Original Communication

Dietary Alpha-Tocopherol Affects Tissue Vitamin E and Malondialdehyde Levels but Does not Change Antioxidant Enzymes and Fatty Acid Composition in Farmed Atlantic Salmon (*Salmo salar* L.)

Mohammad Faizan[1], Ingunn Stubhaug[2], David Menoyo[3], Tuba Esatbeyoglu[1], Anika Eva Wagner[1], Gunvor Struksnæs[2], Wolfgang Koppe[2], and Gerald Rimbach[1]

[1]Institute of Human Nutrition and Food Science, University of Kiel, Germany
[2]Skretting Aquaculture Research Center, ARC, Stavanger, Norway
[3]Departamento de Producción Animal, Universidad Politécnica de Madrid, Spain

Received: September 13, 2013; Accepted: February 20, 2014

Abstract: In this study the effect of increasing dietary alpha tocopherol on vitamin E tissue concentrations, lipid peroxidation (malondialdehyde), antioxidant enzymes, and fatty acid composition has been investigated in farmed Atlantic salmon. To this end fish (initial body weight ~ 193 g, n=70 per group) were fed diets based on fish oil (27.5 %), fish meal (15.0 %), wheat gluten (20.6 %), and soy protein concentrate (24.0 %) for 14 weeks. Diets were supplemented with 0 (negative control), 150, and 400 mg/kg vitamin E as all-rac alpha-tocopheryl acetate. Dietary vitamin E did not affect feed conversion efficiency ratio but significantly ($p<0.05$) increased alpha-tocopherol concentrations in salmon plasma, liver, and fillet (n=8 per group each). The increase in fillet alpha-tocopherol was accompanied by a considerable decrease ($p<0.01$) in malondialdehyde concentrations at the higher supplementation level. Furthermore, we observed an antagonistic interaction between alpha- and gamma-tocopherol in plasma at the highest supplementation level, since high dietary alpha-tocopherol reduced plasma gamma-tocopherol concentrations. Liver antioxidant enzymes, including glutathione peroxidase and superoxide dismutase, remained largely unchanged in response to dietary alpha-tocopherol. Dietary alpha-tocopherol did not affect eicosapentaenoic acid

 DOI 10.1024/0300–9831/a000166

and docosahexaenoic acid concentrations in salmon fillet. Present data suggest that alpha-tocopherol supplementations beyond dietary recommendations may further improve flesh quality and nutritional value of Atlantic salmon fillet as far as malondialdehyde and vitamin E concentrations are concerned.

Key words: Atlantic salmon, alpha-tocopherol, vitamin E, lipid peroxidation, malondialdehyde, antioxidant enzymes, fatty acids, eicosapentaenoic acid, docosahexaenoic acid

Introduction

Vitamin E comprises four tocopherols and four tocotrienols [1]. Among tocopherol isomers, alpha-tocopherol is supposed to be the most potent lipid-soluble, chain-breaking antioxidant in salmon [2, 3]. Alpha-tocopherol is structurally linked to membranes and embedded within the phospholipid-rich membrane bilayer, where it protects unsaturated fatty acids from peroxidation [2, 3]. The antioxidant properties of vitamin E are exerted through its phenolic hydroxyl group, which donates hydrogen to peroxyl radicals, resulting in the formation of stable lipid species [4]. Interestingly salmon contains not only tocopherols but also tocomonoenols [5], which exhibit an antioxidant activity similar to that of alpha-tocopherol [6]. vitamin E is part of a so-called antioxidant network comprising an interlinking set of redox antioxidants (e. g., ascorbic acid, glutathione) as well as antioxidant enzymes such as glutathione peroxidase (GPx) and superoxide dismutase (SOD), exhibiting synergistic activity [7]. However, it has also been shown in laboratory rodents that very high concentrations of alpha-tocopherol may impair the activity of antioxidant enzymes [8]. Besides antioxidant also gene-regulatory activities of alpha-tocopherol have been reported [9, 10].

Salmon is an oily fish and an important source of long-chain polyunsaturated fatty acids (LCPUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are prone to lipid peroxidation [11]. A comprehensive survey regarding the EPA and DHA content of salmon and other fish and fish products has been published by Strobel and co-workers [12].

LCPUFA derived from oily fish, including salmon, exhibit health benefits in humans. Both EPA and DHA mediate anti-inflammatory and anti-atherogenic activity thereby possibly decreasing cardiovascular disease risk [13, 14]. Therefore, feeding strategies are needed which result in high EPA and DHA concentrations in salmon fillet, thereby optimizing the supply with LCPUFA for human nutrition.

The requirement for vitamin E to ensure good growth in salmon, as currently set by the National Research Council (NRC), is 60 mg per kg diet [15]. In this study we addressed the question whether alpha-tocopherol concentrations beyond the NRC values affect tissue vitamin E levels, as well as biomarkers of lipid peroxidation such as malondialdehyde (MDA). Furthermore, there is only limited data available at which dietary alpha-tocopherol concentrations EPA and DHA are most efficiently protected from lipid peroxidation. Therefore, we investigated in the present feeding trial in Atlantic salmon the effect of increasing dietary alpha-tocopherol concentrations on tissue vitamin E, MDA, antioxidant enzymes including GPx and SOD, and fatty acid composition.

We suggest that the present study may contribute to understanding the role of dietary alpha-tocopherol in terms of flesh quality and nutritional value of Atlantic salmon produced in aquaculture.

Material and Methods

Diets and experimental design

Post-smolt Atlantic salmon (age: ~1.5 years) were fed diets based on fish oil, fish meal, wheat gluten, and soy protein concentrate for 14 weeks as detailed in Table I.

The basal diet (analyzed alpha-tocopherol concentration = 22 mg/kg diet) was supplemented with 0 (negative control, Group I), 150 (Group II), and 400 mg/kg (Group III) vitamin E, as all-rac tocopheryl acetate (DSM, Basel, Switzerland). Analyzed alpha-tocopherol in groups II and III was 140 and 310 mg/kg, respectively. There were no differences in dietary lipid peroxide values (7.6–9.4 mg/kg diet) between groups. Diets contained 1.5 mg beta-, 1.0 mg delta-, and 9.5 mg gamma-tocopherol per kg, respectively. All other vitamins and minerals were supplemented according to NRC standards [15].

The feeding trial was performed according to the guidelines of the Norwegian National Animal Re-

Table I: Composition of the basal diet

Ingredients	Diet (% dry weight)
Wheat gluten	20.6
Soy protein concentrate	24.0
Wheat	4.40
Fava beans	5.00
Scandinavian fish meal	15.0
South American fish oil	27.5
Astaxanthin (10%)	0.035
Vitamin and mineral mix	2.30

Notes: Vitamin and mineral pre-mix according to the National Research Council recommendations (NRC 2011). Analyzed dietary composition: crude fat, 30.5%; crude protein, 40.5 %; crude ash, 5.5 %; alpha-tocopherol, 22 mg/kg; astaxanthin, 32.5 mg/kg; eicosapentaenoic acid (EPA) 17.7 mg/g; docosahexaenoic acid (DHA) 7.9 mg/g.

search Authority at Skretting ARC fish trial station, Stavanger, Norway. A total of 210 Atlantic salmon (*Salmo salar*) with initial average weight of ~ 193 g were distributed in 6 circular fiberglass tanks, each with a volume of 500 m^3, and a diameter of 1 meter. Fish were fed experimental diets in duplicate tanks. The tanks were supplied with flow-through seawater with an exchange rate of 12 L/minute at 12 °C. Fish were exposed to continuous light throughout the study. Diets were provided *ad libitum* twice a day and unfed feed was removed, weighed, and recorded.

Sampling

At the end of the trial eight animals per group (four animals per tank) were randomly sampled and further analyzed in terms of tissues vitamin E levels, MDA, and antioxidant enzymes. Furthermore, two pooled samples each comprising four homogenized fillets were used for fatty composition analysis. Fish were anesthetized with MS-222 and killed by a blow to the head. Blood was drawn from the caudal vein into heparinized tubes and centrifuged at 100 × *g* for 10 minutes at 4 °C and plasma was stored at –80 °C until further analysis. Liver and fillet samples were immediately frozen in liquid nitrogen and also stored at –80 °C for vitamin E, fatty acid composition, lipid peroxidation, and antioxidant enzyme analyses.

Analytical methods

Chemicals and reagents

Sodium hydroxide (NaOH), potassium hydroxide, HPLC-grade methanol, HPLC-grade ethanol and concentrated hydrochloric acid (HCl; 37 %) were purchased from Merck (Darmstadt, Germany). Perchloric acid, 2,4-dinitrophenyl-hydrazine (DNPH; 97 %), acetonitrile (HPLC quality), and the malondialdehyde standard 1,1,3,3-tetraethoxypropane (TEP; 96 %) (CAS no. 122 – 31 – 6) were supplied by Sigma Aldrich (Steinheim, Germany). Dl-α-tocopheryl acetate (CAS no. 7695 – 91 – 2) was purchased from DSM Nutritional Products Ltd. (Basel, Switzerland). Sulfuric acid (95 – 97 %) was purchased from J.T. Baker (Deventer, The Netherlands). Phosphate-buffered saline (PBS) was obtained from PAA (Pasching, Austria). Acetic acid was purchased from Roth GmbH (Karlsruhe, Germany) and water was deionized and distilled by an Aquarius automatic water distillation apparatus (Berkefeld, Germany).

Malondialdehyde analysis in fillet and liver by HPLC

Liver and fillet tissues (200 mg) were homogenized in 1 mL of 1 % sulfuric acid in PBS in a 2-mL Eppendorf tube for 4 minutes using a tissue lyzer (Qiagen, Hilden Germany). Homogenates were centrifuged at 9200 × *g* for 30 minutes at 4 °C. Subsequently, supernatants were collected and used for MDA extraction.

Two hundred fifty microliters of the supernatant were transferred into a 1.5-mL Eppendorf tube with 50 µL of 6M NaOH. This mixture was incubated for 30 minutes at 60 °C in a water bath. Protein was precipitated by adding 125 µL of 35 % perchloric acid and centrifuging at 700 × *g* for 10 minutes. Two hundred fifty microliters of the supernatant were transferred into a new 1.5 mL Eppendorf tube and 50 µL of DNPH was added prior to incubation in the dark at room temperature for 1 hour. Following incubation, 20 µL of sample was injected into the HPLC system. Standard solutions of MDA were prepared from 1,1,3,3 tetraethoxypropane (TEP) in 1 % sulfuric acid in PBS and stored at 4 °C in the dark. MDA analysis was performed on a Jasco system (Jasco GmbH Deutschland, Gross-Umstadt, Germany) equipped with an autosampler (Jasco AS-2057), pump (PU-2080), LG-2080 – 02 ternary gradient unit, three-line degasser (DG-2080 – 53), and photodiode array detector. MDA was separated using a Supelco INC water spherisorb ODS2 column

(10 cm × 4.6 mm, 3 μm) along with a guard column, then eluted in isocratic mode with a mobile phase consisting of 0.2 % acetic acid in double distilled water/acetonitrile (42:58, v/v). The flow rate was set at 0.6 mL/minute and the autosampler thermostat at 4 °C. MDA was analyzed at 310 nm.

Tocopherol analysis in plasma, fillet and liver and by HPLC

From each animal, 100 mg of the respective tissues, or 100 μL plasma, were transferred into test tubes with screw caps on ice and ethanol (2 mL) containing 1 % (by weight) ascorbic acid, 700 μL H_2O, and 300 μL saturated aqueous KOH were added. The tubes were closed, vortexed for 30 seconds, the samples saponified at 70 °C in a shaking water bath for 30 minutes, and then cooled on ice. Fifty microliters BHT in ethanol (1 mg/mL) and 2 mL *n*-hexane were added; the samples were mixed by hand-inversion for 60 seconds, and centrifuged (4 °C, 5 minutes, 200 × *g*) to aid phase separation. One mL of the supernatant organic phase was transferred to a clean tube and dried under a stream of nitrogen gas. The residue was dissolved in 250 μL methanol: water (98:2, v/v), vortexed for 15 seconds, and 40 μL of the resulting solution injected into the HPLC system. Plasma samples (100 μL) were processed as described above, but without saponification.

Tocopherols were analyzed using a Jasco (Gross-Umstadt, Germany) HPLC system (pump PU2080Plus, autosampler AS2057Plus, detector FP2020Plus) at room temperature. The separation of tocopherols was performed on a Waters Spherisorb ODS-2 column (100 mm × 4.6 mm; 3 μm) using methanol/water (98:2, v/v) as mobile phase. The fluorescence detector was set to an excitation wavelength of 296 nm and emission wavelength of 325 nm. The concentrations of alpha- and gamma-tocopherols were quantified by use of authentic tocopherols (Calbiochem, Schwalbach, Germany) as external standards. Analyses were performed in duplicates.

Superoxide dismutase and glutathione peroxidase activity measurements

Liver tissue homogenates were prepared by homogenizing 100 mg liver tissue in 1 mL ice-cold PBS followed by a centrifugation step (3000 × *g*, 4 °C, 10 minutes). Measurements were performed in liver homogenate supernatants diluted 1:10 (v/v) in PBS. Superoxide dismutase (SOD) activity was determined according to the method of Marklund and Marklund [16]. Briefly, 50 μL sample was mixed with 2.85 mL Tris/succinate buffer (0.05 mol/L; pH 8.2). The reaction was started by adding 100 μL of 8 mmol/L pyrogallol. Pyrogallol autoxidation is inhibited by SOD activity and was measured at 420 nm over 3 minutes by using a DU 800 spectrophotometer (Beckman Coulter, Krefeld, Germany). One U SOD refers to a 50 % inhibition of pyrogallol autoxidation. Glutathione peroxidase (GPx) activity was measured according to the method of Lawrence and Burk [17]. In brief, 900 μL of assay mix [0.1 mmol/L NADPH, 1 mmol/L GSH, 1 U/mL glutathione reductase in potassium/phosphate buffer (pH 7.0)] was mixed with 50 μL sample and 50 μL of cumene hydroperoxide. GPx activity was detected at 340 nm over 3 minutes by using a DU 800 spectrophotometer (Beckman Coulter, Krefeld, Germany). Results for antioxidant enzyme activities were adjusted for protein content using a commercial kit (BCA Protein Assay; Pierce, Rockford, IL, USA).

Fatty acid analysis

Fatty acid analysis of the experimental diets and fillets were conducted by using gas chromatography and flame ionization detection [18].

Statistical analysis

Data were calculated as means ± SEM. Statistical analysis was performed using PASW Statistics 18 (IBM, Chicago, IL, USA). Data were analyzed for normality of distribution (Kolmogorov-Smirnov and Shapiro-Wilk tests) and equality of variance (Levene's test) prior one-way ANOVA. In case of variance, equality Scheffé-test was applied as a *post hoc* test; otherwise Games-Howell was applied. Values of $p<0.05$ were considered statistically significant.

Results

Feed conversion ratio as well as hemoglobin and hematocrit of Atlantic salmon fed diets supplemented with 0, 150, and 400 mg/kg dietary alpha-tocopherol did not significantly differ between groups as shown in Table II. However, final body weight in salmon fed diets supplemented with 400 mg/kg alpha tocopherol tended to be ~ 10 % higher as compared to unsupple-

Table II: Effect of dietary alpha-tocopherol on body weight, feed conversion ratio, hemoglobin, and hematocrit in salmon. Data are mean ± SEM.

Group[1]	Initial body weight (g)	Final body weight (g)	Feed intake (% body weight/day)	Feed conversion[3] ratio	Hemoglobin (g/dL)	Hematocrit (%)
I[2]	192 ± 2.19 (n=70)	624 ± 34.4 (n=70)	0.869 ± 0.008	0.75 ± 0.026	10.6 ± 0.2 (n=8)	46.4 ± 2.8 (n=8)
II	193 ± 0.19 (n=70)	670 ± 9.3 (n=70)	0.907 ± 0.015	0.73 ± 0.003	10.2 ± 0.2 (n=8)	47.1 ± 0.7 (n=8)
III	194 ± 0.71 (n=70)	695 ± 15.1 (n=70)	0.939 ± 0.024	0.75 ± 0.002	11.0 ± 0.4 (n=8)	46.0 ± 3.1 (n=8)

Notes: [1]Group I (negative control) = 0 mg/kg vitamin E supplemented; Group II=150 mg/kg vitamin E supplemented; Group III=400 mg/kg vitamin E supplemented. [2]The basal diet contained 22 mg alpha-tocopherol per kg. [3]Feed conversion ratio (FCR) = feed consumed (g)/mass gain (g), FCR was calculated on a tank basis. No significant differences among groups.

mented controls, which may be related to a slightly higher feed intake.

Feeding an increasing concentration of dietary alpha-tocopherol resulted in an increase of alpha-tocopherol concentration in plasma, liver, and fillet. This effect was dose-dependent as far as plasma and fillet alpha-tocopherol concentrations are concerned (Table III). Alpha-tocopherol concentrations were highest in the liver, followed by plasma and fillet.

Interestingly we observed significantly ($p \leq 0.01$) lower gamma-tocopherol concentrations in plasma of salmon receiving the highest dose (400 mg/kg) of dietary alpha-tocopherol (Table III). This effect was less pronounced in the fillet and not evident in the liver.

High dose (400 mg/kg) of dietary vitamin E was associated with significantly lower ($p \leq 0.01$) MDA concentrations in the fillet. A similar trend was observed for the liver tissue but differences were not statistically significant (Table III).

The activity of the antioxidant enzymes superoxide dismutase and glutathione peroxidase in salmon liver remained unchanged by the different dietary treatments (Table III).

As expected, fillet EPA and DHA concentrations directly reflected those of the diet. However, dietary alpha-tocopherol did not affect EPA and DHA concentrations in salmon fillet as shown Figure 1.

Discussion

Under the conditions investigated, dietary alpha-tocopherol did not affect feed intake and daily gain of Atlantic salmon. This finding is in accordance with a previous trial feeding Atlantic salmon with similar dietary vitamin E concentrations as in the present study [19]. Anemia has been shown to be a sign of severe vitamin E deficiency in fish [20]. However, hematological parameters including hematocrit and hemoglobin remained unchanged in our salmon, possibly suggesting that fish fed the non-supplemented diet were not fully depleted in terms of vitamin E.

Diets were supplemented with astaxanthin. It is well documented that astaxanthin may prevent tocopherols from oxidation thereby exhibiting a vitamin E-sparing effect [21–23].

Taken together, a salmon diet based on 30.5 % crude fat (fish oil) and 40.5 % crude protein, containing 22 mg/kg alpha-tocopherol from the raw materials and with no other vitamin E supplementation, does not seem to impair animal growth and health.

Increasing dietary alpha-tocopherol resulted in a dose-dependent increase ($r^2=0.82$; $p<0.001$) of alpha-tocopherol in salmon fillet. Thus supplemental dietary alpha-tocopherol may significantly increase the nutritional value of salmon fillet as far as vitamin E is concerned. European human nutrition societies currently recommend about 15 mg vitamin E per day for adults [24]. Our salmon fillet contain up to 4 mg alpha-tocopherol per 100 g. Thus the consumption of 200 g salmon would be sufficient to fulfill roughly 30 % of the dietary vitamin E recommendations. In this context it has been recently shown that increased farmed salmon intake may increase antioxidant defenses in humans [25].

Furthermore, it needs to be established whether different tocopherol concentrations in fillets, as observed in the present study, may affect EPA and DHA fillet concentrations after long-term storage, which was beyond the scope of this study.

Table III: Alpha-tocopherol and gamma-tocopherol concentrations in plasma, liver, and fillet as well as malondialdehyde concentrations in liver and fillet and antioxidant enzyme activities in liver of salmon fed increasing concentrations of α-tocopherol. Data are mean ± SEM (n=8). Significant difference between groups are indicated by * (p≤0.05), ** (p≤0.01) and *** (p≤0.001).

	Tocopherol						Lipid peroxidation MDA (μmol/kg)		Liver antioxidant enzyme activity	
	Plasma (μmol/L)		Liver (μmol/kg)		Fillet (μmol/kg)					
Group[1]	α	γ	α	γ	α	γ	Liver	Fillet	SOD (U/mg protein)	GPx (mU/mg protein)
I[2]	26.5 ± 5.4	2.06 ± 0.22	53.1 ± 15.9	1.19 ± 0.11	1.88 ± 0.28	0.098 ± 0.03	45.7 ± 6.7	4.64 ± 0.35	11.4 ± 4.7	28.3 ± 6.6
II	55.9 ± 3.5	1.69 ± 0.07	117 ± 14.8	1.09 ± 0.08	5.15 ± 1.4	0.102 ± 0.03	37.9 ± 3.2	4.51 ± 0.24	7.93 ± 2.8	34.4 ± 2.2
III	91.7 ± 10.5	1.22 ± 0.10	123 ± 10.9	1.17 ± 0.06	9.74 ± 1.1	0.075 ± 0.02	26.6 ± 1.4	2.60 ± 0.11	16.9 ± 8.5	20.8 ± 7.0
I – II	*		***		*					
I – III	***	*	***		***			**		
II – III	*	**			*			***		

Notes: [1]Group I=0 mg/kg vitamin E supplemented (negative control); Group II=150 mg/kg vitamin E supplemented; Group III=400 mg/kg vitamin E supplemented.
[2]The basal diet A contained 22 mg alpha-tocopherol per kg.

High-dose dietary alpha-tocopherol significantly decreased gamma-tocopherol concentration in plasma. This finding is in line with previous observations in humans [26–28] and rats [29] and is thought to be partly related to the induction of cytochrome P450 enzymes that are involved in the metabolism of tocopherols, and which preferentially degrade the desmethyl vitamers.

Importantly gamma-tocopherol is gaining attention in human nutrition as it exhibits isomer-specific biological properties, including the scavenging of reactive nitrogen species [30] and the inhibition of cyclooxygenase-2 [31]. In salmon gamma-tocopherol is more efficiently deposited in triacylglycerol-rich tissues [3]. In the present study no negative effects of dietary alpha tocopherol level were observed on the gamma tocopherol concentration in the fillet, which is important from a consumer point of view.

We observed a dose-dependent decrease in MDA levels in salmon fillet in response to increasing dietary alpha-tocopherol concentrations. MDA is a product of lipid peroxidation of polyunsaturated fatty acids and an important biomarker for the sensory quality of fish [11]. Since the highest supplementation of dietary alpha-tocopherol (400 mg/kg diet) was accompanied by a further decrease in MDA in salmon fillet, we suggest that high-dose vitamin E beyond the current dietary requirement may make an important contribution in terms of shelf life and sensory quality of salmon fillet as reported by others [3]. It needs to be taken into account that in the present study fish oil was used as the sole fat source. Thus, possibly lower dietary vitamin E levels are needed to prevent lipid peroxidation when plant-derived oils are used in the salmon diet.

We did not observe significant differences in hepatic superoxide dismutase and glutathione peroxidase activities in response to dietary alpha-tocopherol. Also, in our previous study in rats [29], dietary alpha-tocopherol concentrations did not change superoxide dismutase and glutathione peroxidase activity. This may be related to the fact that zinc, copper, manganese, and selenium, all of which are known to be important co-factors of superoxide dismutase and glutathione peroxidase, respectively, were sufficiently supplied through the salmon diet. Furthermore also astaxanthin, present in the experimental diets, may have exhibited antioxidant activity [22].

Both EPA and DHA acid concentrations in salmon fillet were not different among groups. It is well known that alpha-tocopherol protects polyunsaturated fatty acids against lipid peroxidation. At the same time there is evidence suggesting that alpha-tocopherol interferes with the endogenous synthesis of LCPUFA in fish. In fact, dietary vitamin E deficiency significantly increased

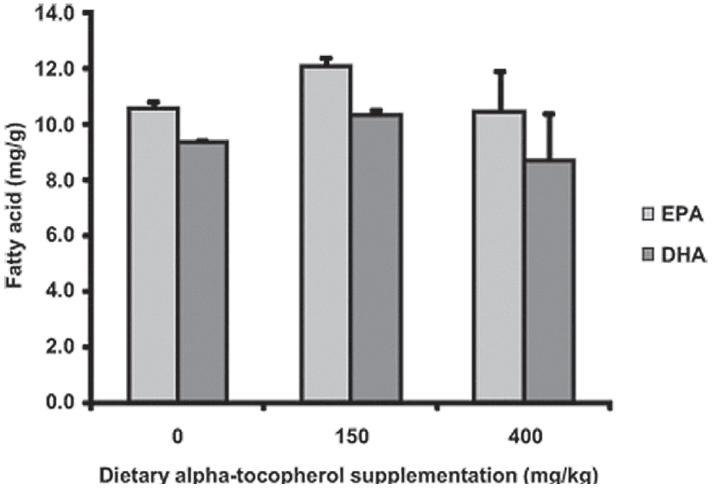

Figure 1: Effect of dietary alpha-tocopherol on eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in salmon fillet. No significant differences among groups (n=8 per group). The basal diet contained 22 mg alpha-tocopherol per kg.

the recovery of desaturated and elongated products, including EPA in isolated hepatocytes derived from Atlantic salmon [22, 32], possibly due an inhibition of delta-6-desaturase. Thus supplemental dietary tocopherol may on the one hand protect LCPUFA in terms of oxidation and may on the other hand also inhibit its endogenous synthesis. Both opposing effects may have led to the observation that EPA and DHA levels remained largely unchanged between groups.

Conclusion

Present data suggest that alpha-tocopherol supplementations beyond the dietary requirement (60 mg per kg diet) may further improve flesh quality and nutritional value of Atlantic salmon fillet as far as MDA and alpha-tocopherol concentrations are concerned, when diets rich in fish oil are fed to salmon. Increasing concentrations of dietary alpha-tocopherol did not, however, change EPA and DHA concentrations in salmon fillet.

References

1. Rimbach, G., Minihane, A.M., Majewicz, J., Fischer, A., Pallauf, J. and Virgli, F. (2002) Regulation of cell signalling by vitamin E. Proc. Nutr. Soc. 61, 415–425.

2. Hamre, K., Berge, R.K. and Lie, O. (1998) Turnover of alpha-, gamma-, and delta-tocopherol and distribution in subcellular and lipoprotein fractions indicate presence of an hepatic tocopherol binding protein in Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. 18, 71–83.

3. Parazo, M.P.M., Lall, S.P., Castell, J.D. and Ackman, R.G. (1998) Distribution of alpha- and gamma-tocopherols in Atlantic salmon (*Salmo salar*) tissues. Lipids 33, 697–704.

4. Rimbach, G., Moehring, J., Huebbe, P. and Lodge, J.K. (2010) Gene-regulatory activity of alpha-tocopherol. Molecules 15, 1746–1761.

5. Yamamoto, Y., Fujisawa, A., Hara, A. and Dunlap, W.C. (2001) An unusual vitamin E constituent (alpha-tocomonoenol) provides enhanced antioxidant protection in marine organisms adapted to cold-water environments. Proc. Natl. Acad. Sci. U S A. 98, 13144–13148.

6. Yamamoto, Y., Maita, N., Fujisawa, A., Takashima, J., Ishii, Y. and Dunlap, W.C. (1999) A new vitamin E (alpha-tocomonoenol) from eggs of the Pacific salmon Oncorhynchus keta. J. Nat. Prod. 62, 1685–1687.

7. Flader, D., Brandsch, C., Hirche, F. and Eder, K. (2003) Effect of mega doses of dietary vitamin E on the antioxidant status of rats fed lard or salmon oil. Int. J. Vitam. Nutr. Res. 73, 275–283.

8. Packer, L., Weber, S. and Rimbach, G. (2001) Molecular aspects of alpha-tocotrienol antioxidant action and cell signalling. J. Nutr. 131, 369–373.

9. Azzi, A. (2007) Molecular mechanism of alpha-tocopherol action. Free Radic. Biol. Med. 43, 16–21.

10. Barella, L., Muller, P.Y., Schlachter, M., Hunziker, W., Stöcklin, E., Spitzer, V., Meier, N., de Pascual-Teresa, S., Minihane, A.M. and Rimbach, G. (2004) Identification of hepatic molecular mechanisms of action of alpha-tocopherol using global gene expression profile analysis in rats. Biochim. Biophys. Acta 1689, 66–74.

11. Menoyo, D., Lopez-Bote, C., Obach, A. and Bautista, J. (2005) Effect of dietary fish oil substitution with linseed oil on the performance, tissue fatty acid profile, metabolism, and oxidative stability of Atlantic salmon. J. Anim. Sci. 83, 2853–2862.

12. Strobel, C., Jahreis, G. and Kuhnt, K. (2012) Survey of n-3 and n-6 polyunsaturated fatty acids in fish and fish products. Lipids Health Dis. 11, 144–153.

13. Calder, P.C. (2011) Fatty acids and inflammation: the cutting edge between food and pharma. Eur. J. Pharmacol. 668 Suppl. 1: 50–58.

14. Elmadfa, I., Stroh, S., Brandt, K. and Schlotzer, E. (1993) Influence of a single parenteral application of a 10 % fish oil emulsion on plasma fatty acid pattern and the function of thrombocytes in young adult men. Ann. Nutr. Metabol. 37, 8–13.

15. Nutrient Requirements of Fish and Shrimp; National Research Council (2011).

16. Marklund, S. and Marklund, G. (1974) Involvement of superoxide anion radical in autoxidation of pyrogallol and a convenient assay for superoxide-dismutase. Eur. J. Biochem. 47, 469–474.

17. Lawrence, R.A. and Burk, R.F. (1976) Glutathione peroxidase-activity in selenium-deficient rat-liver. Biochem. Biophys. Res. Commun. 71, 952–958.

18. Grahl-Nielsen, O. and Barnung, T. (1985) Variation in the fatty acid profile of marine animals caused by environmental and developmental changes. Mar. Environ. Res. 17, 218–221.

19. Hamre, K., and Lie, O. (1995) Minimum requirement of vitamin E for Atlantic salmon, *Salmo salar* L, at first feeding. Aquacult. Res. 26, 175–184.

20. Hamre, K. (2011) Metabolism, interactions, requirements and functions of vitamin E in fish. Aquacult. Nutr. 17, 98–115.

21. Mansour, N., McNiven, M.A. and Richardson, G.F. (2006) The effect of dietary supplementation with blueberry, α-tocopherol or astaxanthin on oxidative stability of Arctic char (Salvelinus alpinus) semen. Theriogenology 66, 373–382.

22. Bell, J.G., McEvoy, J., Tocher, D.R. and Sargent, J.R. (2000) Depletion of alpha-tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. J. Nutr. 130, 1800–1808.

23. Jensen, C., Birk, E., Jokumen, A., Skibsted, L.H. and Betelsen, G. (1998) Effect of dietary levels of fat, α-tocopherol and astaxanthin on colour and lipid peroxidation during storage of frozen rainbow trout (Oncorynchus mykiss) and during chill storage of smoked trout. Z. Lebensm. Forsch. 207, pp. 189–196.

24. Referenzwerte für die Nährstoffzufuhr, DGE, Bonn, ISBN 978–3-86528–143–142.

25. Garcia-Rodriguez, C.E., Mesa, M.D., Olza, J., Vlachava, M., Kremmyda, L. and Diaper, N.D. (2012) Does consumption of two portions of salmon per week enhance the antioxidant defense system in pregnant women? Antioxid. Redox Signal. 16, 1401–1406.

26. Handelman, G.J., Machlin, L.J., Fitch, K., Weiter, J.J. and Dratz, E.A. (1985) Oral alpha tocopherol supplements decrease plasma gamma-tocopherol levels in humans. J. Nutr. 115, 807–813.

27. Melchert, H.U. and Pabel, E. (1998) The tocopherol pattern in human serum is markedly influenced by intake of vitamin E drugs – results of the German national health surveys. Journal of the American Oil Chemists Society 75, 213–216.

28. Huang, H.Y. and Appel, L.J. (2003) Supplementation of diets with alpha-tocopherol reduces serum concentrations of gamma- and delta-tocopherol in humans. J. Nutr. 133, 3137–3140.

29. Gaedicke, S., Zhang, X., Huebbe, P., Boesch-Saadatmandi, C., Lou, Y. and Wiswedel, I. (2009) Dietary vitamin E, brain redox status and expression of Alzheimer's disease-relevant genes in rats. Br. J. Nutr. 102, 398–406.

30. Cooney, RV., Franke, A.A., Harwood, P.J., Hatch-Pigott, V., Custer, L.J. and Mordan, L.J. (1993) Gamma-tocopherol detoxification of nitrogen dioxide: superiority to alpha-tocopherol. Proc. Natl. Acad. Sci. U S A. 90, 1771–1775.

31. Jiang, Q., Elson-Schwab, I., Courtemanche, C. and Ames, B.N. (2000) Gamma-tocopherol and its major metabolite, in contrast to alpha-tocopherol, inhibit cyclooxygenase activity in macrophages and epithelial cells. Proc. Natl. Acad. Sci. U S A. 97, 11494–11499.

32. Mourente, G., Bell, J.G. and Tocher, D.R. (2007) Does dietary tocopherol level affect fatty acid metabolism in fish? Fish Physiol. Biochem. 33, 269–280.

Prof. Gerald Rimbach

Institute of Human Nutrition and Food Science
University of Kiel
Hermann-Rodewald-Strasse 6
24098 Kiel
Germany
rimbach@foodsci.uni-kiel.de