different at $P < 0.05$. Means were tested by ANOVA and ranked by Duncan's multiple range test.

composition during the 9 days of storage at 1 ± 0.3 °C. Fish fillets had higher concentrations of total SFA at 6 and 9 days compared to at days 0 (fresh) and 3 ($P < 0.05$). The MUFA levels of the fillet were similar during the 9 days of storage at 1 ± 0.03 °C. Total n-6 and n-3 PUFA levels of the fillet were the highest on day 0 and the lowest on day 6 ($P < 0.05$). Especially the DHA (22:6n-3) levels decreased from day 0 to day 6 ($P < 0.05$). Furthermore, the total PUFA and unsaturated (PUFA + MUFA) levels decreased from day 0 to day 6 ($P < 0.05$).

Discussion

It was reported that the minimal vitamin E requirement of rainbow trout varied between 27.5 and 50 mg kg^{-1} diet levels (7). In this study, diets containing high levels of α-tocopherol acetate (130, 370 and 580 mg kg^{-1} diet, respectively) were used to see to what extent refrigerated storage (1 ± 0.03 °C) conditions protect fish quality against oxidation.

α-Tocopherol acetate supplementation had no effect on the growth performance of rainbow trout, which is in agreement with the findings of previous studies on rainbow trout (9), sea bass (21), turbot (15,16), hybrid tilapia (19) and channel catfish (13). Tocher et al. (14) reported that growth as determined by body weights and SGR was lower in fish fed diet 0 mg kg^{-1} vitamin E compared with diet 1000 mg kg^{-1} vitamin E. In contrast, there were no significant differences in growth performance among the dietary groups with turbot or halibut.

In the present study, the relationship between α-tocopherol level in fillet and dietary vitamin E level was

linear, which is consistent with the results of previous studies on trout and rainbow trout (5,9-12). Frigg et al. (11) fed trout diets supplemented with 0, 50, 100 and 200 mg kg^{-1} of vitamin E, and at the end of the trial feeding a level of 19.7 µg g-1 of α-tocopherol was found in fillet from fish fed the diet with highest vitamin concentration. Chaiyapechara et al. (9) reported that the vitamin E levels in the fillet of rainbow trout fed a diet containing 300 and 1500 mg kg^{-1} vitamin E were 13.9 and 49.1 mg kg^{-1} fillet, respectively. In addition, previous studies on Atlantic salmon (8), channel catfish (13) and African catfish (17,18) have shown that the concentration of α-tocopherol in muscle is proportional to feed α-tocopherol concentration. Similarly, in the feeding trial using sea bass, Gatta et al. (21) and Pirini et al. (3) fed 139, 254, 493 and 942 mg kg^{-1} α-tocopherol acetate in a diet for 12 weeks, which resulted in a muscle vitamin E content of 9.2, 12.9, 17.8 and 31.4 µg g^{-1}, respectively. The fillet vitamin E levels results of our study were significantly higher than the findings reported by Frigg et al. (11), Gatta et al. (21) and Pirini et al. (3).

In this study, the fillet α-tocopherol levels gradually decreased and the TBARS values increased during storage (days 0, 3, 6 and 9) at 1 ± 0.3 ºC. The reduction in α-tocopherol concentration of fillets during refrigerated storage may be related to loss of vitamin E caused by lipid peroxidation. Increasing α-tocopherol concentration of the fillets significantly lowered the TBARS values during refrigerated storage. This is shown by the significant effect of vitamin E on the oxidative state of the fillets. A similar effect of dietary vitamin E concentration in reducing fillet lipid oxidation was observed in rainbow trout (5,8,9,11), sea bass (21), turbot, halibut and sea bream (14), and hybrid tilapia (19). Similarly, Scaife et al. (2) reported that during long-term frozen storage, quality characteristics of salmon fillets (α-tocopherol concentration and oxidative stability) may decline, and improved oxidative stability during frozen storage were caused by increased muscle concentrations of α-tocopherol through dietary supplementation of α-tocopherol acetate (2). Since vitamin E prevents the development of rancidity, vitamin E levels and TBARS values could be used as indices in fish quality evaluation (11). High levels of fillet α-tocopherol may have the potential to improve fish flesh.

In this trial, there was no effect of dietary α-tocopherol acetate on fatty acid profiles of the fillet samples. These results confirmed previous findings (10-12,28) that different α-tocopherol acetate levels in the diet did not affect the fatty acid composition of salmonids. Watanabe et al. (29) and Bai and Lee (20) suggested that a tocopherol deficiency exerts some effects on fatty acid composition, but the tocopherol supplements exceeding a level required by fish had little effect on fatty acid composition. However, Watanabe and Takashima (30) reported that in most of the tissues of α-tocopherol deficient carp, the percentage of monoenes increased, whereas the percentage of polyenes decreased. They found that the most significant change observed was the marked decrease in 18:2n-6 level in all tissues. In our study, similar results were not observed.

In the present study, the fatty acid composition of the fish fillets generally reflected the fatty acid composition of trial diets. This finding supports those previously reported by Steffens (1). In the trial, the fatty acid composition of the fillets was described to undergo slow changes during the refrigerated storage period. During refrigerated storage total SFA levels increased and total n-6 and n-3 PUFA levels decreased in fish fillets. Similarly, Monetti et al. (31) indicated that the TBARS values found in the same specimens had already shown very slow lipid changes, which were practically negligible. Frigg et al. (11) indicated that when α-tocopherol concentration in rainbow trout fillet increased to approximately 20 µg g^{-1} fillet oxidation in fish tissue was effectively prevented.

The results of the present study showed that the dietary vitamin E levels did not affect the growth performance of rainbow trout (body weight gain, specific growth rate and condition factor). There were no significant effects of increasing dietary vitamin E concentration on the fatty acid composition of fish fillets. However, the increase in dietary level of α-tocopherol acetate resulted in increased concentrations in the fillets. Increasing the levels of α-tocopherol acetate in the diets from 130 to 580 mg kg^{-1} can slow down the level of lipid oxidation in fish fillets during refrigerated storage for 9 days at 1 ± 0.3 ºC.

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