Aquacult Int (2013) 21:843–859
DOI 10.1007/s10499-012-9511-y

The effect of combining linseed oil and sesamin on the fatty acid composition in white muscle and on expression of lipid-related genes in white muscle and liver of rainbow trout (*Oncorhynchus mykiss*)

AnnaLotta Schiller Vestergren · Sofia Trattner · Jinfeng Pan · Pernilla Johnsson · Afaf Kamal-Eldin · Eva Brännäs · Ali A. Moazzami · Jana Pickova

Received: 15 September 2011 / Accepted: 6 February 2012 / Published online: 22 February 2012

Abstract Sesamin (S) is a known lipid modulator and has been shown to increase the conversion of α-linolenic acid (ALA) to docosahexaenoic acid in rainbow trout (*Oncorhynchus mykiss*) fed vegetable oil mixtures including linseed oil. In this study, we evaluated the effects of S supplementation in linseed oil-based diets, content of α- and γ-tocopherols, fatty acid (FA) composition, as well as the gene expression of lipid-related genes. Fish with an average weight of 36.5 g were fed different combinations of commercial linseed oil (LO), purified linseed oil triacylglycerols (TAG) with polar fraction removed and a mixed linseed-sunflower oil (6:4 v/v) (MO). S was added at 0.58 g 100^{-1}g feed and fed to the fish for a period of 58 days. Expression of PPARα was downregulated in white muscle of fish fed S containing diets ($P < 0.05$). The expression of PPARβ1A was not affected by S supplementation except where TAG oil was used. The expression of PPARβ1A declined significantly in TAG + S fed group ($P < 0.05$), which indicates that some minor compounds in linseed oil might suppress the effect of S on the expression of PPARβ1A. The expression of PPARγ(long) declined in LO + S and MO + S fed group ($P < 0.05$). The β-oxidation-related genes CPT1 and ACO were upregulated by vegetable oils compared to fish oil. S decreased percentage of ALA in white muscle of fish fed LO + S ($P < 0.05$). The increased desaturation index and the decreased ALA levels suggest that S may increase the biosynthesis of highly unsaturated FA in rainbow trout.

Keywords ACO · ALA · CPT1 · Δ6 fatty acid desaturase · DHA · Linseed oil · PPARs · Sesamin · Sunflower oil

Abbreviations

AA	Arachidonic acid (20:4n-6)
ACO	Acyl-CoA oxidase

A. S. Vestergren · S. Trattner (✉) · J. Pan · P. Johnsson · A. Kamal-Eldin · A. A. Moazzami · J. Pickova
Department of Food Science, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden
e-mail: sofia.trattner@slu.se

S. Trattner · E. Brännäs
Department of Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences, 901 83 Umeå, Sweden

ALA	Alphalinolenic acid (18:3n-3)
CPT1	Carnitine palmitoyl transferase I
Δ6 fad	Delta-6 fatty acid desaturase
DPA	Docosapentaenoic acid (22:5n-6)
DHA	Docosahexaenoic acid (22:6n-3)
EPA	Eicosapentaenoic acid (20:5n-3)
FA	Fatty acids
HUFA	Highly unsaturated fatty acids
LA	Linoleic acid (18:2n-6)
LO	Linseed oil
LO + S	Linseed oil + sesamin
MO	Mixed oil
MO + S	Mixed oil + sesamin
MUFA	Monounsaturated fatty acids
n-3	Omega-3
n-6	Omega-6
n.s.	Not significant
PPARs	Peroxisome proliferator-activated receptor
PUFA	Polyunsaturated fatty acids
S	Sesamin
SAFA	Saturated fatty acids
TAG	Purified linseed oil triacylglycerols
TAG + S	Purified linseed oil triacylglycerols + sesamin
TLC	Thin layer chromatography

Introduction

Fish is considered a healthy food for humans due to its high content of highly unsaturated long chain n-3 fatty acid (HUFA) mainly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). The aquaculture industry, where fishmeal and fish oil are widely used as aquafeed components, is an increasingly important player in supplying fish for human consumption. However, parallel to the growing aquaculture production, the global production of fishmeal and fish oil remains stable or even decline. As a consequence, the production of fishmeal and fish oil will not be able to meet the aquaculture requirement in the future. This propels steps to find substitute of fish oil in commercial aquafeeds. Complete or partial replacement of fish oil with vegetable oils, such as rapeseed oil, palm oil, linseed oil, or soy oil, in fish diets has been studied intensively and has been shown to influence the lipid composition and fatty acid metabolism of salmonids such as Atlantic salmon (*Salmo salar* L.) (Thomassen and Rosjo 1989; Bell et al. 2004; Torstensen et al. 2005; Jordal et al. 2007) and rainbow trout (*Oncorhynchus mykiss*) (Pettersson et al. 2009; Turchini and Francis 2009). As a natural consequence, inclusions of vegetable oils also affect the expression of lipid-related genes (Jordal et al. 2005; Leaver et al. 2008; Torstensen et al. 2009; Trattner et al. 2008b). The drawback of replacing fish oil with vegetable oils is the lowered content of HUFA (Miller et al. 2008). To counteract the negative effect of a lower content of HUFA, several studies (Trattner et al. 2007, 2008a, b) have been performed to improve the conversion of α-linolenic acid (ALA, 18:3n-3) to HUFA by adding lipid modulators, known to increase the activity and gene

expression of enzymes involved in both mitochondrial and peroxisomal fatty acid oxidation in rodents (Ide et al. 2009; Ashakumary et al. 1999; Lim et al. 2007). For example, the sesame furanofuran lignan, sesamin, known as a naturally occurring peroxisome proliferator that strongly influences the lipid metabolism in rodents (Ashakumary et al. 1999; Schoonjans et al. 1996) as well as in humans (Jeng and Hou 2005), has been shown in equimixture with episesamin to increase synthesis of DHA and alter expression of genes involved in β-oxidation in Atlantic salmon hepatocytes (Trattner et al. 2008a) as well as in white muscle of rainbow trout (Trattner et al. 2008b). The addition of sesamin to linseed oil diet was less efficient in converting ALA to DHA than in a combination of linseeed oil and sunflower oil. The reason for this is not fully explored even though parts can be contributed to the proposed back regulation mechanism of n-3 HUFA biosynthesis by high ALA/AL ratio in the diet (Tocher et al. 2002), since the ALA content was higher in the pure linseed oil diet than in the mixed oil diet. Besides the potential effects of dietary fatty acids (FA), fish lipid quality may also be influenced by the minor components of vegetable oils; the so-called unsaponifiable fraction (Kamal-Eldin 2005). The unsaponifiable portion of linseed oil consists mainly of sterols and tocopherols and makes up about 0.4–1.4% of the total lipid content (Choo et al. 2007). The primary sterols are sitosterol, cycloartenol and campesterol (Schwartz et al. 2008).

Little is known about the effect of vegetable oil components other than the FA themselves. Minor components present in linseed oil may interfere with the desaturation and elongation of ALA to n-3 HUFAs or with other steps in the lipid homoeostasis, such as transcription regulation, mitochondrial- and peroxisomal β-oxidation. Therefore, studies investigating the role of minor vegetable oil components on fish lipids are highly warranted. The impact of new additives such as sesamin on lipid composition and quality in the fish need also to be studied further. Moreover, it is important to monitor the underlying mechanisms in the molecular level to better understand the implications of such an addition on metabolic regulation.

To evaluate the effect of sesamin and minor compounds in linseed oil on lipid homoeostasis, a screening on gene expression level of transcription regulators, rate-limiting enzymes involved in the HUFA biosynthesis, and mitochondrial and peroxisomal β-oxidation was performed. The pleiotropic nuclear receptors peroxisome proliferator-activated receptor (PPAR) family are key regulators of lipid homoeostasis. A wide range of genes and thereby their corresponding enzymes, involved in the desaturation and β-oxidation of FA, are evidently affected by sesamin (Jeng and Hou 2005; Kiso et al. 2005; Kushiro et al. 2002). Many of these effects are correlated with changes in activity of PPARs (Ashakumary et al. 1999; Trattner et al. 2008b; Sirato-Yasumoto et al. 2001). Several isoforms for PPARβ and γ have been described in salmonids (Leaver et al. 2007; Andersen et al. 2000). Most likely PPARα is also present in several isoforms; however, to our knowledge, they have not yet been identified. Delta-6 fatty acid desaturase (Δ6 fad) is the rate-limiting enzyme involved in HUFA biosynthesis responsible for the first step converting ALA to 18:4n-3 as well as in the DHA synthesis from EPA (Vagner and Santigosa 2010). Sesamin/episesamin exposure has been shown to down regulate gene expression of Δ6 fad as well as Δ5- fatty acid desaturase inconsistently coupled to an increased elongation and desaturation of ALA to DHA in A. salmon hepatocytes (Trattner et al. 2008b). It has also been shown to affect Acyl-CoA oxidase (ACO) and carnitine palmitoyl transferase I (CPT1) genes involved in β-oxidation (Trattner et al. 2008b).

This study aims to investigate the effect of linseed oil and stripped linseed oil after removing the minor compounds, often polar, to evaluate the importance of minor compounds of linseed oil and sesamin on rainbow trout lipid metabolism. FA composition in white muscle and the gene expression pattern in both liver and white muscle by studying the

expression of PPARα, PPARβ1A, PPARγ(long form), Δ6 fad, ACO and CPT1 were measured. The gene expression assays for these gene and isoforms were designed to cover the most predominantly expressed forms in liver and white muscle. We aimed to investigate whether sesamin might interact with components in the linseed oil to such a degree that the positive effect of sesamin is impaired when using pure linseed oil. In addition, impact of different plant oil-based diets with or without sesamin with different n-3/n-6 ratio is studied.

Materials and methods

Chemicals and reagents

Sesamin (98% purity) was obtained from KEB Biotech (Beijing, China). A commercial refined food-grade linseed oil was purchased from ZETA (Di Luca and Di Luca AB, Stockholm, Sweden). (+)α-tocopherol (no. T3634) and (+)γ-tocopherol (no. 47785 and T1782) were purchased from Sigma Aldrich (St. Louis, MO, USA). All other chemicals and solvents were purchased from Merck (Darmstadt, Germany).

Purification of linseed oil triacylglycerols

A commercial refined food-grade linseed oil was purified by adsorption chromatography to obtain purified triacylglycerol (TAG) fractions following the method of Fuster et al. (1998). In short, glass columns were packed (3 × 20 cm i.d.) with 150 g of aluminium oxide (Aluminium oxide 90 Active Neutral, Merck, Darmstadt, Germany) that had been activated in 100°C for ca 8 h followed by a period of ca 15 h at 200°C, and *n*-hexane. The oil was dissolved in an equal volume of hexane and passed through the column, which was then washed with *n*-hexane. Analysis of the purified linseed oil triacylglycerols by thin layer chromatography (TLC) using pre-coated silica gel 60 TLC glass plates (mean particle size 10–12 μm, 0.25 mm layer thickness, Merck, Darmstadt, Germany) with hexane/diethyl ether/acetic acid (85:15:1, v/v/v) as mobile phase showed that it mainly contained triacylglycerols together with small amounts of sterol esters and a compound that was eluted after the triacylglycerols. (+)γ-Tocopherol was added to the purified oil/*n*-hexane mixture to protect the oil from oxidation. Hexane was removed by vacuum evaporation followed by 17-h evaporation with nitrogen that was led through the oil while the oil was stirred and heated (≈45°C). When the oil no longer smelled of hexane, it was capped and stored in the freezer until further use. The level of residual hexane in the purified oil was determined by gas chromatographic (GC) analysis to <0.5 mg/kg ± 10% by Aarhus-Karlshanm (AAK, Karslhamn, Sweden). The maximum allowed level for hexane in food in Sweden is 1 mg/kg (SLVFS (1993:17).

Fish and diets

One month before the start of the feeding trial, juvenile rainbow trout were transported from Aquaculture North, Kälarne, Sweden, to the experimental facilities at Umeå Marine Research Station, Sweden. The rainbow trout (mean weight 36.5 g) were acclimatised to laboratory conditions during the 1 week in a 500-L fish tank supplied with 8°C tap water (non-chlorinated) and fed in excess with a commercial diet (Biomar®, 2 mm pelleted trout feed). They were tagged with a PIT-tag (Passive Integrated Transponder) by injecting the tag into the posterior part of the abdomen of each fish. Before handling, all fish were

anaesthetised (ethyleneglycol monophenyl ether 5 mL L^{-1}). The fish were then allowed to recover for 2 weeks. During the 4th week, the fish were gradually acclimatised to the experimental temperature 14.5°C and divided into 6 × 2 groups with each duplicate group consisting of 9 + 10 fish. Each group was placed in a 100-L water tank supplied with flow-through (1L/min) tap water and additional oxygenation. Each group was fed one of the experimental diets in surplus to requirement for 58 days until the average weight of the groups had increased two-fold.

The feeding was done by a computerised feeding system (ITU OY®, Jyväskyle, Finland) that calculates the daily ration based on temperature, biomass fish in each tank and growth model. The diets were prepared as described by (Sanchez-Vazquez et al. 1999) and contained a base of defatted fish meal (TrippleNine, Esbjerg, Denmark) (21.8%), vegetable oil (21.8%), casein (18.6%), starch (15%), dextrin (9.8%), calcium sulphate (4%), sodium alginate (4%), gelatine (3.1%) and vitamin and mineral mixture (0.3%). Sesamin was added at 0.58 g 100^{-1} g feed according to (Trattner et al. 2008b). The diets used were as follows: (1) linseed oil (LO), (2) linseed oil with added sesamin (LO + S), (3) purified linseed oil (TAG), (4) purified linseed oil with sesamin (TAG + S), (5) linseed and sunflower oil mixed 6:4 v/v (MO) and (6) the mixed oil with sesamin (MO + S). Analyses of the six diets are shown in Table 1. The daily growth coefficient (DGC) was calculated as: $DGC = 100 \times (W_2^{1/3} - W_1^{1/3})D^{-1}$ with W_2 being final weight, W_1 the starting weight and D the number of days (Brännäs et al. 2005).

Fatty acid and lipid class analysis

Lipids from white muscle and diets were extracted following the method of Hara and Radin (1978). Triacylglycerols and phospholipids contribution to total lipids in fish white muscle was determined by analysing duplicate samples of total lipids on TLC plates with an automatic CAMAG 4 TLC sampler (Camag, Muttenz, Switzerland). Triacylglycerols and phospholipids were assigned by comparison to an external standard (TLC 18-4A, Nu-Chek Prep, Elysian, MN, USA). Peaks were integrated using winCATS software version 1.4.4. For GC analysis, total lipids of fish white muscle were separated into triacylglycerols and phospholipids according to Pickova et al. (1997). The total lipids from the diets and triacylglycerols and phospholipids from white muscle were methylated according to Appelqvist (1968) and analysed on a Varian CP-3800 gas chromatograph (GC) system (Agilent Technologies, Santa Clara, CA, USA) using the same column and conditions as in Trattner et al. (2008b). Fatty acid peaks were identified by comparison with the standard fatty acid mixture GLC standard 461, Nu-Chek Prep, Elysian, MN, USA) and retention times. Peak area integration was performed using star chromatography workstation software version 5.5 (Varian AB, Stockholm, Sweden).

Adjustment of α-linolenic acid, linoleic acid, α- and γ-tocopherol content in commercial and purified linseed oils

The percentage FA composition of purified linseed oil (TAG) and whole linseed oil was obtained from GC analysis (see above). The ALA and LA content of whole linseed oil was calibrated to that of the purified linseed oil by mixing two batches of commercial oil 1:1 v/v (batch no. L8 102 and L8 135) with different ALA and LA proportions. The content of α- and γ-tocopherol was determined by high-performance liquid chromatography (HPLC) (see below). The concentrations of the two tocopherols were adjusted in the two oils by addition of +α- and +γ-tocopherol.

Table 1 Lipid content, fatty acid profile (% of total identified) and α-tocopherol, γ-tocopherol and sesamin content (mg 100 g^{-1} lipid) of different diets

	Control	LO	LO + S	TAG	TAG + S	MO	MO + S
Lipid %	19.3	20.0	22.6	22.5	22.1	22.1	22.0
Fatty acids							
14:0	7.50	0.19	0.20	0.17	0.17	0.19	0.18
16:0	18.1	6.03	6.16	5.95	5.91	6.39	6.12
16:1n-7	7.0	0.17	0.18	0.16	0.16	0.18	0.18
18:0	2.40	3.54	3.53	3.59	3.59	3.54	3.45
18:1n-9	11.1	21.7	21.9	22.0	22.0	23.8	23.7
18:1n-7	2.30	0.76	0.76	0.76	0.76	0.74	0.81
18:2n-6	1.50	21.3	23.1	20.9	21.1	36.3	36.8
18:3n-3	1.30	37.9	36.1	36.9	36.8	22.9	22.8
20:1n-9	5.20	0.33	0.34	0.39	0.35	0.32	0.32
22:1n-11	8.40	0.11	0.11	0.11	0.10	0.10	0.11
20:5n-3	9.40	0.16	0.15	0.15	0.15	0.16	0.16
22:6n-3	10.2	0.39	0.38	0.37	0.37	0.37	0.40
n-3/n-6	10.8	1.79	1.58	1.78	1.76	0.64	0.63
Vitamins							
α-tocopherol	2	5	4	Trace	Trace	17	20
γ-tocopherol	n.d.	31	20	24	26	18	20
Sesamin	n.d.	n.d.	249	n.d.	273	n.d.	237

LO, linseed oil; LO + S, linseed oil + sesamin; TAG, purified linseed oil; TAG + S, purified linseed oil + sesamin; MO, mixed oil of LO and sunflower oil; MO + S, MO + sesamin; n-3, 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3; n-6 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6; n.d., not detected

Sesamin and tocopherol determinations

For the analysis of sesamin and α- and γ-tocopherols in oils, feed and fish white muscle, lipids were dissolved in hexane and analysed by HPLC using the same system, column and conditions as described by Moazzami and Kamal-Eldin (2006). The concentrations of α- and γ-tocopherol and sesamin were determined by reference to authentical standards using the linear equation obtained from triplicate five-point standard curves. If A is the area response from the analysis and c is the unknown concentration, the concentration of α- and γ-tocopherol was calculated as $A_\alpha = 0.9733 \times c_\alpha + 0.029$ ($r_\alpha^2 = 0.9996$) and $A_\gamma = 1.1203 \times c_\gamma + 0.0224$ ($r_\gamma^2 = 0.9994$), respectively. The concentration of sesamin was calculated by using the relative response factor of sesamin to α-tocopherol ($A_{ses} = 0.9733 \times c_{ses} \times 5.4 + 0.029$).

RNA isolation and quantitative real-time PCR

Total RNA was extracted from fish liver using SV Total RNA Isolation System (Z3105, Promega, Madison, Wisconsin, USA). Total RNA was quantified using a GeneQuant™ 1300 Spectrophotometer (GE Healthcare Life Sciences, Uppsala, Sweden). First strand cDNA was synthesised using the High-Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA, USA). Relative abundance of peroxisome proliferator-activated receptor

(PPAR)-mRNA was assessed using Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). All procedures were undertaken in agreement with the different manufacturers' instructions. Primers for real-time PCR analysis (Table 2) were designed based on available Atlantic salmon sequences from the online version of GenBank®(NCBI) using Primer Express® software (Applied Biosystems, Foster City, CA, USA) (Trattner et al. 2008b), evaluated for similarity with available rainbow trout sequences and custom made at Invitrogen (Carlsbad, CA, USA). The PCR reaction mix (20 µl) consisted of 0.9 µl of each forward and reverse primer (final concentration of 0.5 µM), 2 µl cDNA and 10 µl master mix. The reaction was incubated at 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min followed by a melt curve analysis. Real-time PCR was performed using the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). All samples were run simultaneously for each gene in triplicate with a non-template control on each plate. Elongation factor 1a (EF1α), NADH-ubiquinone oxidoreductase (NUOR), Eukaryotic translation initiation factor 3 (ETiF) and RNA polymerase II polypeptide (RPL2) were evaluated for their stability across all samples, and NUOR was chosen as the most stable reference gene (results not shown) using the DataAssist software version 2.0 (Applied Biosystems, Foster City, CA, USA). The ΔC_T was calculated by subtracting the C_T for NUOR from the C_T for the gene of interest. The relative expression was then calculated comparing the ΔC_T values for fish fed with or without addition of sesamin using the term $2^{-\Delta\Delta CT}$ and reported as arbitrary fold change units (Livak and Schmittgen 2001). For liver samples, the relative expression is relative to the FO group, and for the white muscle samples, the relative expression is relative to the control feed vegetable oil and no sesamin.

Statistical evaluation

All data in tables are presented as mean values ± standard deviation (SD). Difference between values were considered as significant when $P \leq 0.05$. FA was compared using the general linear model (GLM) in SAS statistical software (SAS Institute Inc., Cary, NC, USA, version 9.1). The model included the fixed effect of treatment and random effect of individual. Correlation tests were performed using Minitab 15 statistical software (data not shown) (Minitab, State College, PA, USA). Relative expression of the different genes, in relation to housekeeping genes, was determined, and mean values as well as SD were calculated using StepOne™ software version 2.2 and DataAssist software version 2.0 (Applied Bio Foster City, CA, USA). The 95% confidence interval was calculated and used for statistical discrimination evaluation.

Results

Fish growth

No mortality occurred during the experiment. There was no difference in initial weight, final weight or DGC among the twelve fish groups or the six treatments ($P > 0.05$) (Table 3).

Lipids of fish white muscle

All fatty acids in the three experimental diets differed significantly from the control feed. The lipid content of the white muscle did not show any differences regardless of diet (Table 3).

Table 2 Sequence of primers for real-time PCR analysis

Primer	Forward primer (5′–3′)	Reverse primer (5′–3′)	GenBank Acc. no.	Amplicon length
ACO	CCTTCATTGTACCTCTCCGCA	CATTTCAACCTCATCAAAGCCAA	DQ364432	101
CPT1	GTACCAGCCCCGATGCCTTCAT	TCTCTGTGCGACCCTCTCGGAA	AM230810	124
Δ6	AGAGCGTAGCTGACACAGCG	TCCTCGGTTCTCTCTGCTCC	AY458652	51
EF1α	CACCACCGGCCATCTGATCTACAA	TCAGCAGCCTCCTTCTCGAACTTC	AF321836	77
ETiF	CAGGATGTTGTTGCTGGATGGG	ACCCAACTGGGCAGGTCAAGA	DW542195	102
NUOR	CAACATAGGGATTGGAGAGCTGTACG	TTCAGAGCCTCATCTTGCCTGCT	DW532752	119
PPARα	TCCTGGTGGCCTACGGATC	CGTTGAATTTCATGGCGAACT	DQ294237	111
PPARβ1A	GAGACGGTCAGGGAGCTCAC	CCAGCAACCCGTCCTTGTT	AJ416953	151
PPARγ(long)	CATTGTCAGCCTGTCCAGAC	TTGCAGCCCTCACAGACATG	AJ292963	144
RPL2	TAACGCCTGCCTCTTCACGTTGA	ATGAGGGACCTTGTAGCCAGCAA	CA049789	110

ACO acyl-CoA oxidase, *CPT*1 carnitine palmitoyl transferase I, *Δ6* delta-6 desaturase, *EF1α* elongation factor 1a, ETiF eukaryotic translation initiation factor 3, *NUOR* NADH-ubiquinone oxidoreductase, *PPAR* peroxisome proliferator-activated receptor, *RPL2* RNA polymerase II polypeptide

Table 3 Initial weight (g). final weight (g) and daily growth coefficient (DGC) of rainbow trout and lipid content (lipid %) in white muscle of the fish fed different diets. mean values ± SD

Diet	Initial weight ($n = 19$)	Final weight ($n = 19$)	DGC ($n = 19$)	Lipid content ($n = 6$)
LO	37.5 ± 10.0	88.5 ± 31.5	2.4 ± 0.4	1.3 ± 0.6
LO + S	35.2 ± 11.7	86.3 ± 28.4	2.5 ± 0.6	1.5 ± 0.7
TAG	38.3 ± 13.4	96.4 ± 31.9	2.3 ± 0.3	1.7 ± 0.7
TAG + S	37.6 ± 10.0	82.3 ± 23.1	2.2 ± 0.4	1.7 ± 0.4
MO	37.6 ± 15.6	90.4 ± 38.8	2.4 ± 0.5	1.6 ± 0.3
MO + S	34.8 ± 9.8	85.3 ± 27.3	2.0 ± 0.6	1.3 ± 0.5
Control	–	70.0 ± 12.7	–	1.2 ± 0.3

The ratio between n-3/n-6 FA decreased significantly ($P < 0.05$) in all fish fed vegetable oil diets as a consequence of the higher amount of n-6 FA. The lowest values for n-3/n-6 were observed for fish fed MO and MO + S diets in both the triacylglycerols and the phospholipids as a result of a higher content of linoleic acid (LA) coincided with a lower content of ALA compared to the pure linseed oil diets. The overall n-3 FA content increased in all fish fed LO and TAG diets compared to those fish fed the fish oil-based diet. The composition of fatty acids changed from a high content of highly unsaturated n-3 FA (HUFA) like EPA and DHA towards a high content of ALA. The same trend was seen in n-6 HUFA towards shorter chain FA.

Some of the white muscle FA were significantly affected when sesamin was added to the feed (Table 4). The presence of sesamin in the LO diet significantly decreased ALA level in triacylglycerols ($P < 0.05$). Arachidonic acid (AA) increased in TAG + S in triacylglycerols while in MO + S, AA decreased significantly in phospholipids ($P < 0.05$). No significant difference in content of α-tocopherol or γ-tocopherol was found among fish from the six dietary groups (Table 5).

Gene expression in the liver

Of the three transcription factors tested, PPARβ1A was significantly upregulated ($P < 0.05$) in liver for all treatments compared to FO (Fig. 1a). For PPARα, the relative increase was rather moderate staying around a factor 1.5 when vegetable oil was compared to FO. The only significant changes of PPARα were the upregulation for MO compared to FO and the downregulation when adding sesamin to the MO diet. For PPARγ(long), the increase varied from 1.5 to 2.1, with significant increase ($P < 0.05$) in LO, MO and TAG + S groups compared to FO. Addition of sesamin decreased the relative expression compared to FO groups of PPARγ(long) in MO + S group. For Δ6 fad, a significant upregulation was detected MO, MO + S and TAG + S with a factor of 2.2, 6.9 and 3.2, respectively. Also a significant upregulation ($P < 0.05$) was found when sesamin was added to MO and TAG. In comparison to FO, the expression of CPT1 was significantly ($P < 0.05$) upregulated in all diets expect for LO. When adding sesamin to the LO diet, the relative expression of CPT1 was significantly increased. A striking upregulation of ACO was detected in all diets with a fold change ranging from 40 to 80 (Fig. 1b). When adding sesamin, the expression of ACO was downregulated in MO and upregulated in LO group.

Table 4 Fatty acid profile (% of total identified, mean values ± SD) in triacylglycerols and phospholipids ($n = 6$) from rainbow trout white muscle

Fatty acid	Triacylglycerols						
	Control	LO	LO + S	TAG	TAG + S	MO	MO + S
SAFA	22.7 ± 0.9^{b}	17.3 ± 1.2^{a}	18.8 ± 1.3^{a}	18.2 ± 0.5^{a}	17.5 ± 1.5^{a}	17.8 ± 0.9^{a}	18.8 ± 1.3^{a}
MUFA	27.9 ± 0.6^{a}	29.5 ± 1.8^{ab}	30.5 ± 1.8^{b}	30.9 ± 1.0^{b}	30.8 ± 1.6^{b}	30.9 ± 1.7^{b}	31.4 ± 0.9^{b}
18:2n-6	6.44 ± 0.95^{a}	15.8 ± 0.9^{b}	14.5 ± 1.6^{b}	13.5 ± 0.6^{b}	14.3 ± 1.3^{b}	22.7 ± 2.1^{c}	20.3 ± 1.8^{c}
18:3n-3	1.37 ± 0.12^{a}	17.4 ± 1.4^{d}	14.9 ± 2.6^{c}	14.0 ± 1.0^{c}	14.8 ± 1.9^{c}	9.04 ± 0.89^{b}	8.32 ± 0.56^{b}
18:4n-3	6.81 ± 0.54^{a}	2.51 ± 1.26^{b}	2.40 ± 1.31^{b}	3.63 ± 0.31^{b}	3.07 ± 0.60^{b}	2.35 ± 0.52^{b}	2.38 ± 0.43^{b}
20:2n-6	0.39 ± 0.03^{a}	0.51 ± 0.08^{ab}	0.51 ± 0.14^{ab}	0.44 ± 0.07^{a}	0.39 ± 0.11^{a}	0.76 ± 0.15^{b}	0.60 ± 0.09^{b}
20:3n-6	0.13 ± 0.09^{a}	0.38 ± 0.10^{b}	0.40 ± 0.17^{b}	0.33 ± 0.09^{b}	0.28 ± 0.11^{a}	0.47 ± 0.08^{b}	0.50 ± 0.21^{b}
20:4n-6	0.44 ± 0.03^{c}	0.26 ± 0.05^{a}	0.30 ± 0.03^{a}	0.26 ± 0.03^{a}	0.35 ± 0.05^{bc}	0.39 ± 0.04^{bc}	0.35 ± 0.08^{bc}
20:4n-3	0.00 ± 0.00^{a}	0.60 ± 0.10^{d}	0.53 ± 0.20^{cd}	0.46 ± 0.09^{c}	0.39 ± 0.09^{c}	0.24 ± 0.17^{b}	0.26 ± 0.13^{b}
20:5n-3	3.88 ± 0.34^{d}	1.91 ± 0.12^{a}	2.14 ± 0.44^{abc}	2.39 ± 0.30^{bc}	2.49 ± 0.29^{c}	2.08 ± 0.34^{ab}	2.24 ± 0.29^{abc}
22:5n-3	1.46 ± 0.07^{b}	0.77 ± 0.12^{a}	0.87 ± 0.23^{a}	0.92 ± 0.07^{a}	1.07 ± 0.21^{a}	0.85 ± 0.19^{a}	0.97 ± 0.17^{a}
22:6n-3	10.8 ± 0.6^{b}	5.88 ± 0.82^{a}	6.32 ± 0.80^{a}	5.99 ± 0.57^{a}	6.06 ± 0.66^{a}	5.75 ± 0.30^{a}	6.47 ± 0.93^{a}
PUFA	31.7 ± 0.9^{a}	48.2 ± 1.8^{b}	45.4 ± 3.8^{b}	45.3 ± 1.3^{b}	46.7 ± 1.6^{b}	47.5 ± 2.0^{b}	45.8 ± 1.5^{b}
n-3	24.3 ± 1.0^{b}	30.8 ± 1.3^{c}	29.3 ± 3.0^{c}	30.3 ± 0.8^{c}	30.9 ± 0.7^{c}	22.6 ± 0.4^{a}	23.4 ± 0.9^{ab}
n-6	7.40 ± 0.9^{a}	17.5 ± 1.0^{b}	16.1 ± 1.8^{b}	15.0 ± 0.7^{b}	15.8 ± 1.5^{b}	25.0 ± 2.2^{c}	22.4 ± 2.1^{c}
n-3/n-6	3.34 ± 0.51^{e}	1.77 ± 0.12^{b}	1.83 ± 0.26^{bc}	2.02 ± 0.08^{d}	1.97 ± 0.20 ^{cd}	0.91 ± 0.09^{a}	1.06 ± 0.13^{a}
Fatty acid	Phospholipids						
	Control	LO	LO + S	TAG	TAG + S	MO	MO + S
SAFA	21.8 ± 0.3^{a}	22.4 ± 2.7^{ab}	25.0 ± 2.5^{b}	21.5 ± 1.7^{ab}	22.4 ± 1.9^{ab}	23.6 ± 2.0^{ab}	23.4 ± 4.3^{ab}
MUFA	8.00 ± 0.60^{a}	10.2 ± 0.4^{b}	10.5 ± 1.4^{b}	10.2 ± 1.1^{b}	11.0 ± 0.6^{b}	11.2 ± 1.5^{b}	10.1 ± 0.9^{b}
18:2n-6	2.35 ± 0.43^{a}	6.33 ± 0.59^{c}	5.70 ± 0.35^{bc}	5.85 ± 0.32^{b}	5.51 ± 0.64^{b}	9.33 ± 0.65^{d}	8.60 ± 0.96^{d}
18:3n-3	0.61 ± 0.07^{a}	9.39 ± 0.51^{c}	8.33 ± 0.63^{c}	9.12 ± 0.61^{c}	8.48 ± 0.98^{c}	6.09 ± 0.50^{b}	5.56 ± 0.97^{b}
18:4n-3	1.03 ± 0.53^{a}	1.78 ± 0.61^{ab}	1.42 ± 0.32^{ab}	1.94 ± 0.37^{b}	1.44 ± 0.39^{ab}	1.28 ± 0.46^{ab}	1.39 ± 0.42^{ab}

Table 4 continued

Fatty acid	Phospholipids						
	Control	LO	LO + S	TAG	TAG + S	MO	MO + S
20:2n-6	0.22 ± 0.13^{a}	0.48 ± 0.06^{b}	0.46 ± 0.14^{b}	0.48 ± 0.10^{b}	0.41 ± 0.10^{ab}	0.75 ± 0.16^{c}	0.64 ± 0.20^{bc}
20:3n-6	0.23 ± 0.04^{a}	0.74 ± 0.17^{b}	0.68 ± 0.26^{b}	0.70 ± 0.17^{b}	0.58 ± 0.25^{b}	0.97 ± 0.23^{b}	1.13 ± 0.63^{b}
20:4n-6	0.54 ± 0.51^{a}	0.82 ± 0.15^{a}	0.95 ± 0.17^{ab}	0.81 ± 0.06^{a}	0.96 ± 0.19^{ab}	1.48 ± 0.16^{c}	1.12 ± 0.30^{b}
20:4n-3	0.00 ± 0.00^{a}	0.78 ± 0.12^{c}	0.67 ± 0.17^{c}	0.76 ± 0.17^{c}	0.62 ± 0.11^{c}	0.44 ± 0.14^{b}	0.42 ± 0.11^{b}
20:5n-3	6.84 ± 0.36	5.84 ± 0.74	6.10 ± 0.80	6.25 ± 0.66	6.43 ± 0.75	5.94 ± 0.20	5.59 ± 1.16
22:5n-3	1.98 ± 0.18^{b}	1.48 ± 0.11^{a}	1.50 ± 0.24^{a}	1.71 ± 0.09^{b}	1.77 ± 0.11^{b}	1.51 ± 0.11^{a}	1.49 ± 0.11^{a}
22:6n-3	50.4 ± 1.5^{b}	31.5 ± 2.4^{a}	32.2 ± 3.1^{a}	32.7 ± 2.1^{a}	33.5 ± 1.1^{a}	31.6 ± 2.3^{a}	34.1 ± 2.0^{a}
PUFA	64.2 ± 1.2	62.1 ± 2.8	60.1 ± 3.1	63.3 ± 2.5	62.1 ± 1.4	61.0 ± 3.4	62.4 ± 4.1
n-3	60.9 ± 1.4^{c}	53.5 ± 2.6^{ab}	52.2 ± 3.1^{ab}	55.1 ± 2.6^{ab}	54.4 ± 0.9^{b}	48.1 ± 2.9^{a}	50.7 ± 3.2^{ab}
n-6	3.34 ± 0.99^{a}	8.56 ± 0.74^{b}	7.95 ± 0.48^{b}	8.19 ± 0.32^{b}	7.75 ± 0.95^{b}	12.8 ± 0.9^{c}	11.7 ± 1.5^{c}
n-3/n-6	19.9 ± 7.4^{d}	6.28 ± 0.57^{b}	6.58 ± 0.56^{bc}	6.75 ± 0.51^{bc}	7.10 ± 0.84^{c}	3.77 ± 0.26^{a}	4.36 ± 0.44^{a}

SAFA; Saturated fatty acids (14:0, 16:0, 18:0); MUFA, monounsaturated fatty acids (16:1n-7, 18:1n-7, 18:1n-9, 20:1n-9, 22:1n-11); ALA α-linolenic acid (18:3n-3); LA linoleic acid (18:2n-6); EPA eicosapentaenoic acid (20:5n-3); DHA docosahexaenoic acid (22:6n-3); PUFA, polyunsaturated fatty acids (18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-6, 20:4n-6, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3); Control, group fed fish oil diet, for other abbreviations see Table 1

[a–d] Mean values for the same lipid class (triacylglycerols or phospholipids) with different superscripts (a, b, c, d, e where e > d > c > b > a) are significantly different ($P < 0.05$)

Table 5 Content of tocopherol and sesamin in white muscle from rainbow trout fed different diets (mg 100 g-1 lipid; $n = 6$)

Diet	α-tocopherol	γ-tocopherol	Sesamin
LO	31.7 ± 10.3	17.4 ± 7.6[b]	n.d.
LO + S	36.1 ± 9.4	11.3 ± 5.1[ab]	14.4 ± 7.4
TAG	39.2 ± 9.0	15.3 ± 4.3[b]	n.d.
TAG + S	21.9 ± 2.8	13.6 ± 5.3[ab]	15.3 ± 9.5
MO	30.1 ± 10.8	7.4 ± 4.6[a]	n.d.
MO + S	38.0 ± 14.4	9.0 ± 4.2[a]	11.5 ± 5.9

n.d. means not detected. For other abbreviations see Table 1

[a,b] Mean values in the same columns with different superscripts (a, b, where b > a) are significantly different ($P < 0.05$)

Gene expression in the white muscle

The relative expression (compared to non-sesamin groups) of all analysed genes except CPT1 and PPARβ1A was significantly downregulated when sesamin was added to the LO diet (Fig. 2a). CPT1 was significantly upregulated for the same treatment. The only effect seen when adding sesamin to MO diet was an upregulation of PPARβ1A (Fig. 2b). The sesamin addition to TAG diets downregulated the relative expression of all genes tested except CPT1, which was significantly upregulated (Fig. 2c).

Discussion

The main purpose of this study was to investigate the effect of linseed oil and purified linseed oil, where minor compounds were removed, and the effect of added sesamin. Changes in FA composition in white muscle and the expression of lipid-related genes in liver and white muscle of rainbow trout were investigated. No major effects of sesamin were seen on FA composition in white muscle. However, in the triacylglycerol fraction of LO + S group, ALA was significantly decreased compared to LO group. As expected, the FA composition of muscle reflected the composition of fish feed, with lower proportion of SAFA and higher proportion of PUFA in the triacylglycerol fraction of vegetable oil fed fish compared to FO. These results do not support the findings from our previous study where sesamin increased DHA (Trattner et al. 2008a). We also speculated that the linseed oil may contain substances that could impair elongation and desaturation of ALA towards EPA and DHA (Trattner et al. 2008a), as the ratio of long chain polyunsaturated n-3 fatty acids and ALA was higher in mixed oil diet compared to linseed oil diet, indicating higher conversion of ALA towards EPA and DHA. The present study demonstrates that fish fed TAG diet had higher proportion of EPA and lower proportion of ALA in triacylglycerol fraction and increased level of DPA in phospholipid fraction compared to the LO diet. These results could indicate that the removed polar fraction of the linseed oil has some effect on the metabolism of PUFA. The n-3/n-6 ratio of the linseed oil-based diet in Trattner et al. (2008a) was 3.6 vs the ratio 1.8 in present study, which could have an impact on FA metabolism. Another possible reason for the lower DHA level in this study is that only sesamin was used in our study whereas an equimixture of sesamin and episesamin was used by Trattner et al. (2008a) and episesamin has been reported to be a stronger lipid modulator than sesamin (Jeng and Hou 2005; Kiso et al. 2005; Kushiro et al. 2002). Also,

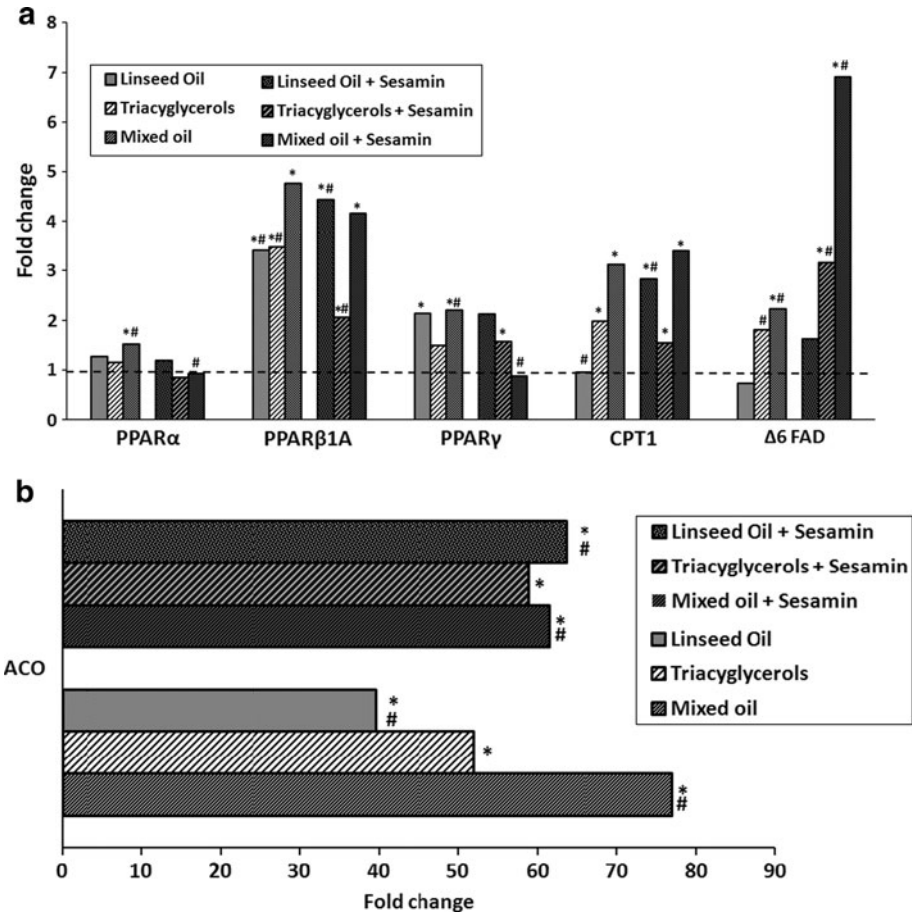

Fig. 1 Relative expression of genes involved in lipid homoeostasis expressed as fold change in liver of fish fed either linseed (LO), purified linseed (TAG) or linseed/sunflower mixed oils (6/4 v/v) (MO) with and without sesamin (S) compared to the expression in control fish fed fish oil-based diet. **a** Peroxisome proliferator-activated receptor alpha, beta, and gamma (PPARα, β1A and γ), delta-6 fatty acid desaturase (Δ6 FAD) and carnitine palmitoyl transferase I (CPT1). **b** Acyl-CoA oxidase (ACO). The *dotted line* indicates the gene expression reference level in livers of control fish, (*Asterisk*) denotes significant difference from fish fed with fish oil-based diet ($P < 0.05$), $n = 6$ and (*Number sign*) denotes significant difference from fish fed vegetable oils without sesamin and fish fed the same diet with sesamin addition ($P < 0.05$), $n = 6$. For other abbreviations, see Table 1

the size of fish could have an impact on the FA metabolism; in this study, the fish were larger that the fish used by Trattner et al. (2008a). Zheng et al. (2005) showed that environmental factors can influence the FA elongation and desaturation. Many mammalian studies have proven that sesamin is metabolised, which is coupled to increases in n-3HUFA/ALA through effects on elongation and desaturation (Fujiyama-Fujiwara et al. 1995; Mizukuchi et al. 2003), peroxisomal β-oxidation or other mechanisms involved in FA turnover (Ashakumary et al. 1999; Jeng and Hou 2005; Sirato-Yasumoto et al. 2001).

The shift in substrate availability for β-oxidation in salmonid species when changing the diet from dietary fish oils to vegetable oils has been thoroughly demonstrated both at the enzymatic and gene expression levels in several studies (Stubhaug et al. 2006; Tocher et al.

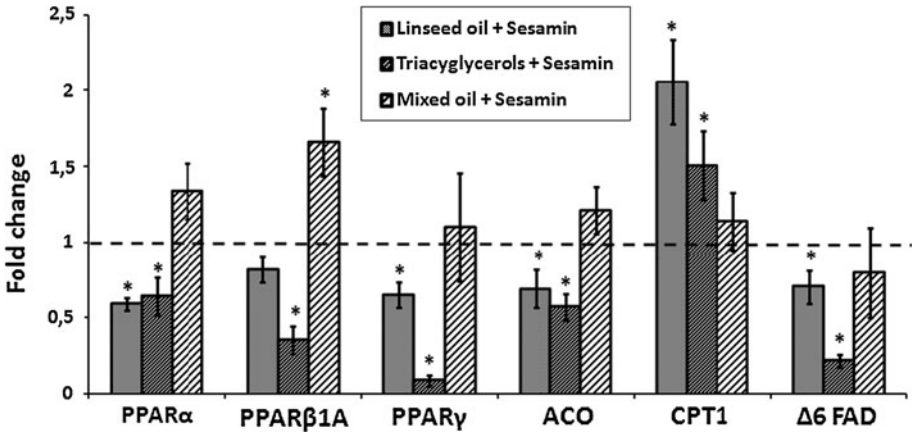

Fig. 2 Relative expression of genes involved in lipid homoeostasis in white muscle of fish fed linseed oil-based diets with sesamin, triacylglycerol-based diets with sesamin and mixed oil-based diets with sesamin. The *dotted line* indicate the gene expression reference level in white muscle of fish fed vegetable oils without sesamin. *Asterisk* denotes significant difference from vegetable oil diets without sesamin ($P < 0.05$), $n = 6$. PPARα, β and γ = peroxisome proliferator-activated receptor alpha, beta and gamma; ACO = acyl-CoA oxidase; CPT1 = carnitine palmitoyl transferase I and Δ6 FAD = delta-6 fatty acid desaturase

2003). Our findings also clearly show an increased mRNA expression in the liver of both ACO and CPT1, involved in peroxisomal and mitochondrial β-oxidation, respectively (Fig. 1a, b). When adding sesamin to the feed, the expression of these genes in the liver was elevated only in the LO + S compared to LO group. The relative expression of CPT1 was upregulated in white muscle of LO and TAG fed fish when sesamin was supplemented to the feed; however, ACO was downregulated. The relative expression of Δ6 fad was upregulated in MO, MO + S and TAG + S compared to FO, in MO + S compared to MO and in TAG + S compared to TAG. The increased expression of Δ6 fad in TAG + S compared to TAG is consistent with the FA results. In the other groups, there were no relation between gene expression levels and FA profile. In white muscle of LO and TAG groups, sesamin significantly downregulated the relative expression of Δ6 fad.

The effects of CPT1 and ACO were pronounce but were not coupled to an expected upregulation in PPARα (Kjaer et al. 2008). Significant down regulation of PPARα was observed in white muscle from fish fed the sesamin diet (Fig. 2a–c). The role of PPARα is thought to be primarily in controlling the reversible induction of β-oxidation in specific tissues, especially liver, as a response to changes in energy requirements and nutritional status. In this study, the most upregulated of PPAR was PPARβ1A being significantly upregulated in all vegetable oils diets compared to FO. Similar increased expression of PPARβ/δ and PPARγ coupled with n-3 PUFA enrichment has been seen in pigs fed a linseed oil diet (Luo et al. 2009). However, the results in our study of PPARβ1A and PPARγ(long) expression level were inconsistent with other studies where no significant changes were found by feeding a vegetable oil in seawater environment (including 15% linseed oil) diet (Jordal et al. 2005). The relative expression of PPARβ1A was upregulated in the liver of all vegetable oil fed groups compared to FO. Also, the addition sesamin upregulated the expression of PPARβ1A in the LO + S group. In white muscle, the sesamin addition upregulated PPARβ1A in the MO + S group and downregulated in the TAG + S group. For PPARγ(long), the relative expression was increased in LO, MO and

TAG + S compared to FO and downregulated in MO + S compared to MO. In white muscle, its expression in LO + S and TAG + S group decreased significantly (Fig. 2a–c) and increased but not significantly in MO + S group (Fig. 2b). The addition of sesamin in diets rendered the expression of both PPARγ(long) and PPARα back to the same levels as if they had been fed a fish oil diet. The downregulation of PPARβ1A and PPARγ(long) after sesamin addition supports the results from Trattner et al. (2008a, b) where sesamin added to a linseed oil diet decreased the expression of PPARα and PPARβ1A as well as PPARγ(long) indicating a difference in response of sesamin in fish compared to mammals. Treatment with synthetic ligands has shown that PPARβ directly controls lipid utilisation through upregulation of genes involved in β-oxidation and energy uncoupling in various mammalian tissues (Dressel et al. 2003), whereas PPARγ is considered to play a critical role in tissue lipid storage and fat accumulation in adipocytes.

In rats, sesamin has been shown to stimulate upregulation of the gene expression for peroxisomal enzymes as late stage enzymes for β-oxidation (Kiso et al. 2005). From these results, an up regulation of PPARα and PPARβ1A would have been expected in the livers of fish fed sesamin added diets. Expression of PPARβ1A declined significantly only in the TAG + S group of our study (Fig. 2c). Otherwise, the increased expression level was maintained regardless if sesamin was added or not. This could suggest an effect on the PPARβ1A expression of some of the minor components present in vegetable oils (Kamal-Eldin 2005), which were removed during purification.

In this study, the gene expression alterations described above were not directly related to a corresponding conversion of ALA to DHA. In Atlantic salmon, Jordal (2006) demonstrated mRNA expression not always being correlated to the protein levels in the given tissue. This lack of correlation between mRNA expression and protein levels may also in turn could have an effect on lipid composition.

Conclusions

Linseed oil and purified linseed oil diet affected fatty acid profile of fish muscle differently. This study shows that plant oils altered genes related to β-oxidation and PPARs. In addition, these oils together with an addition of sesamin showed similar expression levels to the fish oil responses, lowering the PPAR expression to levels comparable to fish fed fish oil, PPARβ1A being the most affected. The addition of sesamin lowered the expression when the minor compounds in linseed oil were removed (TAG + S). It is suggested that minor compounds in sunflower and linseed oil together with sesamin have influence on the expression of the PPARs genes, being possible modulators of FA composition.

Acknowledgments This study was financed by the Swedish Research Council FORMAS. The authors thank Kajsa Brännäs and Lisa Brännäs for valuable technical assistance.

References

Andersen Ø, Eijsink VGH, Thomassen M (2000) Multiple variants of the peroxisome proliferator-activated receptor (PPAR) [gamma] are expressed in the liver of Atlantic salmon (*Salmo salar*). Gene 255(2):411–418

Appelqvist L-Å (1968) Rapid methods of lipid extractions and fatty acid methyl ester preparation for seed and leaf tissue with special remarks on preventing the accumulation of lipids contaminants. Arkiv För Kemi, Royal Swedish academy of Science (Kungliga Svenska Vetenskapsakademien) 28(36):551–570

Ashakumary L, Rouyer I, Takahashi Y, Ide T, Fukuda N, Aoyama T, Hashimoto T, Mizugaki M, Sugano M (1999) Sesamin, a sesame lignan, is a potent inducer of hepatic fatty acid oxidation in the rat. Metab Clin Exp 48(10):1303–1313

Bell JG, Henderson RJ, Tocher DR, Sargent JR (2004) Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. Lipids 39:223–232

Brännäs E, Chaix T, Nilsson J, Eriksson L-O (2005) Has a 4-generation selection programme affected the social behaviour and growth pattern of Arctic charr (Salvelinus alpinus)? Appl Anim Behav Sci 94(1–2):165–178

Choo WS, Birch J, Dufour JP (2007) Physicochemical and quality characteristics of cold-pressed flaxseed oils. J Food Compost Anal 20(3–4):202–211

Dressel U, Allen TL, Pippal JB, Rohde PR, Lau P, Muscat GEO (2003) The peroxisome proliferator-activated receptor β/δ agonist, GW501516, regulates the expression of genes involved in lipid catabolism and energy uncoupling in skeletal muscle cells. Mol Endocrinol 17(12):2477–2493

Fujiyama-Fujiwara Y, Umeda-Sawada R, Kuzuyama M, Igarashi O (1995) Effects of sesamin on the fatty acid composition of the liver of rats fed N-6 and N-3 fatty acids-rich diet. J Nutr SciVitaminol 41:217–225

Fuster MD, Lampi A-M, Hopia A, Kamal-Eldin A (1998) Effects of α- and γ-tocopherols on the autooxidation of purified sunflower triacylglycerols. Lipids 33:715–722

Hara A, Radin NS (1978) Lipid extraction of tissue with low toxicity solvent. Anal Biochem 90:420–426

Ide T, Nakashima Y, Iida H, Yasumoto S, Katsuta M (2009) Lipid metabolism and nutrigenomics—Impact of sesame lignans on gene expression profiles and fatty acid oxidation in rat liver, vol 61

Jeng KCG, Hou RCW (2005) Sesamin and sesamolin: nature's therapeutic lignans. Curr Enzym Inhib 1(1):11–20

Jordal A-EO (2006) Nutrigenomic study of lipid metabolism in Atlantic salmon (*Salmo Salar* L.)—the effects of dietary plant oil inclusions. Dissertation for the degree philosophiae doctor (PhD) at the University of Bergen

Jordal A-EO, Torstensen BE, Tsoi S, Tocher DR, Lall SP, Douglas SE (2005) Dietary rapeseed oil affects the expression of genes involved in hepatic lipid metabolism in Atlantic salmon (*Salmo salar* L.). J Nutr 135:2355–2361

Jordal A-EO, Lie Ø, Torstensen BE (2007) Complete replacement of dietary fish oil with a vegetable oil blend affect liver lipid and plasma lipoprotein levels in Atlantic salmon (*Salmo salar* L.). Aquac Nutr 13:114–130

Kamal-Eldin A (2005) Minor components of vegetable oils. In: Shahidi F (ed) Bailey's industrial oil and fat products, vol III., 6John Wiley, Sussex, pp 483–523

Kiso Y, Tsuruoka N, Kidokoro A, Matsumoto I, Abe K (2005) Sesamin ingestion regulates the transcription levels of hepatic metabolizing enzymes for alcohol and lipids in rats. Alcoholism: clinical and experimental research 29(11Suppl.)

Kjaer MA, Vegusdal A, Gjoen T, Rustan AC, Todorcevic M, Ruyter B (2008) Effect of rapeseed oil and dietary n-3 fatty acids on triacylglycerol synthesis and secretion in Atlantic salmon hepatocytes. Biochim Biophys Acta Mol Cell Biol Lipids 1781(3):112–122

Kushiro M, Masaoka T, Hageshita S, Takahashi Y, Ide T, Sugano M (2002) Comparative effect of sesamin and episesamin on the activity and gene expression of enzymes in fatty acid oxidation and synthesis in rat liver. J Nutr Biochem 13(5):289–295

Leaver MJ, Ezaz MT, Fontagne S, Tocher DR, Boukouvala E, Krey G (2007) Multiple peroxisome proliferator-activated receptor β subtypes from Atlantic salmon (*Salmo salar*). J Mol Endocrinol 38(3–4):391–400

Leaver MJ, Villeneuve LAN, Obach A, Jensen L, Bron JE, Tocher DR, Taggart JB (2008) Functional genomics reveals increases in cholesterol biosynthetic genes and highly unsaturated fatty acid biosynthesis after dietary substitution of fish oil with vegetable oils in Atlantic salmon (*Salmo salar*). BMC Genomics 9(299)

Lim JS, Adachi Y, Takahashi Y, Ide T (2007) Comparative analysis of sesame lignans (sesamin and sesamolin) in affecting hepatic fatty acid metabolism in rats. Br J Nutr 97(1):85–95. doi:10.1017/s0007114507252699

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25(4):402–408

Luo HF, Wei HK, Huang FR, Zhou Z, Jiang SW, Peng J (2009) The effect of linseed on intramuscular fat content and adipogenesis related genes in skeletal muscle of pigs. Lipids 44(11):999–1010

Miller MR, Nichols PD, Carter CG (2008) n-3 Oil sources for use in aquaculture—alternatives to the unsustainable harvest of wild fish. Nutr Res Rev 21:85–96

Mizukuchi A, Umeda-Sawada R, Igarashi O (2003) Effect of dietary fat level and sesamin on the polyunsaturated fatty acid metabolism in rats. J Nutr Sci Vitaminol 49(5):320–326

Moazzami AA, Kamal-Eldin A (2006) Sesame seed is a rich source of dietary lignans. J Am Oil Chem Soc 83(8):719–723

Pettersson A, Johnsson L, Brannas E, Pickova J (2009) Effects of rapeseed oil replacement in fish feed on lipid composition and self-selection by rainbow trout (*Oncorhynchus mykiss*). Aquac Nutr 15(6): 577–586

Pickova J, Dutta PC, Larsson P-O, Kiessling A (1997) Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: comparison between two cod (Gadus morhua) stocks. Can J Fish Aquat Sci 54(10):2410–2416

Sanchez-Vazquez FJ, Yamamoto T, Akiyama T, Madrid JA, Tabata M (1999) Macronutrient self-selection through demand-feeders in rainbow trout. Physiol Behav 66(1):45–51

Schoonjans K, Staels B, Auwerx J (1996) The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. Biochim Biophys Acta—Lipids and Lipid Metab 1302(2):93–109. doi:10.1016/0005-2760(96)00066-5

Schwartz H, Ollilainen V, Piironen V, Lampi AM (2008) Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats. J Food Compost Anal 21(2):152–161

Sirato-Yasumoto S, Katsuta M, Okuyama Y, Takahashi Y, Ide T (2001) Effect of sesame seeds rich in sesamin and sesamolin on fatty acid oxidation in rat liver. J Agric Food Chem 49:2647–2651

SLVFS (1993:17) Livsmedelsverkets föreskrifter om extraktionsmedel vid framställning av livsmedel och livsmedelsingredienser. SLVFS 17

Stubhaug I, Lie Ø, Torstensen BE (2006) β-Oxidation capacity in liver increases during parr-smolt transformation of Atlantic salmon fed vegetable oil and fish oil. J Fish Biol 69(2):504–517. doi:10.1111/j.1095-8649.2006.01122.x

Thomassen MS, Rosjo C (1989) Different fats in feed for salmon—influence on sensory parameters, growth-rate and fatty-acids in muscle and heart. Aquaculture 79(1–4):129–135

Tocher DR, Fonseca-Madrigal J, Bell JG, Dick JR, Henderson RJ, Sargent JR (2002) Effects of diets containing linseed oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes in Atlantic salmon (*Salmo salar*). Fish Physiol Biochem 26(2):157–170

Tocher DR, Bell JG, Dick JR, Crampton VO (2003) Effects of dietary vegetable oil on Atlantic salmon hepatocyte fatty acid desaturation and liver fatty acid compositions. Lipids 38(7):723–732

Torstensen BE, Bell JG, Rosenlund G, Henderson RJ, Graff IE, Tocher DR, Lie Ø, Sargent JR (2005) Tailoring of Atlantic salmon (*Salmo salar* L.) flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend. J Agric Food Chem 53(26):10166–10178

Torstensen BE, Nanton DA, Olsvik PA, Sundvold H, Stubhaug I (2009) Gene expression of fatty acid binging proteins, fatty acid transport proteins (cd36 and FATP) and β-oxidation-related genes in Atlantic salmon (*Salmo salar* L.) fed fish oil or vegetable oil. Aquac Nutr 15:440–451

Trattner S, Pickova J, Park KH, Rinchard J, Dabrowski K (2007) Effects of alpha-lipoic and ascorbic acid on the muscle and brain fatty acids and antioxidant profile of the South American pacu *Piaractus mesopotamicus*. Aquaculture 273(1):158–164

Trattner S, Kamal-Eldin A, Brännäs E, Moazzami AA, Žlábek V, Larsson P, Ruyter B, Gjøen T, Pickova J (2008a) Sesamin supplementation increases white muscle docosahexaenoic acid (DHA) Levels in rainbow trout (*Oncorhynchus mykiss*) fed high alpha-linolenic acid (ALA) containing vegetable oil: metabolic actions. Lipids 43(11):989–997

Trattner S, Ruyter B, Ostbye TK, Gjøen T, Zlabek V, Kamal-Eldin A, Pickova J (2008b) Sesamin increases alpha-linolenic acid conversion to docosahexaenoic acid in Atlantic salmon (*Salmo salar* L.) hepatocytes: role of altered gene expression. Lipids 43(11):999–1008

Turchini GM, Francis DS (2009) Fatty acid metabolism (desaturation, elongation and β-oxidation) in rainbow trout fed fish oil- or linseed oil-based diets. Br J Nutr 102:69–81

Vagner M, Santigosa E (2010) Characterization and modulation of gene expression and enzymatic activity of delta-6 desaturase in teleosts: a review. Aquaculture. doi:10.1016/j.aquaculture.2010.11.031

Zheng XZ, Torstensen BE, Tocher DR, Dick JR, Henderson RJ, Bell JG (2005) Environmental and dietary influences on highly unsaturated fatty acid biosynthesis and expression of fatty acyl desaturase and elongase genes in liver of Atlantic salmon (*Salmo salar*). Biochim Biophys Acta Mol Cell Biol Lipids 1734(1):13–24