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The Study of Fillet Quality and the Growth Performance of Rainbow Trout (*Oncorhynchus mykiss*) Fed with Diets Containing Different Amounts of Vitamin E

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Abstract

This study investigated the effects of adding different amounts of α-tocopherol acetate (ATA, 100, 300 and 500 mg kg^{-1} diet), to a commercial feed on the growth performance, body composition and vitamin E levels in the fillet of rainbow trout (initial weight = 131.3 ±1.0 g) over a period of 58 days. In the analyses of trial diets, average rates of 46.6 ±0.4% crude protein, 13.9±0.1% lipid, and 130.2 (Diet 1), 370.5 (Diet 2) and 580.9 (Diet 3) mg kg^{-1} diet of vitamin E were found, respectively. The results of the 58-day feeding study were that the specific growth rate and condition factors of the fish were not affected by diets containing different levels of ATA ($P>0.05$). Fish that were fed 370.5 and 580.9 mg kg^{-1} ATA diets had significantly lower feed conversion ratios than fish that were fed a 130.2 mg kg^{-1} ATA diet ($P<0.05$). The diets containing different levels of ATA did not affect the fillet's proximate composition, viscerosomatic index, or hepatosomatic index, except for the fillet and liver lipid content. The fillet and liver lipid content and hepatosomatic index of the fish increased with growth. The vitamin E levels in fish fillet reflected dietary ATA levels. The increase in dietary ATA affected ATA accumulation in the fish fillets and whole-body lipids. The high level of ATA in the fish flesh indicated good flesh quality. These results showed that fish on Diets 2 and 3 had significantly better FCR than those on Diet 1, and fish on Diet 3 had significantly higher flesh quality than the others.

Key Words: Rainbow trout, nutrition, α-tocopherol acetate, growth performance, flesh quality.

Introduction

Rainbow trout is widely produced around the world because of its delicious taste. Rainbow trout is considered an important component of human nutrition because of its high polyunsaturated fatty acid content, especially in the n-3 family. In 2000, the total production in the world reached about 500,000 Mt (Anon., 2001). It is desirable for rainbow trout feed to be high in lipid content, with the aim of improving growth performance and the feed conversion ratio (FCR). However, high levels of dietary lipid leads to increased lipid content in the entire body of fish (Nickell and Bromage, 1998; Lie, 2001; Chaiyapechara *et al.*, 2003). The viscerosomatic index (VSI) increases with increasing dietary lipid. Therefore, viscera resulting from digestible feed calories are usually discarded (Jobling *et al.*, 1998; Rasmussen *et al.*, 2000; Chaiyapechara *et al.*, 2003). The dietary lipids for fish culture are high in polyunsaturated fatty acid content, and the polyunsaturated fatty acids are readily incorporated into fillets of fish. Increased lipid content in fish negatively affects the fish quality because of degradation of the fish fillet through lipid oxidation. Therefore, diets should be fortified with appropriate amounts of antioxidants (Jensen *et al.*, 1998; Chaiyapechara *et al.*, 2003).

Vitamin E is a lipid-soluble vitamin and a natural antioxidant. Alpha-tocopherol acetate (ATA) is used as a vitamin E source in fish feed in aquaculture, with the aim of improving fish growth (Hamre *et al.*, 1998; Kaushik *et al.*, 1998; Huo *et al.*, 1999).

The effects of including varying amounts of vitamin E on the quality of fish fillet has been studied for several species such as rainbow trout (Boggio *et al.*, 1985; Frigg *et al.*, 1990; Jensen *et al.*, 1998; Akhtar *et al.*, 1999; Chaiyapechara *et al.*, 2003), channel catfish (Bai and Gatlin, 1993), sea bass (Gatta *et al.*, 2000; Pirini *et al.*, 2000), Atlantic salmon (Hamre *et al.*, 1998; Scaife *et al.*, 2000), turbot (Ruff *et al.*, 2002; Ruff *et al.*, 2003), catfish (Lim *et al.*, 2001; Ng *et al.*, 2003), and hybrid tilapia (Huang *et al.*, 2003). In Turkey, the levels of vitamin E found in the fillet of rainbow trout that were fed a commercial feed have been reported by Köprücü and Özdemir (2002).

This study was carried out to examine the effects of commercial feed containing 3% fish oil and different levels of ATA on the growth performance, body composition and the fillet vitamin E content in rainbow trout.

Materials and Methods

Fish and Experimental Diets

The study was conducted at İstanbul University's Faculty of Fisheries, Sapanca Inland

Waters Research Center in Adapazarı, Turkey. The experimental diets were prepared by adding 3% fish oil (Sürsan Corporation, Samsun, Turkey) and 100 mg kg^{-1} (Diet 1), 300 mg kg^{-1} (Diet 2) or 500 mg kg^{-1} (Diet 3) of DL-α-tocopheryl acetate (Sigma Chemical Company Ltd., Poole, UK) to a commercial rainbow trout diet produced in Turkey (4 mm floating pellets). The actual levels of vitamin E in the experimental diets were 130.2, 370.5 and 580.9 mg kg^{-1}, respectively, as shown in Table 1.

Rainbow trout juveniles with a mean initial weight of 131.3±1.0 g (±SEM, n = 180) were randomly allocated to six 1.2 m^3 fiberglass tanks (30 fish per tank) and fed the experimental diets twice daily for 58 days. The daily feeding rations were adjusted to 2% of 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, respectively.

Growth Performance

In each tank, all fish were individually weighed once every two weeks, and daily rations were determined after each weighing. The feed conversion ratio was calculated using the following equation: FCR = diet given (dry weight) (g) / live weight gain (g). The specific growth rate (SGR) was calculated using the following equation: SGR = [(ln final weight–ln initial weight) x 100 / time in days]. The condition factor (CF) was calculated by CF = [total body weight (g)/ total body length (cm)³ x 100]; the hepatosomatic index by HSI, % = [liver weight (g)/total body weight (g) x 100] and the viscerosomatic index by VSI, % = [viscera weight (g)/body weight (g) x 100]. Values for lipid accumulation in the whole body and liver of the fish (Ricker, 1979) were also calculated.

Table 1. Chemical composition of the experimental diets

Chemical composition[1]	(Mean± SEM)
Dry matter (g / 100g)	92.1± 0.1
Crude protein (g / 100g)	46.6± 0.4
Crude lipid (g / 100g)	13.9± 0.1
Ash (g / 100g)	10.9± 0.2
Crude cellulose (g / 100g)	2.2± 0.2
Carbohydrate (g / 100g)	18.4± 0.5
Gros energy (KJ / g)	19.6± 0.3
Alpha tocopherol acetate (mg kg^{-1})	
Diet 1	130.2
Diet 2	370.5
Diet 3	580.9

[1]:Values are means± SEM, n=6

Sampling Procedure and Storage Conditions

At the end of the feeding trials, five fish from each group were killed, packaged in black nylon bags, and then taken to the laboratory in a freezer. These samples were stored in a freezer until analysis for vitamin E and proximate fillet composition.

Proximate Analysis

Five fish, at the beginning and the end of the feeding trials, were killed and submitted for analysis of fillet composition. The fillet and the liver were separately blended prior to proximate analysis. Moisture, crude protein, lipid, crude fiber and ash contents of the experimental diets and fish fillet were determined by standard methods (AOAC, 1998). Gross energy value was calculated by the method of Halver (1972).

Vitamin E Analysis

Vitamin E (α-tocopherol) was examined using a high performance liquid chromatograph (HPLC) (Shimadzu SCL-10AVP, Kyoto, Japan) with a fluorescence detector, as described in Huo *et al.* (1996). Samples were homogenized in 2 mL of methanol containing 1 mg mL^{-1} BHT (butyl hydroxytoluene), and tocopherol (Eisai, Tokyo, Japan) added as an internal standard, using a Potter Elvehjem tube. The samples were then centrifuged for 2 min at 1500 x g and the supernatant was then transferred to a polypropylene tube. The solid residue was homogenized in 2 mL methanol/BHT, with the extract to be combined with the first extract, and then 1 mL methanol/BHT was used to rinse the potter tube. The combined extracts were centrifuged for 10 min at 12 000 x g, and an aliquot of 100 µL was injected into 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 through at a rate of 1 mL min^{-1}. Peak areas were integrated and α-tocopherol concentrations were calculated from a standard curve derived by chromatographing pure DL-α-tocopherol (Sigma-Aldrich, MO, USA) under similar conditions. Values obtained were expressed in mg kg^{-1} in fish fillet and whole body lipid. Samples were analysed in duplicate.

Statistical Analysis

The difference between the growth performance and body composition among groups were analyzed with one-way analyses of variance (ANOVA) and Duncan's multiple range test with a statistical package program (SSPS version 10.0) for $P<0.05$ at the end of feeding trials.

Results

Chemical composition of the diets is shown in Table 1. Values were found to be similar across the board, except for the vitamin E levels.

The final body weight (FBW), body weight gain (BWG) and specific growth rate (SGR) of rainbow trout fed diets supplemented with 100 mg kg^{-1}, 300 mg kg^{-1} and 500 mg kg^{-1} ATA did not differ significantly from one another (P>0.05, Table 2 and Figure 1). The CF was not affected by dietary ATA supplementation (P>0.05). However, the CF was significantly higher (P<0.05) compared to initial values (Table 2).

The feed consumption was not significantly different among all experimental groups (P>0.05). Fish on Diet 1 had significantly higher FCR than those on Diets 2 and 3 (P<0.05) (Table 2).

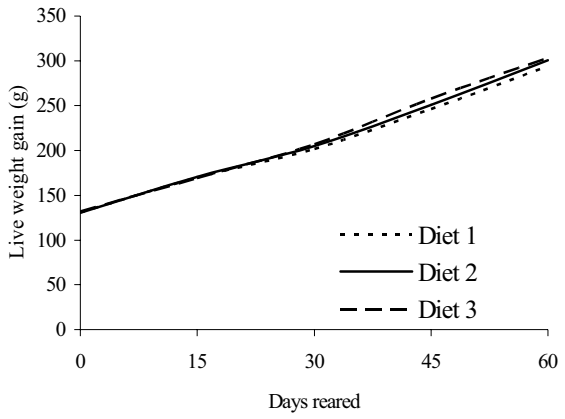

Figure 1. Live weight gain of fish fed with experimental diets.

The moisture, crude protein and crude ash content of fish fillets were not affected significantly (P>0.05) by changes in dietary vitamin E level (Table 3). The fillet moisture decreased and fillet crude ash increased in comparison to initial values (P<0.05). The fillet lipid level of experimental fish on Diet 1 was lower (P<0.05) than for those on Diets 2 and 3 (Table 3). Fillet lipid increased from an initial value of 1.7±0.0% to 7.6±0.0%, 8.5±0.1%, and 8.8±0.0%, respectively (P<0.05). The highest amount of liver lipid was found in the fish on Diet 1 (4.5±0.0%) and the lowest amount was found in the fish on Diet 2 (3.2± 0.1%). The liver lipid of all experimental groups increased with growth (P<0.05).

There was no significant effect of dietary vitamin E concentration on VSI or HSI (P>0.05). However, the final HSI values were significantly higher than the initial values (P<0.05).

Vitamin E in the fish fillet and whole-body lipid after the 58 days of feeding is shown in Table 3. Vitamin E level increased with increasing dietary ATA levels. The fillet vitamin E content of groups on Diets 1, 2, and 3 were 30.1±0.2, 34.3±0.1, and 40.1±0.1 mg kg^{-1} diet, respectively (P<0.05). The fillet vitamin E levels increased (P<0.05) with growth (Table 3). There was a strong positive correlation between dietary ATA and vitamin E in the fillet (r = 0.91).

Discussion

The present study showed that fillet vitamin E and lipid content of rainbow trout was significantly enhanced when different amounts (100, 300 and 500 mg kg^{-1} diet) of ATA and 3% fish oil were added to the commercial feed. It has previously been reported that the rainbow trout needs 50 mg vitamin E in kg diet (NRC, 1993). Fish on all diets showed high growth performance (Lindhorst-Emme, 1990).

Table 2. Growth performance and nutrient utilization of rainbow trout fed with the experimental diets.

Measurements	Groups		
	Diet 1	Diet 2	Diet 3
Initial body weight (g)	131.1 ± 0.9[a]	130.8 ± 1.0[a]	132.0 ± 1.0[a]
Final body weight (g)	293.8 ± 6.1[a]	300.5 ± 6.4[a]	303.5 ± 5.7[a]
Body weight gain (g)	162.6 ± 5.2[a]	169.7 ± 5.5[a]	171.5 ± 4.8[a]
Specific growth rate	1.4 ± 0.0[a]	1.4 ± 0.0[a]	1.4 ± 0.0[a]
Initial fish length (cm)	22.8 ± 0.1[a]	22.5 ± 0.1[a]	22.7 ± 0.1[a]
Final fish total length (cm)	27.4 ± 0.2[a]	27.7 ± 0.2[a]	27.6 ± 0.2[a]
Initial condition factor	1.1 ± 0.0[a]	1.2 ± 0.0[a]	1.1 ± 0.0[a]
Final condition factor	1.4 ± 0.0[a]	1.4 ± 0.0[a]	1.4 ± 0.0[a]
Total diet given (g/fish)	193.9 ± 3.3[a]	195.3 ± 3.0[a]	197.0 ± 3.3[a]
Feed conversion ratio	1.2 ± 0.0[a]	1.2 ± 0.0[b]	1.2 ± 0.0[b]

[1]:Values are means ± SEM, n=60

Values in each row with different superscript differ at P<0.05. Mean were tested by ANOVA and ranked by Duncan's multiple range test

Table 3. Body composition, liver fat, viscerosomatic and hepatosomatic index values and vitamin E concentration of rainbow trout fed with the experimental diets

		Groups		
	Initial	Diet 1	Diet 2	Diet 3
Proximate composition (%)[1]				
Fillet				
Moisture	77.1 ± 0.1[a]	72.4 ± 0.0[b]	71.2 ± 0.0[b]	71.5 ± 0.0[b]
Crude protein	18.3 ± 0.0[a]	18.1 ± 0.0[a]	18.7 ± 0.0[a]	18.5 ± 0.0[a]
Crude lipid	1.7 ± 0.0[c]	7.6 ± 0.0[b]	8.5 ± 0.1[a]	8.8 ± 0.0[a]
Ash	1.2 ± 0.0[b]	1.5 ± 0.0[a]	1.4 ± 0.0[a]	1.5 ± 0.0[a]
Liver lipid	1.1 ± 0.1[d]	4.5 ± 0.0[a]	3.2 ± 0.1[c]	3.9 ± 0.1[b]
Viscerosomatic index (VSI) and hepatosomatic index (HSI)[2]				
VSI (%)	16.6 ± 1.8[a]	17.9 ± 2.1[a]	16.8 ± 0.8[a]	16.2 ± 1.2[a]
HSI (%)	1.3 ± 0.9[b]	1.7 ± 0.2[a]	1.9 ± 0.3[a]	1.8 ± 0.3[a]
Vitamin E concentration[3]				
mg/kg fillet	10.1 ± 0.1[d]	30.1 ± 0.2[c]	34.3 ± 0.1[b]	40.1 ± 0.1[a]
mg/kg lipid	-	367.8 ± 1.9[b]	370.7 ± 1.3[b]	418.0 ± 1.3[a]

[1]:Values are means ± SEM, n=2
[2]:Values are means ± SEM, n=3
[3]:Values are means ± SEM, n=2
Values in each row with different superscript differ at P<0.05. Mean were tested by ANOVA and ranked by Duncan's multiple range test.

The final weight, BWG, SGR and CF were not affected by increasing ATA level. Similarly, the trial diets did not affect the feed consumption, but supplementation of the diet with more than 370.5 mg of ATA per kilogram of diet improved FCR. In previous studies, the growth performance of rainbow trout (Jensen *et al.*, 1998; Chaiyapechara *et al.*, 2003), sea bass (Gatta *et al.*, 2000), turbot (Ruff *et al.*, 2002; Ruff *et al.*, 2003), hybrid tilapia (Huang *et al.*, 2003), and channel catfish (Bai and Gatlin, 1993) was not affected when the fish were fed diets containing different amounts of vitamin E (20–1500 mg kg^{-1} diet). Similarly, Lygren *et al.* (2000) found no differences in the SGR of Atlantic salmon fed three different levels of dietary vitamin E. On the other hand, juvenile Korean rockfish fed a diet without vitamin E showed a significantly lower weight gain and FCR than those fed diets containing 20–120 mg vitamin E per kilogram of diet (Bai and Lee, 1998). Chaiyapechara *et al.* (2003) reported that the FCR of rainbow trout (average final weight 433 g) fed a diet containing 1500 mg ATA in kg diet was better than for those on a diet containing 300 mg ATA. In the present study, the finding that diets containing higher than 370.5 mg ATA in kg diet showed better FCR agreed with the results of Chaiyapechara *et al.* (2003).

During the last few years, the importance of understanding the effects of high dietary vitamin E on fillet composition and flesh quality has been demonstrated (Pirini *et al.*, 2000; Scaife *et al.*, 2000; Chaiyapechara *et al.*, 2003). For example, supplementation of diets with vitamin E has been shown to have positive effects on seafood quality (Gatta *et al.*, 2000). It is well established that tissue ATA has a protective role against lipid peroxidation (Scaife *et al.*, 2000). Baker (1997) states that α-tocopherol is the most important factor in maintaining the post mortem membrane stability of fish fillet. Similarly, Ruff *et al.* (2003) report that increasing vitamin E levels in the fish fillet led to higher quality in the fish fillet. In this study, fish on Diet 3 had a good flesh quality because the amount of vitamin E in their diet was significantly higher than in the others.

In the current study, the diets containing various amounts of ATA did not affect the proximate composition of fillet, their HSI, or VSI. Lygren *et al.* (2000) report that adding different levels of vitamin E to the diets of Atlantic salmon did not affect their HSI. In the present study, the lowest level of dietary ATA (130.2 mg) significantly increased liver lipid. The HSI, fillet lipid level, and liver lipid level increased with growth. Weatherup and McCracken (1999) states that lipid level in fish increases with growth. Generally, lipid accumulation in fish increases with higher levels of dietary lipid (Nickell and Bromage, 1998; Jobling *et al.*, 1998; Rasmussen *et al.*, 2000; Scaife *et al.*, 2000; Lie, 2001; Chaiyapechara *et al.*, 2003). As reported for several fish species such as rainbow trout (Chaiyapechara *et al.*, 2003), sea bass (Gatta *et al.*, 2000; Pirini *et al.*, 2000) and Atlantic salmon (Hamre *et al.*, 1998), the present study results did not show dietary ATA to have influenced the proximate composition of rainbow trout.

The fillet vitamin E content of the fish reflected their dietary ATA levels in the present study. This positive correlation between dietary and fillet vitamin E content is also reported for rainbow trout and other fish species (Boggio *et al.*, 1985; Frigg *et al.*, 1990; Jensen *et al.*, 1998; Akhtar *et al.*, 1999; Chaiyapechara *et al.*, 2003; Gatta *et al.*, 2000; Pirini *et al.*, 2000; Hamre *et al.*, 1998; Bai and Gatlin, 1993;

Lim *et al.*, 2001; Ng *et al.*, 2003). Köprücü and Özdemir (2002) state that the level of vitamin E was 8.7 μg g^{-1} diet in the fillet of rainbow trout fed with a Turkish commercial feed. Chaiyapechara *et al.* (2003) report that the fillet vitamin E levels of rainbow trout on diets containing 15% lipid and 300 and 1500 mg per kg of diet were 13.9 and 49.1 mg kg^{-1} fillet, respectively; the corresponding vitamin E levels were 173.6 and 604.7 mg kg^{-1} lipid. The present findings more or less corresponded to the results.

In conclusion, the present study demonstrated that diets containing different ATA did not affect the growth performance but led to increased vitamin E content in the fillets. The high vitamin E content in the fish flesh may be an indicator of the good flesh quality. These results suggest that feeding the fish diets containing more than 370.5 mg ATA per kilogram of diet results in higher flesh quality.

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