JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2011, *17*(3), 295-302

Journal of Agroalimentary Processes and Technologies

Effect of dietary alpha-tocopheryl acetate on alpha-tocopherol content of novel omega-3-enhanced farmed rainbow trout (*Oncorhynchus mykiss*) fillets

G.L.Mihalca[1*], O. Tiţa[1], Mihaela Tiţa[1], Ana Mihalca[2]

[1] *"Lucian Blaga" University, 550012,Str. Ion Raţiu, Nr.5-7, Sibiu, România*
[2] *"Ţara Bârsei" College of Prejmer, Prejmer, str. Brasovului, nr. 160A, Romania*

Received: 12 May 2011; Accepted: 22 July 2011

Abstract

A trout diet was supplemented with 0, 8.5, or 15 g/100 g of flaxseed oil (FO). To prevent lipid oxidation of fillets, FO-supplemented diets were also enhanced with 0, 400, and 900 mg/kg of alpha-tocopheryl acetate (α-TA). α-tocopherol content of fillets were determined following fish harvest on days 0, 30, 60, 90, and 120. FO supplementation resulted in increased ($P<0.05$) concentration of omega-3 fatty acid (ω3 FA) in fillets, mainly due almost two-fold increase ($P<0.05$) of α-linolenic acid, while docosahexaenoic and eicopentaenoic acids slightly decreased ($P<0.05$). The highest ($P<0.05$) α-tocopherol content in fillets was determined when supplementing trout with 900 mg/kg of α-TA at day 120. Our results indicate that regardless of FO level in trout diet, 900 mg/kg of α-TA can prevent lipid deterioration of fillets.

Keywords: Trout fillets; Aquatic foods; α-tocopherol; Omega-3 fatty acids

1. Introduction

Frecently, low-value fish meal and oil have been successfully converted to a high-value human food [32,40]. The aquaculture sector has greatly increased its production and currently contributes nearly 50% of the global annual catch [41], making up for the shortfall [30]. Barlow estimated that aquaculture feeds will consume 90% of the world fish oil supply. Fish and fish food products provide essential FA as a part of human diet [4]. Therefore, seeking alternative sources of dietary lipids for aquacultured fish will be necessary in order to maintain the sustainable growth of the aquaculture industry for the ever-growing human population.

Castell et al. reported that supplementing trout fingerlings with linoleic (LN, 18:2ω6) and α-linolenic (ALA, 18:3ω3) fatty acids increased the concentration of these fatty acids and that of EPA and DHA in the fillets[8].

However, they did not determine the effects of the increased concentration of ω3 PUFA on lipid oxidation or human health. Vitamin E is one of the most important lipid-soluble antioxidants that is used in the food industry [7]. The a-tocopherol has the greatest antioxidant activity among four homologue pairs (α, β, γ, δ-tocopherols and tocotrienols) [1-6].

Increasing α-TA levels in the diet of rainbow trout did not show any antioxidant effect on the white muscle lipids, a slight reduction of lipid oxidation in the dark muscle did occur [28-30]. The lower bioavailability of synthetic vitamin E (dl-α-TA) when compared to the natural vitamin E (d-α-tocopherol) was demonstrated [10,11]. However, dl-α-TA is fairly stable during storage [12]; therefore, commonly added to fish feed. The dl-α-TA becomes active as an antioxidant following hydrolysis of the acetate group in the fish body [9].

Corresponding author: *e-mail:* mihalca.liviu@yahoo.com

Chaiyapechara et al. (2003) designed fish diets supplemented with 300 and 1500 mg of dl-α-TA per kg of feed. They showed that dl-α-TA at 1500 mg/kg of feed reduced the rate of lipid oxidation [9].

2. Materials and methods

*Feeding trial and fish diets:*The experiment took place at Hărman Farm of Doripesco SA. A gravity-fed flow-through raceway system composed of four levels was used for this study. Each level had two parallel lanes and each lane had five tanks. Tanks were stocked with 75 rainbow trout fingerlings (age 11–12 months, average weight 240 g/fish, and average length 27 cm) per tank (size 91 x 122 x 91 cm). Rainbow trout were fed dry pelleted diets formulated with 0 (basal diet, Table 1), 8.5, or 15.0 g/100 g of FO supplementation. (Table 2). Each level of the FO supplementation was also enhanced with 0, 400, or 900 mg/kg α-tocopheryl acetate (α-TA).

Hence, there were nine treatment diets. The dietary treatments were randomly assigned to the tanks in each level of the raceway system. The basal diet was supplemented with 0, 8.5, and 15.0 g/100 g FO. The supplementation did not affect the protein content in the diets, and therefore, the diets were isonitrogenous. Fish were hand fed to satiation twice a day for 120 days. Feed was stored at 4°C.

Approximately 1500 l/min of spring water flowed through the raceway system. It was aerated entering the system and half the way through the system to maintain a dissolved oxygen concentration above 70% of saturation. Water temperature was approximately 12°C during the feeding trial. Fish were maintained on a natural photoperiod.

Sample preparation: One fish in each tank per level was harvested randomly on days 0, 30, 60, 90, and 120 and then killed by a blow to the head. All harvested fish were stored at 4°C before trout were filleted to obtain boneless and skinless butterfly fillets. Fillets were homogenized in a laboratory blender. Homogenized samples were placed in nylon vacuum pouches, labeled, vacuum packed and stored at -80°C until analyzed. These sample preparation steps were performed on the same day when fish were harvested.

*Lipid extraction and fatty acid analysis:*Lipids were extracted from a fillet sample using methodology described by Folch, Lees, and Sloane (1957) [14] and the extracted lipids were used for analysis of fatty acid profile. According to the procedure of Fritsche and Johnston (1990) [15-17], fatty acids were transmethylated by the addition of 4ml of 40 g/l methanolic H_2SO_4 and heated in a 90°C water bath for 60 min. The mixture was saponified by transferring through a Na_2SO_4 filled glass Pasteur pipette and subsequent drying under N_2 in a 60°C water bath for 60 min. The fatty acid methyl esters (FAME) were resuspended in filtered isooctane. The FAME were analyzed by using a gas chromatograph (Agilent 6890N - GC) and a flame ionization detector fitted with a OB 225 capillary column (30 m length, 0.25 mm inside diameter). Injection and detection temperature was maintained at 220°C and column temperature was 190°C. The fatty acids were identified by comparing their retention times with known standards and references. Peak area and the amount of each fatty acid were computed by an integrator using the Agilent Camp Station software.

*Measurement of α-tocopherol content:*The content of α-tocopherol in trout fillets was measured by a modified saponification method using high pressure liquid chromatography (HPLC) described by Liu and Lee (1998) [24]. An aliquot of 0.25 g of L-ascorbic acid and 0.01 g of TBHQ (tert-butylhydroquinone) were added to approximately 1 g of a fillet sample in a test tube. Exact weight of the fillet sample was recorded and used in calculations. A freshly prepared 7.3 ml digestion solution (110 g/l potassium hydroxide, 550 ml/ml ethanol, 450 ml/ml distilled and deionized water) was added to the fillet sample, followed by vortexing (Euro Turrax T 20b) for 30 s to dissolve the ascorbic acid. The tubes were incubated at 80°C for 20 min with continuous and gentle shaking in order to digest the fillet sample. Immediately following the incubation, the tubes were cooled in ice slush for 10 min. An aliquot of 4 ml of iso-octane added to the cooled solution and the tubes were vortexed for 2 min. Then, the tubes were allowed to stand in the ice slush for 5 min. An aliquot of 1ml of the top clear isooctane layer was transferred to a small tube and stored at -80°C until the solution was injected into the HPLC (Waters Alliance System) using a Rheodyne injector (Waters).

The HPLC was equipped with a Waters Resolve C-18 spherical silica column (5µm, 3.9 x150 mm and a spectrophotometric detector. A mixture of iso-octane/tetrahydrofuran (96/4, ml/ ml) was prepared and filtered (0.45 µm) daily and the mixture was used as a mobile phase. The flow rate was 1ml/min and the injection volume was 30 µl. The α-tocopherol was detected at excitation wavelength of 296 nm and emission wavelength of 325 nm. The blank was run as described above, but without the fillet sample.

Various isomeric forms of tocopherol were also run on the HPLC as standards as well as the extraction efficiency of α-tocopherol was determined and used in calculations. An experimental standard curve was used to calculate a-tocopherol content in the trout fillets, which is reported as mg of α-tocopherol per kg of fillets.

*Statistical analysis:*The experiments were conducted using a 3x3 factorial design [35-36]. The interaction effect (FO x α-TA) and main effect (FO and α-TA) were analyzed. A significant difference was used at 0.05 probability level and differences between treatments were tested using the least significant difference (LSD) test. At least twelve fillets (n = 12) from each treatment (three fillets per level in the raceway system composed of four levels) were randomly obtained and analyzed. At least six diets (n = 6) from each treatment were randomly obtained and analyzed. All statistical analyses of data were performed using SAS Institute (2002) [31-34].

3. Results and discussion

FO and α-TA did not (P>0.05) show any interactions for the α-tocopherol content in trout fillets. The supplemental levels of FO did not (P>0.05) affect the α-tocopherol content in trout fillets (Table 3).

Table 1. Major ingredients of the trout basal diet (g/kg)

Ingredients	**(g/kg)**
Wheat middlings	280
Fish meal	250
Hydrolyzed feather meal	100
Dehulled soybean meal	100
Blood meal	100
Ground extruded whole soybean	60
Corn gluten meal	50
Minerals	25
Vitamins	15
Soy lecithin	10
Yeast culture	10
Nutrient contents	
Crude protein (g/kg)	420
Fat (g/kg)	70
Metabolizable energy (MJ/kg)	12.6

Table 2. Total fat and fatty acid composition of experimental diets

Parameter	**FO supplementation (g/100 g of diet)**		
	0	**8.5**	**15.0**
	g/100 g of total fatty acids		
18:3n3	3.47±1.14 c	33.30±2.98 b	46.22±1.89 a
20:5n3	10.93±0.64 a	2.87±0.52 b	1.22±0.32 c
22:6n3	12.72±0.77 a	3.68±0.60 b	1.62±0.15 c
18:2n6	20.88±1.08 a	24.22±2.02 a	21.60±0.90 a
20:4n6	0.27±0.17 a	0.47±0.38 a	0.33±0.03 a
Total unsaturates	60.98±1.01 c	76.38±2.11 b	82.50±1.39 a
Total saturates	9.02±1.01 a	23.62±2.11 b	17.50±1.39 c
Total ω-3	27.47±1.44 c	40.08±2.07 b	49.07±2.13 a
Total ω-6	22.17±1.08 b	26.42±1.06 a	22.57±0.86 b
ω-3/ω-6	1.27±0.10 c	1.50±0.04 b	2.17±0.04 a
Total fat (g/100 g, dry basis)	14.43±2.32 a	13.59±0.82 a	24.86±0.65 b

Data are given as mean±SEM (n = 6). Mean values in horizontal rows with different letters indicate significant differences (Least Squared Difference test; P<0.05).

Table 3. α-tocopherol content in trout fillets as affected by feed supplementation with flaxseed oil (FO)

Feeding period (day)	**FO supplementation (g/100 g of diet)**		
	0	**8.5**	**15.0**
	mg of α-tocopherol/kg of fillets		
0	165.80±7.50	163.42±5.85	163.02±5.89
30	194.20±14.61	165.28±10.00	193.34±14.02
60	189.58±9.02	162.89±8.64	179.67±9.53
90	203.40±14.66	198.04±19.72	181.36±10.83
120	240.88±27.55	197.21±13.24	201.62±23.30

Data are given as mean ± SEM (n = 12). Values are given as mg of α-tocopherol/kg of fillet. Mean values in horizontal rows with different letters indicate significant differences (No (P>0.05) interaction effects between FO and α-TA on α-tocopherol content; therefore, data reported per FO supplementation; Least Squared Difference test; P<0.05).

Table 4. α-tocopherol content in trout fillets as affected by feed supplementation with α-tocopheryl acetate (α-TA)

Feeding period (day)	**α-TA (mg/kg of diet)**		
	0	**400**	**900**
	mg of α-tocopherol/kg of fillets		
0	152.26±4.47	171.27±8.02	168.72±3.85
30	183.78±13.64	185.47±17.02	183.58±10.09
60	169.67±9.63	173.99±6.08	188.48±11.77
90	177.49±11.14	183.00±10.70	222.31±19.13
120	185.80±8.87 b	181.11±11.47 b	272.80±27.30 a

Data are given as mean ± SEM (n=12). Values are given as mg of α-tocopherol/kg of fillet. Mean values in horizontal rows with different letters indicate significant differences (No (P>0.05) interaction effects between FO and α-TA on α-tocopherol content; therefore; data reported per α-TA supplementation; Least Squared Difference test; P<0.05).

Table 5. Omega-3 fatty acids (ω-3 FA) in trout fillets as affected by feed supplementation with flaxseed oil (FO)

Feeding period (day)	**FO supplementation (g/ 100 g of diet)**	**g/100 g of total fatty acids**				
		C18:3ω3	C20:3ω3	C20:5ω3	C22:6ω3	Total ω3
0	0	1.92±0.74	0.26±0.24	6.26±1.08	21.29±3.40	29.72±1.14
	8.5	2.13±0.19	0.17±0.21	6.39±0.51	22.46±2.31	31.15±0.69
	15.0	1.96±0.33	0.22±0.21	5.86±0.88	19.90±4.45	27.94±1.15
30	0	5.05±2.46c	0.38±0.22	6.33±0.82a	22.41±3.26a	34.16±1.21a
	8.5	9.96±3.70b	0.75±0.14	4.94±0.67b	18.44±2.76b	34.10±0.80a
	15.0	18.26±3.83a	0.80±0.12	4.69±0.78b	18.13±3.72b	41.89±1.09b
60	0	5.35±2.25c	0.59±0.13	5.73±1.24a	19.59±4.28a	31.26±1.38a
	8.5	15.91±2.60b	0.92±0.14	3.62±0.62b	15.18±3.53b	35.64±1.45b
	15.0	23.33±2.90a	0.87±0.08	3.41±1.23b	12.85±2.72b	40.47±1.32c
90	0	6.29±0.85c	0.75±0.45	6.49±1.51a	20.79±2.79a	34.16±0.92a
	8.5	17.27±1.20b	1.30±0.72	3.79±0.71b	15.25±3.56b	37.61±1.31b
	15.0	25.91±2.27a	0.93±0.37	3.30±0.71b	11.64±2.68c	41.79±1.02c
120	0	2.64±0.53c	0.58q±0.26	4.74±0.81a	16.70±2.48a	24.66±2.11a
	8.5	14.82±2.82b	0.79±0.23	2.27±0.65b	8.14±2.04b	26.02±1.85a
	15.0	26.66±5.06a	1.02±0.30	2.63±0.47b	9.05±1.50b	39.36±1.89b

Data are given as mean±SEM (n = 12). Values are given as g/100 g of total fatty acids. Mean values in the columns with different letters indicate significant differences (Least Squared Difference test; P<0.05) within a feeding period.

Table 6. Omega-6 fatty acids (ω-6 FA) in trout fillets as affected by feed supplementation with flaxseed oil (FO)

Feeding period (day)	**FO supplementation (g/100 g of diet)**	**g/100 g of total fatty acids**					
		C18:2ω6	C18:3ω6	C20:3ω6	C20:4ω6	C22:2ω6	Total ω6
0	0	14.81±1.89	0.08±0.11	0.26±0.23	0.52±0.56	1.20±1.30	16.87±0.46
	8.5	15.35±0.69	0.04±0.09	0.22±0.23	0.19±0.41	2.29±2.28	18.10±0.55
	15.0	14.21±1.66	0.04±0.09	0.40±0.23	0.19±0.43	1.64±1.81	16.49±0.71
30	0	15.78±1.52b	0.21±0.15	0.50±0.19	0.39±0.51a	1.10±0.70	17.98±0.58b
	8.5	16.89±1.80b	0.23±0.18	0.60±0.10	0.27±0.41a	1.27±0.24	19.27±0.71ab
	15.0	18.00±1.34a	0.23±0.13	0.48±0.09	0.28±0.39a	1.23±0.66	20.22±0.33a
60	0	14.40±1.24b	0.30±0.07	0.55±0.11	0.56±0.50a	1.21±0.57	17.01±0.52
	8.5	15.70±2.18ab	0.24±0.11	0.61±0.07	0.29±0.36ab	1.13±0.54	17.97±0.70
	15.0	16.26±1.47a	0.24±0.13	0.46±0.09	0.16±0.30b	1.32±0.21	18.45±0.51
90	0	13.86±4.07b	0.27±0.20	0.92±0.43	0.26±0.48	2.18±0.56	17.49±1.01
	8.5	15.40±6.76ab	0.51±0.14	0.92±0.36	0.15±0.29	2.25±1.01	19.23±1.73
	15.0	18.83±3.47a	0.30±0.16	0.58±0.15	ND*	1.90±0.53	21.61±1.16
120	0	12.46±2.39b	0.20±0.08	0.51±0.09	0.63±0.31a	0.51±0.51	14.31±0.72b
	8.5	14.08±2.44ab	0.20±0.13	0.58±0.12	0.18±0.20b	0.72±0.53	15.77±1.13b
	15.0	15.32±2.55a	0.14±0.06	0.51±0.11	0.20±0.23b	0.96±0.75	17.14±0.83a

Data are given as mean ± SEM (n = 12). Values are given as weight g/100 g of total fatty acids. Mean values in the columns with different letters indicate significant differences (Least Squared Difference test; $P<0.05$) within a feeding period. *ND: not detectable.

Generally, the highest α-tocopherol contents in trout fillets were obtained in the group supplemented with 900 mg/kg of α-TA, followed by 400 mg/kg of α-TA, and the non-α-TA supplemented diet (Table 4).

Although higher α-tocopherol concentrations in trout fillets were measured for the 900 mg/kg supplemented group, these differences were insignificant until day 120 (Table 4). According to Stephan et al (1995), the α-tocopherol concentration in the muscle of turbot (*Scophthalmus maximus*) fed peanut oil was higher than those fed cod liver oil [37-40]. Therefore, a source of the dietary fatty acids may have an effect on the deposition of α-tocopherol in fillets. However, our data suggest that rainbow trout fed for up to 120 days diets supplemented with the FO up to 15 g/100 g did not have a significant effect on the deposition of the α-tocopherol in the fish fillets.

We measured increased ($P<0.05$) α-tocopherol concentration in the fillets in the group supplemented with 900 mg/kg α-TA after 120 days of feeding (Table 4). Higher α-tocopherol content in trout fillets has been reported by several investigators as a function of increased level of dietary α-TA [13].

However, liver as opposed to the muscle was the major organ where α-tocopherol was accumulated. Unlike Akhtar et al. [1] feeding trial, our experiment was conducted for 4 months.

Likely, extension of our feeding trial for longer than 4 months could have resulted in higher deposition of α-tocopherol in trout fillets [19]. Table 4 also shows that feeding trout a diet supplemented with 900 mg/kg of α-TA resulted in a significant increase ($P<0.05$) of α-tocopherol in trout fillets at 4 months (120 days) of feeding. Jittinandana et al. [20] fed rainbow trout diets supple-mented with α-TA at 200 and 5000 mg/kg for up to nine weeks.

The 5000 mg/kg supplementation significantly increased α-tocopherol in fillets starting at 4 weeks of feeding and continued increasing at 9 weeks. However, the diet supplemented at 200 mg/kg did not increase α-tocopherol in fillets, which was similar to our data for fillets recovered from trout fed diet supplemented at 400 mg/kg (Table 4). In addition, Jittinandana et al. [21] demonstrated similarly to Akhtar et al. [2] that α-tocopherol is accumulated in the liver at much higher concentration than in the muscle.

Therefore, it is likely that trout initially accumulate α-tocopherol in the liver and then in the muscle, which in turn increases concentration of α-tocopherol in the fillets [22,23,25-27].

This is probably why feed supplementation with α-TA at 900 mg/kg, as in our research, may require longer feeding times than 4 months to obtain more pronounced increase of α-tocopherol in trout fillets.

Tables 5 and 6 show omega-3 (ω3) and omega-6 (ω6) fatty acid (FA) profiles, respectively, of rainbow trout that were fed FO-supplemented diets (Table 2) for up to 4 months (120 days). The total concentration of ω3 FA in fillets increased significantly ($P<0.05$) for trout supplemented with FO at 15.0 g/100 g for 120 days. However, the increase was mainly due to the significant increase ($P<0.05$) of α-linolenic acid (ALA, 18:3ω3) in the fillets, while the concentration of eicosapentaenoic (EPA, 20:5ω3) and docosahexaenoic (DHA, 22:6ω3) acids significantly decreased ($P<0.05$) for fillets obtained from trout supplemented with FO at 8.5 and 15.0 g/100 g for up to 120 days.

Overall, the decrease in the EPA and DHA was compensated by the increase in the ALA; and therefore, the total concentration of ω3 FA in trout fillets increased for the 15.0 g/100 g FO group (Table 5). The concentration of total ω6 FA also significantly increased ($P<0.05$) in the fillets obtained from trout supplemented with 15.0 g/ 100 g of FO for 120 days (Table 6). However, this increase was not as pronounced as for the total ω-3 FA (Table 5). The increase of total ω6 FA was mainly due to increased concentration of linoleic acid (LN, 18:2ω6) (Table 6). The LN is a precursor for arachidonic acid (AN, 20:4ω6). However, the concentration of AN was significantly lower ($P<0.05$) for fillets obtained from trout supplemented with FO at 8.5 and 15.0 g/100 g. Therefore, trout probably inefficiently converted LN from the dietary FO to AN. Several investigators have reported that dietary vegetable oils including FO increase concentration of total ω6 FA in fish fillets [18,42,43]. Therefore, our data is in general agreement with those studies.

4. Conclusions

Regardless of supplemental levels of FO, the dietary supplementation of trout with α-TA at 900 mg/kg significantly increased the α-tocopherol content of the fillets after 120 days of feeding.

Trout fillets containing higher α-tocopherol content had lower lipid oxidation level. The lipid oxidation of ω-3-enhanced trout fillets was alleviated by dietary supplementation of trout with α-TA at 900 mg/kg starting at 60 days of feeding.

However, synergistic effect of α-tocopherol with other antioxidants and aerobic packaging on lipid stability of trout fillets should be further investigated in order to reduce rancidity development. The dietary supplementation of trout with 15.0 g/100 g of FO resulted in almost 1.6-fold increase of total ω3 FA mainly due to increased concentration of ALA with a concurrent decrease of EPA and DHA fatty acids.

Acknowledgements

Researches realised within the project POSDRU/6/1.5/S/26 funded of the Social European Fund by the Sectorial Operating Programm "DEVELOPMENT OF THE HUMAN RESOURCES 2007 – 2013". Thanks to Mr. Dorin Crizbăşan , the owner of Doripesco SA Hălchiu for his support in this study. Thanks to "HAKI" Institute for Fisheries, Aquaculture and Irrigation Szarvas Hungary, for technical assistance and specially to Mrs. Zsuzsana Sandor for expert technical assistance with gas chromatography and high-performance liquid chromatography.

References

1. Akhtar, P., Gray, J. I., Cooper, T. H., Garling, D. L., & Booren, A. M., Dietary pigmentation and deposition of a-tocopherol and carotenoids in rainbow trout muscle and liver tissue, *Journal of Food Science,* **1999**, *64*(2), 234–239, doi: 10.1111/j.1365-2621.1999.tb15872.x
2. Alvarez, M. J., Lopez-Bote, C. J., Diez, A., Corraze, G., Arzel, J., Dias, J., et al., Dietary fish oil and digestible protein modify susceptibility to lipid peroxidation in the muscle of rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*)., *British Journal of Nutrition*, **1998**, *80*(3), 281–289
3. Association of Official Analytical Chemists. *Official methods of analysis* (16th ed.). Washington, DC: Association of Official Analytical Chemists, 1995, USA.
4. Barlow S., Fishmeal and oil: Sustainable feed ingredients for aquafeeds, *Global Aquaculture Advocate*, **2000**, *4*, 85–88.
5. Bell, J. G., Henderson, R. J., Tocher, D. R., McGhee, F., Dick, J. R., Porter, A., et al., Substituting fish oil with crude palm oil in the diet of Atlantic salmon (Salmo salar) affects muscle fatty acid composition and hepatic fatty acid metabolism, *Journal of Nutrition*, **2002**, *132*(2), 222–230, http://jn.nutrition.org/content/132/2/222.full

6. Burton, G. W., Ingold, K. U., Autoxidation of biological molecules. 1. Antioxidant activity of vitamin E and related chainbreaking phenolic antioxidants in vitro, *Journal of American Chemical Society*, **1981**, *103*(21), 6472–6477, doi: 10.1021/ja00411a035
7. Caballero, M. J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M., Izquierdo, M. S., Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*., *Aquaculture*, **2002**, *214*(1-4), 253–271, doi:10.1016/S0044-8486(01)00852-3
8. Castell, J. D., Lee, D. J., Sinnhuber, R. O. (1972). Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*), lipid metabolism and fatty acid composition,. *Journal of Nutrition*, **1972**, *102*(1), 93–100.
9. Chaiyapechara, S., Casten, M. T., Hardy, R. W., & Dong, F. M., Fish performance, fillet characteristics, and health assessment index of rainbow trout (*Oncorhynchus mykiss*) fed diets containing adequate and high concentration of lipid and vitamin E. *Aquaculture*, **2003**, *219*(1-4), 715–738, doi:10.1016/S0044-8486(03)00025-5
10. Charmley, E., Hidiroglou, N., Ochoa, L., McDowell, L. R., & Hidiroglou, M., Plasma and hepatic a-tocopherol in cattle following oral or intramuscular supplementation, *Journal of Dairy Science*, **1992**, *75*(3), 804–810, doi:10.3168/jds.S0022-0302(92)77819-9
11. Chen, Y. C., Nguyen, J., Semmens, K., Beamer, S., Jaczynski, J, Enhancement of omega-3 fatty acid content in rainbow trout (*O. mykiss*) fillets, *Journal of Food Science,* **2006**, *71*(7), 383–389, doi: 10.1111/j.1750-3841.2006.00115.x
12. Chung, Y. K., Mahan, D. C., Lepine, A. J., Efficacy of dietary da-tocopherol and dl-a-tocopheryl acetate for weanling pigs, *Journal of Animal Science*, **1992**, *70*, 2485–2492
13. Cowey, C. B., Adron, J. W., Youngson, A. The vitamin E requirement of rainbow trout (*Salmo gairdneri*) given diets containing polyunsaturated fatty acids derived from fish oil, *Aquaculture*,**1983**, *30*(1-4), 85–93, doi:10.1016/0044-8486(83)90154-0
14. Folch, J., Lees, M., Sloane, S., A simple method for isolation and purification of total lipids from animal tissues, *Journal of Biological Chemistry*, **1957**, *226*, 497–509.
15. Fritsche, K. L., Johnston, P. V., Effect of dietary a-linolenic acid on growth, metastasis, fatty acid profile and prostaglandin production of two murine mammary adenocarcinomas, *Journal of Nutrition*, **1990**, *120*(12), 1601–1609
16. Gelineau, A., Corraze, G., Boujard, T., Larroquet, L.,, Kaushik, S. Relation between dietary lipid level and voluntary feed intake, growth, nutrient gain, lipid deposition and hepatic lipogenesis in rainbow trout, *Reproduction Nutrition Development*, **2001**, *41*(6), 487–503, doi.org/10.1051/rnd:2001103
17. Greene, D. H. S., Selivonchick, D. P., Effects of dietary vegetable, animal and marine lipids on muscle lipid and hematology of rainbow trout (*Oncorhynchus mykiss*), *Aquaculture*, **1990**, *89*(2), 165–182, doi:10.1016/0044-8486(90)90308-A
18. Guillou, A., Soucy, P., Khalil, M., Adambounou, L., Effects of dietary vegetable and marine lipid on growth, muscle fatty acid composition and organoleptic quality of flesh of brook charr (*Salvelinus fontinalis*), *Aquaculture*, **1995**, *136*(3-4), 351–362.
19. Jaczynski, J., Park, J. W., Physicochemical properties of surimi seafood as affected by electron beam and heat., *Journal of Food Science*, **2003**, *68*(5), 1626–1630, doi: 10.1111/j.1365-2621.2003.tb12303.x
20. Jittinandana, S., Kenney, P. B., Slider, S. D., Kamireddy, N., Hankins, High dietary vitamin E affects storage stability of frozenrefrigerated trout fillets, *Journal of Food Science*, **2006**, *71*(2), C91–C96, doi: 10.1111/j.1365-2621.2006.tb08888.x
21. Jittinandana, S., Kenney, P. B., Slider, S. D., Hankins, J. A., Effect of high dietary vitamin E on lipid stability of oven-cooked and hot-smoked trout fillets, *Journal of Food Science*, **2006**, *71*(3), C130–C136, doi: 10.1111/j.1365-2621.2006.tb15608.x
22. Ke, P. J., Cervantes, E., Robles-Martinez, C., Determination of thiobarbituric acid reactive substances (TBARS) in fish tissue by an improved distillation spectrophotometric method, *Journal of the Science of Food and Agriculture*, 1984, *35*(11), 1248–1254, doi: 10.1002/jsfa.2740351117
23. Lee, K. J., Dabrowski, K., Interaction between vitamin C and E affects their tissue concentrations, growth, lipid oxidation, and deficiency symptoms in yellow perch (*Perca flavescens*), *British Journal of Nutrition,* **2003**, *89*(5), 589–596, doi: 10.1079/BJN2003819
24. Liu, J. F., Lee, Y. W., Vitamin C supplementation restores the impaired vitamin E status of Guinea pigs fed oxidized frying oil, *Journal of Nutrition*, **1998**, *128*(1), 116–122.
25. Monahan, F. J., Buckley, D. J., Morrisey, P. A., Lynch, P. B., Gray, J. I., Influence of dietary fat and a-tocopherol supplementation on lipid oxidation in pork, *Meat Science*, **1992**, *31*(2), 229–241, doi:10.1016/0309-1740(92)90042-3

26. Morris, P. C., Gallimore, P., Handley, J., Hide, G., Haughton, P., Black, A., Full-fat soya for rainbow trout (*Oncorhynchus mykiss*) in freshwater: Effects on performance, composition and flesh fatty acid profile in absence of hind-gut enteritis, *Aquaculture*, **2005**, *248*(1-4), 147–161, doi:10.1016/j.aquaculture.2005.04.021
27. Nurnberg, K., Kuchenmeister, U., Nurnberg, G., Ender, K., Hackl, W., Influence of exogenous application of n-3 fatty acids on meat quality, lipid composition, and oxidative stability in pigs, *Arch Tierernahr.,* **1999**, *52*(1), 53–65
28. Pozo, R., Lavety, J., Love, R. M. The role of dietary a-tocopherol (vitamin E) in stabilizing the canthaxanthin and lipids of rainbow trout muscle, *Aquaculture*, **1988**, *73*(1-4), 165–175, doi:10.1016/0044-8486(88)90051-8
29. Regost, C., Arzel, J., Cardinal, M., Laroche, M., Kaushik, S. J., Fat deposition and flesh quality in seawater reared, triploid brown trout (*Salmo trutta*) as affected by dietary fat levels and starvation, *Aquaculture*, **2001**, *193*(3-4), 325–345, doi.org/10.1016/S0044-8486(00)00498-1
30. Sargent, J. R., Tacon, A. G. J., Development of farmed fish: A nutritionally necessary alternative to meat, *Proceedings of the Nutrition Society*, **1999**, *58*(2), 377–383, doi: 10.1017/S0029665199001366
31. SAS Institute, *SAS/STAT guide for personal computers*, version 8.1.Cary, NC: SAS Institute Inc., 2002
32. Sathivel, S., Bechtel, P. J., Babbitt, J., Prinyawiwatkul, W., Negulescu, I. I., & Reppond, K. D., Properties of protein powders from arrowtooth flounder (*Atheresthes stomias*) and herring (*Clupeaharengus*) byproducts, *Journal of Agricultural and Food Chemistry*, **2004**, *52*(16), 5040–5046, doi: 10.1021/jf0351422
33. Singh, R. B., Niaz, M. A., Sharma, J. P., Kumar, R., Rastogi, V., Moshiri, M., Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infraction: The Indian experiment of infarct survival-4, *Cardiovascular Drugs and Therapy*, **1997**, *11*(3), 485–491, doi: 10.1023/A:1007757724505
34. Skonberg, D. I., Rasco, B. A., Dong, F. M., Fatty acid composition of salmonid muscle changes in response to a high oleic acid diet., *Journal of Nutrition*, **1994**, *124*(9), 1628–1638.
35. Steel, R. G. D., Torrie, J. H., Principles and procedures of statistics: A biometrical approach (2nd ed.). New York: McGraw-Hill Book, 1980, USA
36. Steffens, W., Rennert, B., Wirth, M., Krueger, R., Effect of two lipid levels on growth, feed utilization, body composition and some biochemical parameters of rainbow trout, *Oncorhynchus mykiss*. *Journal of Applied Ichthyology*, **1999**, *15*(6), 159–164.
37. Stephan, G., Guillaume, J., Lamour, F., Lipid peroxidation in turbot (*Scophthalmus maximus*) tissue: Effect of dietary vitamin E and dietary n-6 or n-3 polyunsaturated fatty acids, *Aquaculture*, **1995**, *130*(2-3*)*, 251–268.
38. Tichivangana, J. Z.,Morrissey, P. A., Metmyoglobin and inorganic metals as prooxidants in raw and cooked muscle systems, *Meat Science*, **1985**, *15*(2), 107–116.
39. Toyoshima, K., Noguchi, R., Hosokawa, M., Fukunaga, K., Nishiyama, T., Takahashi, R., et al., Separation of sardine oil without heating from surimi waste and its effect on lipid metabolism in rats, *Journal of Agricultural and Food Chemistry*, **2004**, *52*(8), 2372–2375, doi: 10.1021/jf0306439
40. Vannuccini, S., Overview of fish production, utilization, consumption and trade. Rome: *Food and Agriculture Organization Fisheries Information*, Data and Statistics Unit, 2004
41. Wang, J. T., Liu, Y. J., Tian, L. X., Mai, K. S., Du, Z. Y., Wang, Y., et al., Effect of dietary lipid level on growth performance, lipid deposition, hepatic lipogenesis in juvenile cobia (*Rachycentron canadum canadum*), *Aquaculture*, **2005**, *249*(1-4), 439–447, doi:10.1016/j.aquaculture.2005.04.038
42. Wonnacott, E. J., Lane, R. L., Kohler, C., Influence of dietary replacement of menhaden oil with canola oil on fatty acid composition of sunshine bass, *North American Journal of Aquaculture*, **2004**, *66*(4), 243–250, doi:10.1577/A03-049.1
43. Zhao, G., Etherton, T. D., Martin, K. R., West, S. G., Gillies, P. J.,Kris-Etherton, P. M., Dietary a-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women, *Journal of Nutrition,* **2004***, 134*(11), 2991–2997.