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Influence of Dietary Sesamin, a Bioactive Compound on Fatty Acids and Expression of Some Lipid Regulating Genes in Baltic Atlantic Salmon (*Salmo salar* L.) Juveniles

S. TRATTNER[1,3], B. RUYTER[2], T. K. ØSTBYE[2], A. KAMAL-ELDIN[1], A. MOAZZAMI[1], J. PAN[1], T. GJØEN[4], E. BRÄNNÄS[3], V. ZLABEK[1,5], J. PICKOVA[1,5]

[1]Department of Food Science, SLU, Uppsala, Sweden, [2]Nofima AS, Ås, Norway, [3]Department of Wildlife, Fish, and Environmental Studies, SLU, Umeå, Sweden, [4]Department of Pharmaceutical Bioscience, School of Pharmacology, University of Oslo, Oslo, Norway, [5]Research Institute of Fish Culture and Hydrobiology, University of South Bohemia in Ceske Budejovice, Vodnany, Czech Republic

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Summary

The effects of inclusion of sesamin / episesamin in Baltic Atlantic salmon (*Salmo salar* L.) diets based on vegetable oils were studied. The study was designed as a dose response study with two control diets, one diet based on fish oil (FO) and one diet based on a mixture of linseed and sunflower oil (6:4 by vol.) (MO). As experimental diets three different levels of inclusion of sesamin / episesamin (hereafter named sesamin) to the MO based diet and one diet based on sesame oil and linseed oil (SesO) (1:1 by vol.) were used. The dietary oils were mirrored in the fatty acid profile of the white muscle. Sesamin significantly decreased the levels of 18:3n-3 in the white muscle phospholipid (PL) fraction of all groups fed sesamin, no significant differences were found in the triacylglycerol fraction (TAG). Slightly increased levels of docosahexaenoic acid (22:6n-3, DHA) in PL and TAG were found in some of the sesamin fed groups. Sesamin significantly affected the expression of peroxisome proliferator-activated receptor α, scavenger receptor type B and hormone sensitive lipase, in agreement with previous studies on rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar* L.) hepatocytes published by our group. No significant effects on toxicological response measured as ethoxyresorufin O-deethylase activity was found. The total cytochrome P450 enzymes were significantly higher in MO 0.29 and SesO group. The amount of α- and γ-tocopherols in liver and the amount of γ-tocopherol in white muscle were significantly lower in fish fed the FO diet compared to the MO diet, but no difference after inclusion of sesamin was found in this study. Increased inclusion of sesamin increased the levels of sesamin and episesamin in the liver, but did not affect the amounts in white muscle.

Key words

Sesamin • Episesamin • Tocopherols • Cytochrome P450 • Peroxisome proliferator-activated receptor • Hormone sensitive lipase • Scavenger receptor type B

Corresponding author

Sofia Trattner, Department of Food Science, SLU, Uppsala, Sweden. Fax: 46-18-672995. E-mail: Sofia.Trattner@lmv.slu.se

Introduction

Sesamin, a minor component of sesame oil (Moazzami and Kamal-Eldin 2006), is a potent lipid modulator in mammals. It has been shown to affect enzymatic activity and expression of genes involved in lipid metabolism e.g. acyl-CoA oxidase (ACO) and carnitine palmitoyltransferase (CPT) (Kushiro *et al.* 2002, Jeng and Hou 2005, Kiso *et al.* 2005). In the fungus *Mortierella alpina* and in primary rat hepatocytes, sesamin was shown to reduce Δ-5 desaturation index and enzymatic activity of Δ-5 desaturase (Shimizu *et al.* 1991). To our knowledge, only two studies from our group have investigated the effects of dietary sesamin in

fish. In these studies, it was shown that sesamin increased docosahexaenoic acid (22:6n-3, DHA) in rainbow trout (*Oncorhynchus mykiss*) white muscle phospholipid (PL) and triacylglycerol (TAG) fraction and decreased the expression of peroxisome proliferator-activated receptor α (PPARα) in liver (Trattner *et al.* 2008a). In Atlantic salmon (*Salmo salar* L.) hepatocytes, it was shown that sesamin increased elongation and desaturation of radiolabelled 18:3n-3 towards DHA. It increased the levels of β-oxidation products and the relative expression of cluster of differentiation 36 (CD36), scavenger receptor (SRB) type B, PPARα and γ (Trattner *et al.* 2008b). The metabolic effects of sesamin have been suggested to be caused through the activation of PPARs and sterol regulatory element binding protein-1 (SREBP-1) (Ashakumary *et al.* 1999, Ide *et al.* 2004). Furthermore, sesamin has been reported to inhibit cholesterol absorption and synthesis, and tocopherol hydroxylation and clearance in rats and humans (Jeng and Hou 2005). Other compounds known to modulate lipids are 3-thia fatty acids, conjugated linoleic acid and Lipoic acid (Berge *et al.* 2001, Huong and Ide 2008, Kennedy *et al.* 2009).

The methylenedioxyphenyl group of sesamin is known to affect cytochrome P450-dependent drug oxidation (Murray 2000). Cytochrome P450 (CYP) enzymes are known to play a central role in the oxidative metabolism and biotransformation of a wide range of endogenous and exogenous compounds (Nelson *et al.* 1996). Among the numerous CYP families identified, primarily CYP 1-3 are involved in biotransformation of xenobiotics. The CYP1A subfamily is reported to be expressed in the liver of both mammals and fish (Murray 2000, Jönsson *et al.* 2006). Due to the role of CYP1A isoenzymes in the metabolism and bioactivation of foreign compounds, alteration of the expression of hepatic CYP1A may affect the potential risk of xenobiotics (Williams *et al.* 1998). CYP1A is readily inducible by aryl hydrocarbon (Ah) receptor agonist, thus the activity of CYP1A, measured as ethoxyresorufin O-deethylase (EROD) activity, is used as a biomarker for exposure to xenobiotic compounds in fish (Havelkova *et al.* 2007).

Traditionally, carnivorous farmed fish has been fed diets based on fish ingredients. At present there is an overuse of marine raw materials for aquaculture feed production and at the same time aquaculture is the fastest growing food production industry (Tacon 2005, FAO 2007). Therefore, alternative fish feed ingredients are being investigated. Vegetable oil is used as a replacement of fish oil (up to 50 %) without affecting growth and production yield (Torstensen *et al.* 2005). One well known drawback of replacement with vegetable oils in fish feed are the decreased amounts of n-3 highly unsaturated fatty acids (HUFA) in fish tissues (Torstensen *et al.* 2005, Pettersson *et al.* 2009). The n-3 HUFA are known to have positive health effects in man. In terms of human health, it is important to preserve the beneficial fatty acid (FA) composition of fish (Mozaffarian and Rimm 2006). It is necessary to find alternatives to fish oil use, without decreasing the content of n-3 HUFA in fish.

To achieve more n-3 HUFA in fish fed vegetable oils, bioactive compounds can be added in the fish diet. It is interesting to study the effects on sesamin in common aquaculture species, on the nutritional quality of muscle as human food in terms of FA, tocopherols and sesamin content. Furthermore, it is important to investigate how sesamin is metabolized in the fish and how it affects fish welfare. To investigate the effects of sesamin in wild strain Baltic Atlantic salmon (*Salmo salar* L.) juveniles, a dose response study was designed and the FA composition, the relative expression of nine lipid related genes, the content of tocopherols, sesamin and episesamin, EROD activity and the total level of CYP were analyzed.

Material and Methods

Chemicals and reagents

Sesamin/episesamin mixture (1:1, w/w) was a kind gift from Takemoto Oil and Fat Co., Ltd. (Gamagori Aichi, Japan). Fatty acid peaks were identified by comparison with the standard mixture GLC-68 A (Nu-check Prep, Inc, Elysian, Minnesota, USA). Tocopherol standards were purchased as an isomer kit (article number 15496) from Merck (Darmstadt, Germany). All solvents and other chemicals for FA, tocopherols and sesamin analysis were also purchased from Merck and were used without further purification.

Animals and diets

Baltic Atlantic salmon was fed six different diets, fifteen fish per group. Prior the experiment all fish were fed the same commercial diet. Four groups were fed experimental diets based of vegetable oils and sesamin/episesamin mixture (1:1, w/w) (hereafter named sesamin), while one group was fed a diet based on fish

ingredients, and one group was fed a diet based on vegetable oil. The diets were prepared according to the method of Sanchez-Vazquez (1999), the ingredients are shown in Table 1. The diets differed in oil composition and the content of sesamin as follow; 1) mixed linseed : sunflower oil, 6:4 by vol. (MO), 2) fish oil (FO), 3) sesame oil : linseed oil, 1:1 by vol. (SesO), 4) MO + sesamin 0.29 g 100 g^{-1}, 5) MO + sesamin 0.58 g 100 g^{-1}, 6) MO + sesamin 1.16 g 100 g^{-1}. The FA composition and the tocopherol content of the diets are shown in Table 2. The fish were tagged individually and the individual weight increase was calculated as daily growth gain. Fishes were kept at a water temperature of 10 ºC and were fed *ad libitum* for 77 days. Before the experiment started, the fish were tagged with a PIT-tag (Passive Integrated Transponder) by injecting the tag into the posterior part of the abdomen. Before handling, all fish were anaesthetized (ethyleneglycol monophenyl ether 5 ml l^{-1}). The daily growth rate (DGC) were calculated as: DGC = 100 x ($W_2^{1/3} – W_1^{1/3}$) D^{-1} with W_2 being final weight, W_1 the starting weight and *D* the number of days (Table 3.).

Table 1. Basic feed ingredients in the experimental diets.

Ingredient	g 100 g^{-1}
Casein	17.7
Gelatin	3.0
Fish meal	20.7
Dextrin	9.3
*Oil**	27.0
Vitamins + minerals	0.3
Ca_3PO_4	3.8
Cellulose	14.3
Na alginate	3.8

* The oil used was a mixture of linseed and sunflower oil (6:4) in the MO diets, in the SesO diet sesame oil : linseed oil (1:1) was used and in the FO diet fish oil was used. Sesamin / episesamin was added at a level of 0.29, 0.58 and 1.16 g 100 g^{-1} diet to the MO 0.29, MO 0.58 and MO 1.16 diet.

At sacrifice, the muscle was divided in red and white. The muscles, intestine and liver were frozen at –80 ºC until analyzed. From each group six individuals were used for fatty acid, tocopherols, EROD and CYP analysis and another six individuals were used for gene expression analysis.

Lipid analysis

White muscle (2 g) and diets (1 g) were extracted following the method of (Hara and Radin 1978). The lipid content was measured gravimetrically. Total lipids of tissues were separated into PL and TAG according to Pickova *et al.* (1997). Total lipids in the diets, and the PL and TAG lipid fractions of tissues were methylated following the procedure of (Appelqvist 1968) and the FA were analysed by gas chromatograph CP3800 (Varian AB, Stockholm, Sweden) equipped with flame ionisation detector (FID) and split injector and fitted with a fused silica capillary column BPX 70 (SGE, Austin, Tex.), length 50 m, id. 0.22 mm, 0.25 µm film thickness. The column temperature was programmed to start at 158 °C hold 5 min and then increase 2 °C/min from 158 °C to 220 °C and remain at 220 °C for 8 min. The carrier gas was helium (0.8 ml/ min) and make up gas was nitrogen. The injector and detector temperatures were 230 °C and 250 °C, respectively. FA were identified by comparison with the standard FA mixture GLC-68. Peak areas were integrated using Varian Star chromatography workstation software version 5.5.

Tocopherols, sesamin and episesamin analyses

For the analysis of tocopherols in the diets, and the tocopherols, sesamin and episesamin in the tissues, the lipid extracts were dissolved in hexane and analysed with high performance liquid chromatography (HPLC). The mobile phase used was hexane/1,4-dioxane (94:4, vol/vol). The HPLC system was equipped with a Bischoff HPLC pump (Bischoff Analysentechnik und geräte GmbH, Leonberg, Germany) and Agilent 1100 series fluorescence detector (Agilent Technologies, Waldbronn, Germany). The HPLC column was Alltech SI 5U silica column (4.6 x 250 mm; Alltech Associates Inc., Deerfield, IL). The fluorescence detector was operated at an excitation wavelength of 296 nm and an emission wavelength of 324 nm as described by Moazzami and Kamal-Eldin (2006). Identification and quantification was achieved by comparison to external standards.

RNA analysis

Total RNA was purified from livers, muscle and intestine from each group (n=6) and analyzed in duplicate, using Trizol® (Invitrogen), followed by DNase treatment (TURBO DNA-free, Ambion). All protocols were according to the manufacturer's instructions. RNA

Table 2. Fatty acid composition of the diets, (% of total FA), and tocopherols (µg/g lipid), duplicate analyses.

	MO	FO	SesO	MO 0.29	MO 0.58	MO 1.16
14:0	0.22	7.4	0.18	0.20	0.19	0.19
16:0	6.47	17.2	8.13	6.30	6.17	6.16
16:1	0.18	6.88	0.20	0.18	0.17	0.17
18:0	3.46	2.28	4.87	3.49	3.49	3.50
18:1n-9	21.3	11.8	28.0	21.3	21.3	21.2
18:1n-7	0.65	2.22	0.73	0.65	0.64	0.65
18:2n-6	33.7	1.66	29.7	33.7	33.7	33.6
18:3n-3	30.0	1.31	24.8	29.9	29.9	29.9
20:1	0.22	6.00	0.23	0.23	0.22	0.23
22:1	0.05	9.55	0.11	0.11	0.05	0.00
20:5n-3	0.16	8.38	0.16	0.15	0.15	0.17
22:5n-3	0.00	0.78	n.d	n.d	n.d	n.d
22:6n-3	0.32	9.79	0.34	0.34	0.31	0.36
SAFA	10.5	27.2	13.7	10. 6	10.4	10.3
MUFA	22.5	37.4	29.2	22.6	22.6	22.5
PUFA	64.3	22.8	55.1	64.1	64.1	64.2
n-3	30.5	20.4	25.3	30.4	30.4	30.5
n-6	33.9	2.40	29. 8	33.7	33.7	33.8
n-3/n-6	0.90	8.49	0.85	0.90	0.90	0.90
α-tocopherol	1900	600	1000	2200	2500	2100
γ-tocopherol	2300	n.d	4000	2300	2600	2500

SAFA = saturated fatty acids (20:0, 20:2, 22:0, 24:0), MUFA = monounsaturated fatty acids (14:1, 18:1 n-5, 24:1), PUFA = polyunsaturated fatty acids. MO = mixed oil, FO= fish oil, SesO = sesame oil : linseed oil, MO 0.29 = mixed oil with sesamin addition 0.29 g 100 g^{-1}diet, MO 0.58 = mixed oil with sesamin addition 0.58 g 100 g^{-1}diet, MO 1.16 = mixed oil with sesamin addition 1.16 g 100 g^{-1}diet.

Table 3. Daily growth coefficient (n=6) and range between the smallest and largest value during 77 days feeding period.

	FO	SesO	MO	MO 0.29	MO 0.58	MO 1.16
Average ± StDev	0.9 ± 0.14	0.9 ± 0.28	0.8 ± 0.20	0.7 ± 0.28	0.7 ± 0.14	0.7 ± 0.10
Range	0.66 - 1.03	0.54 - 1.20	0.53 - 1.11	0.29 - 1.16	0.50 - 0.91	0.55 - 0.84

Abbreviations see Table 2.

quality and quantity were determined spectrophotometrically ($A_{260/280}$) using NanoDrop® (ND-1000 Spectrophotometer, NanoDrop Technologies, Wilmington, Delaware, USA). Samples were stored in RNase-free water at –80 °C.

The cDNA was synthesized from 2.4 µg RNA, a modified protocol from the Taq Man Reverse Transcription Reagents kit (Applied Biosystems). The Oligo $d(T)_{16}$ primers were used. The reaction was performed by incubating the samples at 25 °C for 10 min, 48 °C for 6 min, 95 °C for 50 min and was terminated by reducing the temperature to 10 °C. Primers for Real-Time PCR analysis (Table 4) were designed using the Primer Express® software based on available salmon sequences in the GenBank® and purchased from Invitrogen (CA, USA). Real-Time PCR was performed in a Prism® 7000 system by using gene-specific primers. A 2 x SYBR® Green PCR Mastermix (ABI) was used in the PCR reaction mix of 25 µl with 1 µl primers (final concentration of 0.5 µM), and 5 µl cDNA. All samples

Table 4. Sequences of primers used for real time PCR analysis.

Primer	Forward primer (5'-3')	Reverse primer (5'-3')	Efficiency
RPL2	TAACGCCTGCCTCTTCACGTTGA	ATGAGGGACCTTGTAGCCAGCAA	1.95
EF1A	CACCACCGGCCATCTGATCTACAA	TCAGCAGCCTCCTTCTCGAACTTC	1.97
PPARα	CGTTGAATTTCATGGCGAACT	TCCTGGTGGCCTACGGATC	1.90
PPARβ	CCAGCAACCCGTCCTTGTT	GAGACGGTCAGGGAGCTCAC	2.04
PPARγ	CATTGTCAGCCTGTCCAGAC	ATGTGACATTCCCACAAGCA	1.95
SRB-I	AACTCAGTGAAGAGGCCAAACTTG	TGCGGCGGTGATGATG	1.79
CD36	GGATGAACTCCCTGCATGTGA	TGAGGCCAAAGTACTCGTCGA	1.76
HSL3	AACGTAGATCAGCCAGTCACCC	ACGTTAGCCGCTTCCCTAGTCT	1.88
Δ5	GAGAGCTGGCACCGACAGAG	GAGCTGCATTTTTCCCATGG	1.77
Δ6	AGAGCGTAGCTGACACAGCG	TCCTCGGTTCTCTCTGCTCC	1.90

RPL2 = RNA polymerase II polypeptide, EF1A = Elongation factor 1α, PPAR = peroxisome proliferator-activated receptor, SRB-I = scavenger receptor type B, CD 36 = cluster of differentiation 36, HSL3 = hormone sensitive lipase, Δ5 = Δ5 desaturase, Δ6 = Δ6 desaturase.

were analyzed in duplicate with a non-template control on each plate. The reference genes used were elongation factor 1α (EF1A) and RNA polymerase II polypeptide (RPL II). The reaction was performed by incubating the samples at 50 °C for 2 min, 95 °C 10 min and 50 cycles of 95 °C for 10 s and 60 °C for 15 s. Standard curves were made for each primer pair and efficiencies (E) were calculated $E=10^{(-1/slope)}$.

Total content of CYP and EROD activity in liver

For the analysis of total CYP content and EROD activity, six liver samples from each group were analysed. The liver tissue was homogenized in ice-cold homogenization buffer (0.25 M sucrose and 0.1 mM EDTA in 0.01 M TRIS buffer, pH 7.4) using a Potter-Elvehjem homogeniser. The homogenate was centrifuged for 15 min at 10,000×*g* (4 °C) and the resulting supernatant was spun down for 1 h at 105,000×*g* (4 °C). The microsomal pellets were resuspended in the homogenization buffer and stored at –80 °C until used. The total CYP content was determined spectrophotometrically by the Co- and dithionite difference method (Shimanzu UV-1601PC, Columbia, USA) according to (Omura and Sato 1964).

Hepatic EROD activity was determined according to a modified method (Jönsson *et al.* 2006). Standard solutions of resorufin (0-50 μM) and protein (BSA; 1 mg BSA ml^{-1}) were prepared in HEPES-Cortland buffer pH 8. The HC buffer was prepared by dissolving 0.38 g KCl, 7.74 g NaCl, 0.23 g $MgSO_4 \cdot 7H_2O$, 0.23 g $CaCl_2 \cdot 2H_2O$, 0.41 g $NaH_2PO_4 \cdot H_2O$, 1.43 g HEPES, and 1 g glucose in 1 l of distilled water. Microsome suspensions were further diluted in the same buffer (1:5 and 1:10). Aliquots of the microsome suspensions (50 μl) and of the standard solutions (40 μl of resorufin and 10 μl of BSA) were added in duplicate wells in 96-well plate. A 160 μl aliquot of 7-ethoxyresorufin (12.7 μM) and NADPH (2.1 mM) in HC buffer was rapidly added to all wells. The plate was then immediately placed in a microplate reader (Wallac 1420 VICTOR^2, Turku, Finland) and the resorufin fluorescence was monitored for 10 min by repeated measurements at 544 nm (ex) and 590 nm (em). EROD activity was calculated and expressed as pmol of resorufin formed per mg protein and minute. The protein contents of the microsomes were assayed by the (Smith *et al.* 1985), adapted for microplate readers.

Data analysis

Fatty acids, EROD, CYP, tocopherols, sesamin and episesamin data are presented as mean values ± standard deviation. The General Linear Model (GLM) of SAS (SAS Institute Inc., Cary, N.C., USA, version 8.2) was used to compare the physiological responses of the different diets. The model included the fixed effect of treatment and random effect of individual. Relative expression of the different genes, in relation to housekeeping genes were determined by using the Relative Expression Software Tool (REST-384©-version 1) for group wise comparison and statistical analysis of relative expression results in real-time PCR (Pfaffl *et al.* 2002).

Results

Survival

The total mortality during the study was 5 fish, of which three belonged to the FO-group, one to the MO group and one to the MO 0.28 group.

There was no difference in starting weight, final weight or daily growth coefficient (DGC) between the MO group and the groups with added sesamin. For the FO group both start and end weight were lower (6.9 and 13.8, respectively) than in the other groups (range 8.7-20.7 g). The DGR is shown for the six fish from each group which were analysed for fatty acid composition. The variation between individuals is large in all groups as seen from the data in Table 3.

Fatty acid composition and lipid content

The groups did not differ in lipid content. The FA composition of white muscle clearly reflected the FA profile of the oils used in the diets (Table 5). The FO group was characterized by high proportions of EPA and DHA in PL and TAG. The vegetable oil groups had higher proportions of 18:3n-3 and lower proportions of n-3HUFA. The response to the change in dietary FA composition was faster in TAG than PL. The levels of 18:3n-3 were significantly lower in PL of all three groups fed sesamin compared to the MO group without sesamin ($P<0.05$). The level of docosapentaenoic acid (DPA, 22:5n-3) and DHA in PL of the MO groups with added sesamin were higher than in the MO group, however the difference was only significant for DPA in the MO 0.58 group ($P<0.05$). In the PL of MO 0.58 group DHA increased to 36.6 ($P=0.16$). Similar to the PL fraction 18:3n-3 decreased and DPA and DHA increased in the TAG of MO 0.58 and MO 1.16 group (non significant changes).

Table 5. Fatty acid composition of the white muscle PL and TAG fraction, duplicate analyses, $n=6$ (% of total FA).

	FO	**SesO**	**MO**	**M 0.29**	**M 0.58**	**MO 1.16**
Lipid (%)	2.00±0.43	2.06±0.54	1.92±0.55	1.74±0.33	1.82±0.51	1.92±0.31
Phospholipids						
14:0	1.52±0.26^{z}	0.41±0.09^{y}	0.52±0.19^{z}	0.40±0.03^{z}	0.41±0.09^{z}	0.43±0.03^{z}
16:0	17.6±5.28^{z}	16.1±1.46$^{z\,y}$	16.9±1.19$^{z\,y}$	14.5±1.19^{y}	15.5±1.33^{zy}	15.8±1.15^{zy}
16:1	1.75±0.21	0.34±0.03	0.45±0.21	0.36±0.02	0.37±0.08	0.40±0.04
18:0	4.05±0.21^{z}	5.85±0.47^{y}	5.59±0.31^{y}	5.69±0.28^{y}	5.76±0.40^{y}	5.88±0.26^{y}
18:1n-9	7.05±1.21^{z}	9.70±2.79^{y}	7.68±1.21^{z}	7.29±0.82^{z}	7.40±1.07^{z}	6.98±0.76^{z}
18:1n-7	2.40±0.14^{z}	1.20±0.18^{y}	1.28±0.11^{y}	1.21±0.04^{y}	1.33±0.06^{y}	1.30±0.11^{y}
18:1n-5	0.52±0.08	0.53±0.18	0.44±0.04	0.56±0.14	0.43±0.02	0.50±0.12
18:2n-6	1.25±0.24^{z}	8.53±0.99^{y}	8.46±0.49^{y}	8.59±0.87^{y}	8.05±0.31^{y}	8.37±0.65^{y}
18:3n-3	0.68±0.12^{z}	5.55±0.50^{x}	6.45±0.53^{y}	5.81±0.28^{x}	5.77±0.22^{x}	5.97±0.32^{x}
20:0	0.11±0.12	0.12±0.03	0.07±0.04	0.09±0.01	0.08±0.04	0.07±0.02
18:4n-3	0.66±0.27^{z}	2.24±0.46^{y}	2.16±0.34^{y}	2.05±0.43^{y}	1.87±0.21^{y}	2.07±0.09^{y}
20:1	0.53±0.45^{z}	0.26±0.13^{y}	0.25±0.20^{y}	0.20±0.09^{y}	0.21±0.11^{y}	0.18±0.03^{y}
20:2n-6	0.17±0.03^{z}	0.43±0.04^{y}	0.36±0.03^{y}	0.44±0.06^{y}	0.43±0.04^{y}	0.40±0.07^{y}
20:3n-6	0.07±0.03^{z}	0.89±0.10^{y}	0.67±0.08^{x}	0.87±0.04^{yu}	0.77±0.06^{v}	0.79±0.09^{vu}
20:4n-6	1.51±0.21^{z}	1.47±0.14^{z}	1.31±0.19^{y}	1.51±0.11^{z}	1.44±0.10^{z}	1.50±0.08^{z}
20:3n-3	0.09±0.02^{z}	0.38±0.06^{yx}	0.35±0.03^{y}	0.43±0.07^{x}	0.42±0.07^{yx}	0.40±0.08^{yx}
22:1	1.02±0.12	1.30±0.05	1.21±0.15	1.36±0.13	1.28±0.12	1.28±0.11
20:5n-3	8.99±0.84^{z}	5.87±0.46^{y}	6.38±0.67^{y}	6.34±0.48^{y}	6.14±0.26^{y}	6.39±0.23^{y}
24:1	0.16±0.05	0.11±0.05	0.12±0.08	0.08±0.02	0.13±0.05	0.07±0.04
22:5n-3	1.80±0.31^{zx}	1.30±0.21^{yx}	1.26±0.10^{y}	1.39±0.11^{yx}	1.48±0.10^{x}	1.38±0.16^{yx}
22:6n-3	40.7±2.55^{z}	33.2±4.71^{y}	34.1±1.69^{y}	35.7±2.91^{y}	36.6±1.73^{y}	34.7±2.79^{y}
SAFA	23.5±5.59	22.6±2.13	23.1±1.32	20.8±1.34	21.8±1.73	22.2±1.21
MUFA	13.6±1.04^{zx}	14.5±1.50^{x}	12.6±0.82^{y}	13.1±1.13^{y}	12.0±1.01^{z}	12.8±0.92^{y}
PUFA	56.0±3.00^{z}	59.0±3.12^{y}	60.5±1.08^{yx}	61.1±2.36^{yx}	62.2±1.31^{x}	59.9±2.49^{yx}
n3	52.8±2.85^{z}	47.7±3.97^{y}	49.5±1.38^{zx}	49.7±3.09^{zyx}	51.3±1.33^{yx}	48.8±2.82^{yx}
n6	3.17±0.33^{z}	11.6±1.04^{y}	11.0±0.51^{y}	11.4±0.85^{y}	10.9±0.45^{y}	11.1±0.83^{y}
n3/n6	16.8±1.53^{z}	4.15±0.75^{y}	4.51±0.31^{y}	4.39±0.61^{y}	4.70±0.25^{y}	4.44±0.50^{y}
n3HUFA/18:3n3	77.8±9.57	7.61±1.07	6.73±0.64	7.58±0.88	7.89±0.37	7.20±0.75

	FO	SesO	MO	M 0.29	M 0.58	MO 1.16
Triacylglycerols						
14:0	4.62±0.40^{z}	1.14±0.29^{y}	1.25±0.26^{y}	1.19±0.35^{y}	1.39±0.27^{y}	1.33±0.26^{y}
16:0	12.0±0.18^{z}	8.40±0.63^{y}	7.49±0.46^{x}	7.42±0.70^{x}	7.84±0.99^{yx}	7.55±0.43^{x}
16:1	7.01±0.46^{z}	1.37±0.36^{y}	1.42±0.30^{y}	1.34±0.44^{y}	1.61±0.34^{y}	1.48±0.34^{y}
18:0	2.85±0.23^{z}	4.36±0.25^{y}	3.72±0.13^{x}	3.71±0.14^{x}	4.09±0.26^{v}	3.77±0.08^{x}
18:1n-9	15.8±0.42^{z}	25.1±2.58^{y}	20.8±0.51^{x}	21.3±0.48^{x}	21.2±0.32^{x}	21.0±0.60^{x}
18:1n-7	3.02±0.21^{z}	2.06±1.76^{y}	1.26±0.08^{y}	1.22±0.14^{y}	1.37±0.16^{y}	1.28±0.11^{y}
18:1n-5	0.22±0.02^{zv}	0.08±0.07^{yx}	0.13±0.05^{zxv}	0.18±0.01^{vu}	0.11±0.09^{xu}	0.23±0.14^{v}
18:2n-6	4.13±0.64^{z}	25.0±1.81^{y}	27.7±0.9^{x}	27.2±1.65^{xy}	26.3±1.35^{xy}	26.5±1.52^{xy}
18:3n-3	1.78±0.25^{z}	11.9±0.71^{y}	14.2±0.66^{x}	13.7±1.01^{v}	12.9±0.86^{xv}	13.6±1.29^{xv}
18:4n-3	2.68±0.53^{z}	5.96±0.66^{y}	6.24±0.48^{y}	6.57±1.20^{y}	5.89±0.78^{y}	6.47±0.19^{y}
20:1	3.78±1.31^{z}	1.09±0.28^{y}	1.13±0.06^{y}	1.10±0.29^{y}	1.38±0.23^{y}	1.20±0.28^{y}
20:2n-6	0.32±0.04^{z}	0.44±0.05^{y}	0.39±0.01^{y}	0.41±0.06^{y}	0.46±0.06^{y}	0.42±0.06^{y}
20:3n-6	0.14±0.05^{z}	0.47±0.11^{y}	0.47±0.03^{y}	0.50±0.04^{y}	0.49±0.09^{y}	0.47±0.05^{y}
20:4n-6	0.62±0.07^{z}	0.25±0.02^{y}	0.22±0.03^{y}	0.23±0.03^{y}	0.27±0.01^{y}	0.25±0.02^{y}
20:3n-3	0.14±0.09^{z}	0.26±0.04^{y}	0.26±0.01^{y}	0.28±0.05^{y}	0.29±0.05^{y}	0.29±0.08^{y}
20:4n-3	6.03±0.49^{z}	1.31±0.27^{y}	1.15±0.37^{y}	1.24±0.54^{y}	1.67±0.24^{y}	1.40±0.43^{y}
22:1	0.41±0.06	0.12±0.03	0.27±0.36	0.13±0.03	0.16±0.02	0.14±0.03
20:5n-3	6.05±0.43^{z}	1.31±0.18^{y}	1.41±0.21^{y}	1.29±0.30^{y}	1.48±0.13^{y}	1.47±0.28^{y}
24:1	0.56±0.05^{z}	0.19±0.03^{y}	0.21±0.04^{y}	0.19±0.04^{y}	0.24±0.04^{y}	0.21±0.02^{y}
22:5n-3	2.90±0.24^{z}	0.71±0.17^{y}	0.79±0.17^{yx}	0.71±0.17^{y}	0.95±0.10^{x}	0.83±0.17^{yx}
22:6n-3	17.1±0.92^{z}	4.20±0.59^{y}	4.09±0.49^{y}	4.00±0.84^{y}	4.73±0.41^{y}	4.51±0.85^{y}
SAFA	19.7±0.42^{z}	14.3±0.97^{x}	12.9±1.35^{y}	12.7±1.13^{y}	13.7±1.40^{yx}	13.05±0.72^{y}
MUFA	30.9±0.65^{z}	30.0±3.32^{x}	25.6±0.60^{y}	25.6±0.51^{y}	26.1±0.95^{y}	25.67±0.85^{y}
PUFA	41.9±1.55^{z}	52.3±2.52^{x}	56.8±1.40^{y}	56.4±1.89^{y}	55.8±1.53^{y}	56.2±1.73^{y}
n3	36.7±1.40^{z}	25.6±1.04^{x}	28.1±0.65^{y}	27.8±1.08^{y}	27.9±0.76^{y}	28.6±1.04^{y}
n6	5.25±0.68^{z}	26.7±1.71^{y}	28.7±0.85^{x}	28.5±1.60^{y}	28.0±0.86^{yx}	27.6±1.57^{yx}
n3/n6	7.07±0.88^{z}	0.96±0.05^{y}	0.98±0.02^{y}	0.98±0.07^{y}	1.00±0.02^{y}	1.04±0.08^{y}
n-3HUFA/18:3n3	19.9±3.24^{z}	1.17±0.14^{y}	0.99±0.07^{y}	1.04±0.18^{y}	1.17±0.09^{y}	1.11±0.19^{y}

Abbreviations: see Table 2, n-3HUFA = n-3 highly unsaturated fatty acids. $^{u\text{-}z}$ Mean values across the row not sharing a common superscript are significantly different by $P<0.05$.

Table 6. Content of vitamin E, sesamin and episesamin in white muscle and liver (μg g^{-1}).

	FO	SesO	MO	MO 0.29	MO 0.58	MO 1.16
White muscle						
α-Tocopherol	1.36±0.58	1.00±0.19	1.09±0.73	1.46±0.20	1.50±0.26	1.53±0.23
γ-Tocopherol	0.15±0.04^{u}	0.80±0.50^{uv}	1.01±0.97^{vz}	1.74±0.36^{xy}	1.14±0.61^{vyz}	1.87±0.36^{x}
Episesamin	–	0.32±0.23	–	0.40±0.21	0.37±0.21	0.32±0.10
Sesamin	–	0.20±0.09	–	0.20±0.12	0.14±0.08	0.16±0.04
Liver						
α-Tocopherol	87.8±37.7^{y}	146.4±28.3^{y}	234.7±83.5^{z}	195.5±56.4^{z}	231.74±87.8^{z}	188.0±63.6^{z}
γ-Tocopherol	1.73±1.29^{y}	11. 9±4.05^{z}	11.3±3.00^{z}	8.30±2.30^{z}	9.46±3.11^{z}	9.37±2.63^{z}
Episesamin	–	2.43±0.95^{yz}	–	1.69±0.78^{y}	2.32±0.52^{yz}	3.14±0.75^{z}
Sesamin	–	1.90±1.17^{x}	–	0.67±0.27^{z}	0.81±0.08^{zy}	1.53±0.43^{xy}

Abbreviations: see Table 2. MO, n=4; FO, n=3; SesO, n=4; MO 0.29 n=5; MO 0.58 n=4; MO 1.16 n=5. $^{u\text{-}z}$ Mean values across the row not sharing a common superscript are significantly different by $P<0.05$.

Tocopherols and sesamin content

The contents of α- and γ- tocopherols in the liver were significantly lower (P<0.05) in FO group compared to the MO groups with or without addition of sesamin. In the white muscle, the γ-tocopherol content was significantly lower in the FO group than in the MO groups (P<0.05). No differences were found in the content of sesamin and episesamin in white muscle, in the liver sesamin and episesamin levels were higher in the MO 1.16 group, the difference was significant compared to the MO 0.29 group (P=0.03, P<0.01 for sesamin and episesamin respectively), but was higher than in the SesO (P=0.3, P=0.2 for sesamin and episesamin respectively) and MO 0.58 (P=0.1 both for sesamin and episesamin) group as well (Table 6).

Relative expression of target genes

The relative expressions of target genes in the experimental groups, compared to MO group, after normalization to the reference gene are shown in Figure 1. In the MO 1.16 group, PPARα (P=0.05) was upregulated compared to control group. In the MO 0.29 group, SRB (P=0.03) and HSL3 (P<0.01) were upregulated, and in the MO 0.58 group, HSL3 (P=0.03) was upregulated compared to the MO group.

Total content of CYP and EROD activity in the liver

The EROD activity did not differ significantly among groups (Figure 2). The CYP (Figure 2) levels were significantly higher in the MO 0.29 group (P=0.02) and slightly higher in the MO 1.16 group (P=0.07) compared to MO group and significantly lower in MO 0.58 group (P<0.01) compared to MO 0.29 group. The CYP response was also significantly higher in the SesO group compared to the MO group. There was a large variation in individual response detected, with CV values within groups from 16 up to 125 %.

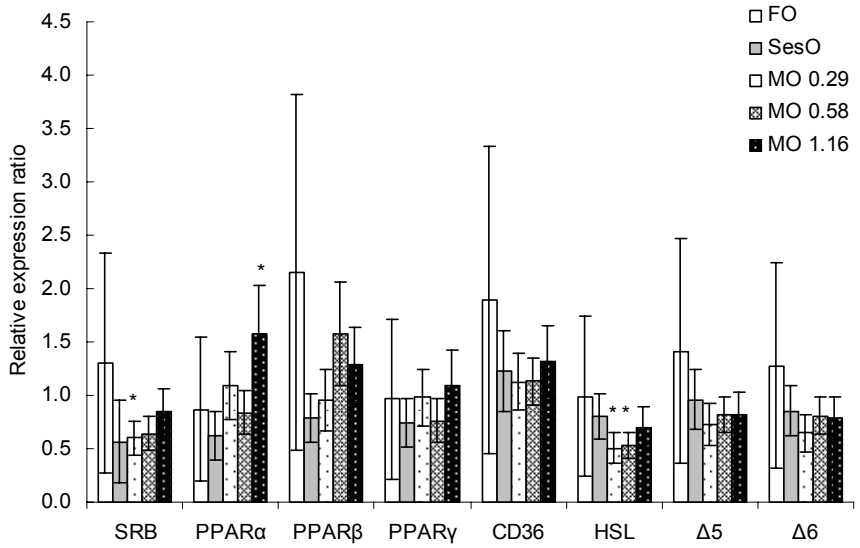

Fig. 1. Relative expression ratio compared to the MO group of the analyzed genes in the FO, SesO, MO 0.29, MO 0.58 and MO 1.16 groups. Genes with significantly different expression ratio to the MO group are indicated with an asterisk (P<0.05). For abbreviations see Table 2 and 3.

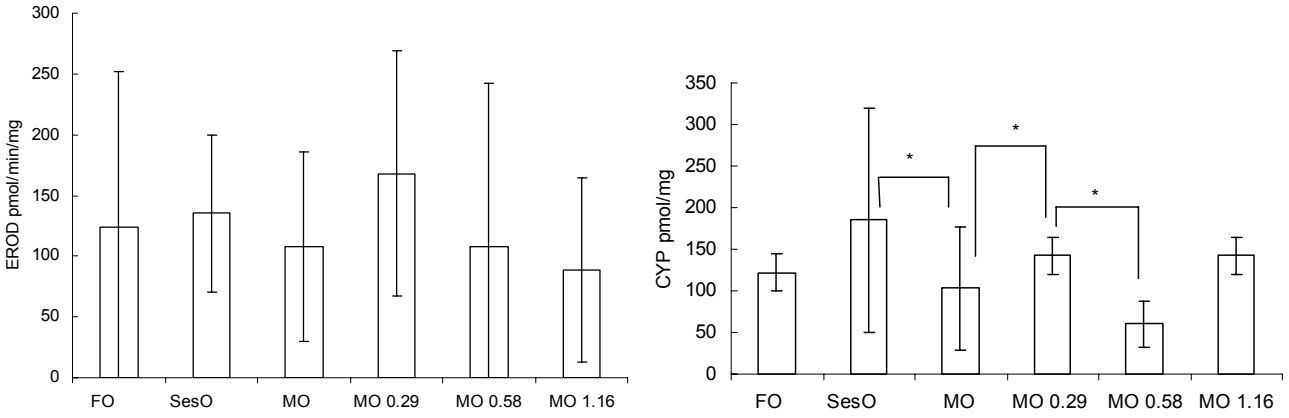

Fig. 2. Ethoxyresorufin O-deethylase activity, EROD (pmol.min^{-1}.mg^{-1} protein) and total cytochrome P450, CYP (nM/mg microsomal protein) in the liver. MO, n=4; FO, n=3; SesO, n=4; MO 0.29 n=5; MO 0.58 n=4; MO 1.16 n=5. Significantly different CYP content are indicated with an asterisk (P<0.05).

Discussion

In general, the results of different analyses in this study showed a large variation caused by a broad individual response. The reason for the different weight in the fish group is most likely caused by the low number of individuals as the fish were divided between groups according to stastistical methods. In Sweden, the long breeding history of Baltic Atlantic salmon smolts is aimed for release purposes as a replacement for the wild spawners being hindered the enter the rivers by hydro-electric power dams and the large number of parents is a prerequisite. Therefore, this fish had a wide range of genetic background compared to the fish used in our previous studies on rainbow trout and Atlantic salmon hepatocytes. Schlechtriem *et al.* (2007) found inter individual variations in FA composition of Atlantic salmon smolt, and suggested individual variation in elongation and desaturation capacity as a likely explanation. Other known factors affecting the capacity of elongation and desaturation are environmental factors and life stage, e.g. salmon prior seawater transfer had higher relative expression of Δ-5 and Δ-6 desaturase (Zheng *et al.* 2005). Addition of bioactive compounds such as 3-thia fatty acids, conjugate linolenic acid and lipoic acid can also influence lipid metabolism. The 3-thia fatty acids increased β-oxidation capacity and the levels of DHA (Moya-Falcon *et al.* 2006) and reduced mRNA expression of PPARα and apolipoproteinAI (ApoAI) (Kleveland *et al.* 2006) in Atlantic salmon. Conjugated linoleic acid decreased adipocytes by elevating energy expenditure (Kennedy *et al.* 2009). Dietary lipoic acid was shown to increase EPA levels in pacu (*Piractus mesopotamicus*) muscle (Trattner *et al.* 2007).

In our previous study on rainbow trout, significantly increased levels of DHA and decreased proportions of 18:3n-3 in the TAG and PL fractions were found after sesamin addition to the fish diet. Also the total level of polyunsaturated fatty acids (PUFA) was decreased, possibly due to increased β-oxidation of PUFA in sesamin fed fish (Trattner *et al.* 2008a). These results were confirmed in an in vitro study on Atlantic salmon hepatocytes incubated with radiolabelled 18:3n-3 with or without sesamin addition to the media. It was shown that the amount of 18:3n-3 elongated and desaturated to DHA was increased after sesamin incubation. It was also shown that sesamin increased the total level of β-oxidation products, in particular acetate, which indicated peroxisomal β-oxidation (Trattner *et al.* 2008b). Sesamin also decreased secretion of lipids (mainly TAG) in Atlantic salmon hepatocytes, in agreement with the lipid lowering effects reported as reduced TAG and VLDL levels in rat serum (Umeda-Sawada *et al.* 1998, Kamal-Eldin *et al.* 2000). In the present study, sesamin significantly decreased levels of 18:3n-3 in the PL of all sesamin fed groups, also the average DHA level was (not significantly) increased in the groups after sesamin addition to the diet.

In the rainbow trout study, two different oils, sunflower: linseed oil mixture (MO) and linseed oil (LO) were used. In that study the effects of sesamin on gene expression and FA composition were greater in MO diet than in LO diet. The use of 100 % linseed oil in the LO group decreased desaturation index (n-3HUFA/18:3n-3) compared to the use of a mixture of vegetable oil as in the MO group, indicating less efficient conversion of 18:3n-3 to DHA when linseed oil is included at higher levels in the diet. There are a number of studies showing decreased desaturation index with increased inclusion of linseed oil, a summary of results from studies are presented in Table 7. In a study on Atlantic salmon, it was suggested that increased inclusion of linseed oil due to its high content of 18:3n-3 inhibited elongation and desaturation of 18:3n-3 in hepatocytes, and increased oxidation of 18:3n-3 in enterocytes (Tocher *et al.* 2002). The replacement of fish oil with linseed oil, decreased the DHA levels in the liver 4-fold (Tocher *et al.* 2002), whereas replacement of the fish oil with rapeseed oil decreased the DHA levels to half (Bell *et al.* 2001). (Leaver *et al.* 2008) showed increased activity of fatty acyl Δ-6 desaturase in the liver of Atlantic salmon fed vegetable oils, the increased activity was highest for rapeseed oil followed by soybean oil and last linseed oil. The expression of *elov15b* and *elovl2* elongases were also significantly higher in liver of vegetable oil fed fish compared to fish oil fed fish, with lower expression in linseed oil fed fish than in rapeseed oil and soybean oil fed fish (Morais *et al.* 2009). In agreement with these studies, we also found decreased desaturation index in the MO group, which had higher 18:3n-3 content in the diet compared to the SesO group.

The differences in tocopherols (Table 6) are due to the difference in tocopherols in the diet, the vegetable oil had a higher content of tocopherols than the fish oil. The FO diet had low levels of α-tocopherol and levels below detection limit for γ-tocopherol. In contrast to our results, in rats and humans, it has been shown that sesamin increased the levels of γ-tocopherol and reduced

Table 7. An overview of the desaturation index in Atlantic salmon and rainbow trout fed different oils.

Reference	18:3n3 (%) diet	Oil source	Desat. index	Species	Tissue	Lipid fraction
Leaver *et al.* (2008)	1.2	FO	77.2	A. salmon	Liver	Total lipid
	44.9	LO	1.3			
	8.1	RO	11.5			
	5.7	SO	8.6			
Menoyo *et al.* (2007)	41.7	LO 100	1.73	A. salmon	Muscle	Polar lipids
	30.2	LO 75	2.71			
	25.8	LO 50	2.85			
	12.1	LO 25	5.20			
	41.7	LO 100	0.44	A. salmon	Muscle	Neutral lipids
	30.2	LO 75	0.56			
	25.8	LO 50	0.65			
	12.1	LO 25	1.27			
Trattner et al. (2008a)	31.7	MO	10.4	R. trout	Muscle	Phospholipid
	53.4	LO	5.0			
	31.7	MO	1.3			Triacylglycerol
	53.4	LO	0.7			
Rosenlund et al. (2001)	10.2	RO	1.8	A. salmon	Muscle	Total lipid
	22.6	LO	0.9			
	17.0	SO	1.0			

Desat. index = Desaturation index = (n-3HUFA > 18C) / 18:3n-3; FO = fish oil, LO = linseed oil, RO = rapeseed oil, SO = soybean oil, LO100 = 100 % linseed oil, LO75 = 75 % linseed oil and 25 % sunflower oil, LO50 = 50 % linseed oil and 50 % sunflower oil, LO25 = 25 % linseed oil and 75 % sunflower oil, MO = mixture of linseed oil and sunflower oil (6:4).

the urine extraction of its metabolites (Frank *et al.* 2004, Kamal-Eldin *et al.* 1995). In the liver, the sesamin and episesamin contents were increased with increased content in the diet. Even though the sesamin : episesamin ratio was 1:1 in the feed, episesamin was detected at higher levels in muscle and liver. This finding is in agreement with the study on rainbow trout and has also been found in rats (Trattner *et al.* 2008a, Umeda-Sawada *et al.* 1999).

Interestingly, even if there were no significant differences in the composition of n-3 HUFA in TAG, it was found that the MO 1.16 g group with increased relative expression of PPARα also had higher proportions of DPA and DHA in white muscle PL and TAG. A result well corresponding with the increased levels of DHA in the in vivo rainbow trout livers and the in vitro Atlantic salmon hepatocytes and increase β-oxidation in Atlantic salmon hepatocytes. The relative expression of PPARα in rainbow trout liver and Atlantic salmon hepatocytes, was also found previously to be effected by sesamin, although in these previous cases, this expression was downregulated. The highest levels of DPA and DHA were found in PL and TAG of the MO 0.58 group, which also had increased relative expression of HSL3. HSL activate intracellular hydrolysis of TAG, which can then be used for β-oxidation (Watt *et al.* 2003). The increase in DHA could also indicate increased β-oxidation as DHA is produced through β-oxidation of longer n-3 fatty acids (Voss *et al.* 1991).

The average values for each group in the EROD analysis indicate that the activity decreases with increased levels of sesamin and for the CYP analysis the content is lower at intermediate doses than at low and high dose of sesamin (Figure 2). However, due to the large variation within groups, it is difficult to draw any conclusions. It would be interesting to study the dose dependent response under in vitro conditions. The large individual response of enzymes involved in the defence against

xenobiotic compounds also support the above mentioned suggestion that individual fish react differently to bioactive compounds in the diet, also in terms of lipid metabolism.

To improve lipid metabolism in farmed fish may be a useful tool in the future to meet the demands for production of farmed fish with a healthy FA composition produced on less amounts of fish based raw materials. This study indicates that the response of Atlantic salmon (Swedish Baltic origin) to sesamin is less than in rainbow trout in vivo (Trattner *et al.* 2008a) and in Atlantic salmon in vitro (Trattner *et al.* 2008b). This difference deserves further evaluation and may be utilised for selection to improve desaturation capacity in farmed fish fed vegetable oils.

Conflict of Interest

There is no conflict of interest.

Abbreviations

ACO	Acyl-CoA oxidase
CD 36	Cluster of differentiation 36
CPT	Carnitine palmitoyltransferase
CYP	Cytochrome P450
Δ5	Δ5 desaturase
Δ6	Δ6 desaturase
DHA	Docosahexaenoic acid (22:6n-3)
DPA	Docosapentaenoic acid (22:5n-3)
EF1A	Elongation factor 1α
EPA	Eicosapentaenoic acid (20:5n-3)
EROD	Ethoxyresorufin O-deethylase
FA	Fatty acid
FO	Fish oil
HSL3	Hormone sensitive lipase
HUFA	Highly unsaturated fatty acids
MO	Linseed and sunflower oil 6:4 by vol
MUFA	Monounsaturated fatty acids
PL	Phospholipids
TAG	Triacylglycerol
TLC	Thin-layer chromatography
SesO	Sesame oil and linseed oil 1:1 by vol
SRB-I	Scavenger receptor type B
RPL2	RNA polymerase II polypeptide
PCR	Polymerase chain reaction
PPAR	Peroxisome proliferator-activated receptor
PUFA	Polyunsaturated fatty acids
SREBP	Sterol regulatory element binding protein
VLDL	Very low-density lipoprotein

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