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1 Influence of dietary sesamin, a bioactive compound on fatty acids and expression of some
2 lipid regulating genes in Baltic Atlantic salmon (*Salmo salar* L.) juveniles
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Abstract

The effects of including 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 from 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

44 **Abbreviations**

45	ACO	Acyl-CoA oxidase
46	CD 36	Cluster of differentiation 36
47	CPT	Carnitine palmitoyltransferase
48	CYP	Cytochrome P450
49	Δ5	Δ5 desaturase
50	Δ6	Δ6 desaturase
51	DHA	Docosahexaenoic acid (22:6n-3)
52	DPA	Docosapentaenoic acid (22:5n-3)
53	EF1A	Elongation factor 1α
54	EPA	Eicosapentaenoic acid (20:5n-3)
55	EROD	Ethoxyresorufin O-deethylase
56	FA	Fatty acid
57	FO	Fish oil
58	HSL3	Hormone sensitive lipase
59	HUFA	Highly unsaturated fatty acids
60	MO	Linseed and sunflower oil 6:4 by vol
61	MUFA	Monounsaturated fatty acids
62	PL	Phospholipids
63	TAG	Triacylglycerol
64	TLC	Thin-layer chromatography
65	SesO	Sesame oil and linseed oil 1:1 by vol
66	SRB-I	Scavenger receptor type B
67	RPL2	RNA polymerase II polypeptide
68	PCR	Polymerase chain reaction
69	PPAR	Peroxisome proliferator-activated receptor
70	PUFA	Polyunsaturated fatty acids
71	SREBP	Sterol regulatory element binding protein
72	VLDL	Very low-density lipoprotein

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74

75

76 **Introduction**

77 Sesamin, a minor component of sesame oil (Moazzami and Kamal-Eldin 2006), is a
78 potent lipid modulator in mammals. It has been shown to affect enzymatic activity and
79 expression of genes involved in lipid metabolism e.g. acyl-CoA oxidase (ACO) and
80 carnitine palmitoyltransferase (CPT) (Kushiro et al. 2002; Jeng and Hou 2005; Kiso et al.
81 2005). In the fungus *Mortierella alpina* and in primary rat hepatocytes, sesamin was
82 shown to reduce Δ-5 desaturation index and enzymatic activity of Δ-5 desaturase
83 (Shimizu et al. 1991). To our knowledge, only two studies from our group have
84 investigated the effects of dietary sesamin in fish. In these studies, it was shown that
85 sesamin increased docosahexaenoic acid (22:6n-3, DHA) in rainbow trout
86 (*Oncorhynchus mykiss*) white muscle phospholipid (PL) and triacylglycerol (TAG)
87 fraction and decreased the expression of peroxisome proliferator-activated receptor α
88 (PPARα) in liver (Trattner et al. 2008). In Atlantic salmon (*Salmo salar* L.) hepatocytes,
89 it was shown that sesamin increased elongation and desaturation of radiolabelled 18:3n-3
90 towards DHA. It increased the levels of β-oxidation products and the relative expression
91 of cluster of differentiation 36 (CD36), scavenger receptor (SRB) type B, PPARα and γ
92 (Trattner et al. 2008). The metabolic effects of sesamin have been suggested to be caused
93 through the activation of PPARs and sterol regulatory element binding protein-1
94 (SREBP-1) (Ashakumary et al. 1999; Ide et al. 2004). Furthermore, sesamin has been
95 reported to inhibit cholesterol absorption and synthesis, and tocopherol hydroxylation and
96 clearance in rats and humans (Jeng and Hou 2005). Other compounds known to modulate
97 lipids are 3-thia fatty acids, conjugated linoleic acid and Lipoic acid (Berge et al. 2001);
98 (Huong and Ide 2008); Kennedy et al. 2009).

99

100 The methylenedioxyphenyl group of sesamin is known to affect cytochrome P450-
101 dependent drug oxidation (Murray 2000). Cytochrome P450 (CYP) enzymes are known
102 to play a central role in the oxidative metabolism and biotransformation of a wide range
103 of endogenous and exogenous compounds (Nelson et al. 1996). Among the numerous
104 CYP families identified, primarily CYP 1-3 are involved in biotransformation of
105 xenobiotics. The CYP1A subfamily is reported to be expressed in the liver of both
106 mammals and fish (Murray 2000; Jönsson et al. 2006). Due to the role of CYP1A

107 isoenzymes in the metabolism and bioactivation of foreign compounds, alteration of the
108 expression of hepatic CYP1A may affect the potential risk of xenobiotics (Williams et al.
109 1998). CYP1A is readily inducible by aryl hydrocarbon (Ah) receptor agonist, thus the
110 activity of CYP1A, measured as ethoxyresorufin O-deethylase (EROD) activity, is used
111 as a biomarker for exposure to xenobiotic compounds in fish (Havelkova et al. 2007).
112
113 Traditionally, carnivorous farmed fish has been fed diets based on fish ingredients. At
114 present there is an overuse of marine raw materials for aquaculture feed production and at
115 the same time aquaculture is the fastest growing food production industry (Tacon 2005;
116 FAO 2007). Therefore, alternative fish feed ingredients are being investigated. Vegetable
117 oil is used as a replacement of fish oil (up to 50%) without affecting growth and
118 production yield (Torstensen et al. 2005). One well known drawback of replacement with
119 vegetable oils in fish feed are the decreased amounts of n-3 highly unsaturated fatty acids
120 (HUFA) in fish tissues (Torstensen et al. 2005; Pettersson et al. 2009). The n-3 HUFA are
121 known to have positive health effects in man. In terms of human health, it is important to
122 preserve the beneficial fatty acid (FA) composition of fish (Mozaffarian and Rimm
123 2006). It is necessary to find alternatives to fish oil use, without decreasing the content of
124 n-3 HUFA in fish.
125
126 To achieve more n-3 HUFA in fish fed vegetable oils, bioactive compounds can be added
127 in the fish diet. It is interesting to study the effects on sesamin in common aquaculture
128 species, on the nutritional quality of muscle as human food in terms of FA, tocopherols
129 and sesamin content. Furthermore, it is important to investigate how sesamin is
130 metabolized in the fish and how it affects fish welfare. To investigate the effects of
131 sesamin in wild strain Baltic Atlantic salmon (*Salmo salar* L.) juveniles, a dose response
132 study was designed and the FA composition, the relative expression of nine lipid related
133 genes, the content of tocopherols, sesamin and episesamin, EROD activity and the total
134 level of CYP were analyzed.
135

136 Material and method

137 *Chemicals and Reagents*

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

144 *Animals and diets*

145 Baltic Atlantic salmon was fed six different diets, fifteen fish per group. Prior the
146 experiment all fish were fed the same commercial diet. Four groups were fed
147 experimental diets based of vegetable oils and sesamin/episesamin mixture (1:1, w/w)
148 (hereafter named sesamin), while one group was fed a diet based on fish ingredients, and
149 one group was fed a diet based on vegetable oil. The diets were prepared according to the
150 method of Sanchez-Vazquez (1999), the ingredients are shown in Table 1. The diets
151 differed in oil composition and the content of sesamin as follow; 1) mixed linseed :
152 sunflower oil, 6 : 4 by vol. (MO), 2) fish oil (FO), 3) sesame oil : linseed oil, 1:1 by vol.
153 (SesO), 4) MO + sesamin 0.29 g $100g^{-1}$, 5) MO + sesamin 0.58 g $100g^{-1}$, 6) MO +
154 sesamin 1.16 g $100g^{-1}$. The FA composition and the tocopherol content of the diets are
155 shown in Table 2. The fish were tagged individually and the individual weight increase
156 was calculated as daily growth gain. Fishes were kept at a water temperature of 10 ºC and
157 were fed *ad libitum* for 77 days. Before the experiment started, the fish were tagged with
158 a PIT-tag (Passive Integrated Transponder) by injecting the tag into the posterior part of
159 the abdomen. Before handling, all fish were anaesthetized (ethyleneglycol monophenyl
160 ether 5 mL L^{-1}). The daily growth rate (DGC) were calculated as:
161 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
162 *D* the number of days (Table 3.).
163 At sacrifice, the muscle was divided in red and white. The muscles, intestine and liver
164 were frozen at –80 ºC until analyzed. From each group six individuals were used for fatty
165 acid, tocopherols, EROD and CYP analysis and another six individuals were used for
166 gene expression analysis.

167

168 *Lipid analysis*

169 White muscle (2 g) and diets (1 g) were extracted following the method of (Hara and
170 Radin 1978). The lipid content was measured gravimetrically. Total lipids of tissues were
171 separated into PL and TAG according to Pickova *et al*. (1997). Total lipids in the diets,
172 and the PL and TAG lipid fractions of tissues were methylated following the procedure of
173 (Appelqvist 1968) and the FA were analysed by gas chromatograph CP3800 (Varian AB,
174 Stockholm, Sweden) equipped with flame ionisation detector (FID) and split injector and
175 fitted with a fused silica capillary column BPX 70 (SGE, Austin, Tex.), length 50 m. id.
176 0.22 mm, 0.25 μm film thickness. The column temperature was programmed to start at
177 158°C hold 5 min and then increase 2°C/min from 158°C to 220°C and remain at 220°C
178 for 8 min. The carrier gas was helium (0.8 ml/ min) and make up gas was nitrogen. The
179 injector and detector temperatures were 230°C and 250°C, respectively. FA were
180 identified by comparison with the standard FA mixture GLC-68. Peak areas were
181 integrated using Varian Star chromatography workstation software version 5.5.

182

183 *Tocopherols, sesamin and episesamin analyses*

184 For the analysis of tocopherols in the diets, and the tocopherols, sesamin and episesamin
185 in the tissues, the lipid extracts were dissolved in hexane and analysed with high
186 performance liquid chromatography (HPLC). The mobile phase used was hexane/1,4-
187 dioxane (94:4, vol/vol). The HPLC system was equipped with a Bischoff HPLC pump
188 (Bischoff Analysentechnik und geräte GmbH, Leonberg, Germany) and Agilent 1100
189 series fluorescence detector (Agilent Technologies, Waldbronn, Germany). The HPLC
190 column was Alltech SI 5U silica column (4.6 x 250 mm; Alltech Associates Inc.,
191 Deerfield, IL). The fluorescence detector was operated at an excitation wavelength of 296
192 nm and an emission wavelength of 324 nm as described by Moazzami and Kamal-Eldin
193 (2006). Identification and quantification was achieved by comparison to external
194 standards.

195

196 *RNA analysis*

197 Total RNA was purified from livers, muscle and intestine from each group (n = 6) and
198 analyzed in duplicate, using Trizol® (Invitrogen), followed by DNase treatment (TURBO
199 DNA-free, Ambion). All protocols were according to the manufacture's instructions.
200 RNA quality and quantity were determined spectrophotometrically ($A_{260/280}$) using
201 NanoDrop® (ND-1000 Spectrophotometer, NanoDrop Technologies, Wilmington,
202 Delaware, USA). Samples were stored in RNase-free water at –80˚C.
203
204 The cDNA was synthesized from 2.4µg RNA, a modified protocol from the Taq Man
205 Reverse Transcription Reagents kit (Applied Biosystems). The Oligo $d(T)_{16}$ primers were
206 used. The reaction was performed by incubating the samples at 25 ˚C for 10 min, 48 ˚C
207 for 6 min, 95 ˚C for 50 min and was terminated by reducing the temperature to 10 ˚C.
208 Primers for Real-Time PCR analysis (Table 4) were designed using the Primer Express®
209 software based on available salmon sequences in the GenBank® and purchased from
210 Invitrogen (CA, USA). Real-Time PCR was performed in a Prism® 7000 system by
211 using gene-specific primers. A 2 x SYBR® Green PCR Mastermix (ABI) was used in the
212 PCR reaction mix of 25 µl with 1 µl primers (final concentration of 0.5 µM), and 5 µl
213 cDNA. All samples were analyzed in duplicate with a non-template control on each
214 plate. The reference genes used were elongation factor 1α (EF1A) and RNA polymerase
215 II polypeptide (RPL II). The reaction was preformed by incubating the samples at 50˚C
216 for 2 min, 95˚C 10 min and 50 cycles of 95˚C for 10 s and 60˚C for 15 s. Standard curves
217 were made for each primer pair and efficiencies (E) were calculated $E=10^{(-1/slope)}$.
218
219 *Total content of CYP and EROD activity in liver*
220 For the analysis of total CYP content and EROD activity, six liver samples from each
221 group were analysed. The liver tissue was homogenized in ice-cold homogenization
222 buffer (0.25 M sucrose and 0.1 mM EDTA in 0.01 M TRIS buffer, pH 7.4) using a
223 Potter-Elvehjem homogeniser. The homogenate was centrifuged for 15 min at 10,000×*g*
224 (4 °C) and the resulting supernatant was spun down for 1 h at 105,000×*g* (4 °C). The
225 microsomal pellets were resuspended in the homogenization buffer and stored at - 80 °C
226 until used. The total CYP content was determined spectrophotometrically by the Co- and

227 dithionite difference method (Shimanzu UV-1601PC, Columbia, USA) according to
228 (Omura and Sato 1964).
229
230 Hepatic EROD activity was determined according to a modified method by (Jönsson et
231 al. 2006). Standard solutions of resorufin (0–50µM) and protein (BSA; 1 mg BSA ml^{-1})
232 were prepared in HEPES-Cortland buffer pH 8. The HC buffer was prepared by
233 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
234 $NaH_2PO_4 \cdot H_2O$, 1.43 g HEPES, and 1 g glucose in 1 l of distilled water. Microsome
235 suspensions were further diluted in the same buffer (1:5 and 1:10). Aliquots of the
236 microsome suspensions (50 µl) and of the standard solutions (40 µl of resorufin and 10 µl
237 of BSA) were added in duplicate wells in 96-well plate. A 160 µl aliquot of 7-
238 ethoxyresorufin (12.7 µM) and NADPH (2.1 mM) in HC buffer was rapidly added to all
239 wells. The plate was then immediately placed in a microplate reader (Wallac 1420
240 VICTOR^2, Turku, Finland) and the resorufin fluorescence was monitored for 10 min by
241 repeated measurements at 544 nm (ex) and 590 nm (em). EROD activity was calculated
242 and expressed as pmol of resorufin formed per mg protein and minute. The protein
243 contents of the microsomes were assayed by the (Smith et al. 1985), adapted for
244 microplate readers.

245

246 *Data analysis*
247 Fatty acids, EROD, CYP, tocopherols, sesamin and episesamin data are presented as
248 mean values ± standard deviation. The General Linear Model (GLM) of SAS (SAS
249 Institute Inc., Cary, N.C., USA, version 8.2) was used to compare the physiological
250 responses of the different diets. The model included the fixed effect of treatment and
251 random effect of individual. Relative expression of the different genes, in relation to
252 housekeeping genes were determined by using the Relative Expression Software Tool
253 (REST-384©-version 1) for group wise comparison and statistical analysis of relative
254 expression results in real-time PCR (Pfaffl et al. 2002).
255
256 **Results**

257 *Survival*

258 The total mortality during the study was 5 fish, of which three belonged to the FO-group,
259 one to the MO group and one to the MO+0.28 group.
260 There was no difference in starting weight, final weight or daily growth coefficient
261 (DGC) between the MO group and the groups with added sesamin. For the FO group
262 both start and end weight were lower (6.9 and 13.8, respectively) than in the other groups
263 (range 8.7 - 20.7 g). The DGR is shown for the six fish from each group which were
264 analysed for fatty acid composition. The variation between individuals is large in all
265 groups as seen from the data in Table 3.
266
267
268 *Fatty acid composition and lipid content*

269 The groups did not differ in lipid content. The FA composition of white muscle clearly
270 reflected the FA profile of the oils used in the diets (Table 5). The FO group was
271 characterized by high proportions of EPA and DHA in PL and TAG. The vegetable oil
272 groups had higher proportions of 18:3n-3 and lower proportions of n-3HUFA. The
273 response to the change in dietary FA composition was faster in TAG than PL. The levels
274 of 18:3n-3 were significantly lower in PL of all three groups fed sesamin compared to the
275 MO group without sesamin (P<0.05). The level of docosapentaenoic acid (DPA, 22:5n-3)
276 and DHA in PL of the MO groups with added sesamin were higher than in the MO
277 group, however the difference was only significant for DPA in the MO 0.58 group
278 (P<0.05). In the PL of MO 0.58 group DHA increased to 36.6 (P = 0.16). Similar to the
279 PL fraction 18:3n-3 decreased and DPA and DHA increased in the TAG of MO 0.58 and
280 MO 1.16 group (non significant changes).
281
282 *Tocopherols and sesamin content*

283 The contents of α- and γ- tocopherols in the liver were significantly lower (P< 0.05) in
284 FO group compared to the MO groups with or without addition of sesamin. In the white
285 muscle, the γ- tocopherol content was significantly lower in the FO group than in the MO
286 groups (P< 0.05). No differences were found in the content of sesamin and episesamin in
287 white muscle, in the liver sesamin and episesamin levels were higher in the MO 1.16

288 group, the difference was significant compared to the MO 0.29 group (*P* = 0.03, *P*<0.01
289 for sesamin and episesamin respectively), but was higher than in the SesO (*P* = 0.3, *P* =
290 0.2 for sesamin and episesamin respectively) and MO 0.58 (*P* =0.1 both for sesamin and
291 episesamin) group as well (Table 6).
292
293 *Relative expression of target genes*
294 The relative expressions of target genes in the experimental groups, compared to MO
295 group, after normalization to the reference gene are shown in Figure 1. In the MO + 1.16
296 group, PPARα (*P* = 0.05) was upregulated compared to control group. In the MO + 0.29
297 group, SRB (*P*= 0.03) and HSL3 (*P*<0.01) were upregulated, and in the MO + 0.58
298 group, HSL3 (*P*= 0.03) was upregulated compared to the MO group.
299
300 *Total content of CYP and EROD activity in the liver*
301 The EROD activity did not differ significantly among groups (Figure 2). The CYP
302 (Figure 2) levels were significantly higher in the MO 0.29 group (*P* = 0.02) and slightly
303 higher in the MO 1.16 group (*P* = 0.07) compared to MO group and significantly lower in
304 MO 0.58 group (*P* < 0.01) compared to MO 0.29 group. The CYP response was also
305 significantly higher in the SesO group compared to the MO group. There was a large
306 variation in individual response detected, with CV values within groups from 16 up to
307 125 %.
308
309 **Discussion**
310 In general, the results of different analyses in this study showed a large variation caused
311 by a broad individual response. The reason for the different weight in the fish group is
312 most likely caused by the low number of individuals as the fish were divided between
313 groups according to stastistical methods. In Sweden, the long breeding history of Baltic
314 Atlantic salmon smolts is aimed for release purposes as a replacement for the wild
315 spawners being hindered the enter the rivers by hydro-electric power dams and the large
316 number of parents is a prerequisite. Therefore, this fish had a wide range of genetic
317 background compared to the fish used in our previous studies on rainbow trout and
318 Atlantic salmon hepatocytes. Schlechtriem et al. (2007) found inter individual variations

319 in FA composition of Atlantic salmon smolt, and suggested individual variation in
320 elongation and desaturation capacity as a likely explanation. Other known factors
321 affecting the capacity of elongation and desaturation are environmental factors and life
322 stage, e.g. salmon prior seawater transfer had higher relative expression of Δ-5 and Δ-6
323 desaturase (Zheng et al. 2005). Addition of bioactive compounds such as 3-thia fatty
324 acids and lipoic acid can also influence lipid metabolism. The 3-thia fatty acids increased
325 β-oxidation capacity and the levels of DHA (Moya-Falcon et al. 2006) and reduced
326 mRNA expression of PPARα and apolipoproteinAI (ApoAI) (Kleveland et al. 2006) in
327 Atlantic salmon. Conjugated linoleic acid decreased adipocytes by elevating energy
328 expenditure (Kennedy et al. 2009). Dietary lipoic acid was shown to increase EPA levels
329 in pacu (*Piractus mesopotamicus*) muscle (Trattner et al. 2007).
330
331 In our previous study on rainbow trout, significantly increased levels of DHA and
332 decreased proportions of 18:3n-3 in the TAG and PL fractions were found after sesamin
333 addition to the fish diet. Also the total level of polyunsaturated fatty acids (PUFA) was
334 decreased, possibly due to increased β-oxidation of PUFA in sesamin fed fish (Trattner et
335 al. 2008a). These results were confirmed in an in vitro study on Atlantic salmon
336 hepatocytes incubated with radiolabelled 18:3n-3 with or without sesamin addition to the
337 media. It was shown that the amount of 18:3n-3 elongated and desaturarated to DHA was
338 increased after sesamin incubation. It was also shown that sesamin increased the total
339 level of β-oxidation products, in particular acetate, which indicate peroxisomal β-
340 oxidation (Trattner et al. 2008). Sesamin also decreased secretion of lipids (mainly TAG)
341 in Atlantic salmon hepatocytes, in agreement with the lipid lowering effects reported as
342 reduced TAG and VLDL levels in rat serum (Umeda-Sawada et al. 1998; Kamal-Eldin et
343 al. 2000). In the present study, sesamin significantly decreased levels of 18:3n-3 in the PL
344 of all sesamin fed groups, also the average DHA level was (not significantly) increased in
345 the groups after sesamin addition to the diet.
346
347 In the rainbow trout study, two different oils, sunflower: linseed oil mixture (MO) and
348 linseed oil (LO) were used. In that study the effects of sesamin on gene expression and
349 FA composition were greater in MO diet than in LO diet. The use of 100% linseed oil in

350 the LO group decreased desaturation index (n-3HUFA/18:3n-3) compared to the use of a
351 mixture of vegetable oil as in the MO group, indicating less efficient conversion of 18:3n-
352 3 to DHA when linseed oil is included at higher levels in the diet. There are a number of
353 studies showing decreased desaturation index with increased inclusion of linseed oil, a
354 summary of results from studies are presented in Table 7. In a study on Atlantic salmon,
355 it was suggested that increased inclusion of linseed oil due to its high content of 18:3n-3
356 inhibited elongation and desaturation of 18:3n-3 in hepatocytes, and increased oxidation
357 of 18:3n-3 in enterocytes (Tocher et al. 2002). The replacement of fish oil with linseed
358 oil, decreased the DHA levels in the liver 4-fold (Tocher et al. 2002), whereas
359 replacement of the fish oil with rapeseed oil decreased the DHA levels to half (Bell et al.
360 2001). (Leaver et al. 2008) showed increased activity of fatty acyl Δ-6 desaturase in the
361 liver of Atlantic salmon fed vegetable oils, the increased activity was highest for rapeseed
362 oil followed by soybean oil and last linseed oil. The expression of *elov15b* and *elovl2*
363 elongases were also significantly higher in liver of vegetable oil fed fish compared to fish
364 oil fed fish, with lower expression in linseed oil fed fish than in rapeseed oil and soybean
365 oil fed fish (Morais et al. 2009). In agreement with these studies, we also found decreased
366 desaturation index in the MO group, which had higher 18:3n-3 content in the diet
367 compared to the SesO group.
368
369 The differences in tocopherols (Table 6) are due to the difference in tocopherols in the
370 diet, the vegetable oil had a higher content of tocopherols than the fish oil. The FO diet
371 had low levels of α-tocopherol and levels below detection limit for γ-tocopherol. In
372 contrast to our results, in rats and humans, it has been shown that sesamin increased the
373 levels of γ-tocopherol and reduced the urine extraction of its metabolites (Frank et al.
374 2004); (Kamaleldin et al. 1995). In the liver, the sesamin and episesamin contents were
375 increased with increased content in the diet. Even though the sesamin : episesamin ratio
376 was 1:1 in the feed, episesamin was detected at higher levels in muscle and liver. This
377 finding is in agreement with the study on rainbow trout and has also been found in rats
378 (Trattner et al. 2008a; (Umeda-Sawada et al. 1999).
379

380 Interestingly, even if there were no significant differences in the composition of n-3
381 HUFA in TAG, it was found that the MO+1.16g group with increased relative expression
382 of PPARα also had higher proportions of DPA and DHA in white muscle PL and TAG. A
383 result well corresponding with the increased levels of DHA in the in vivo rainbow trout
384 livers and the in vitro Atlantic salmon hepatocytes and increase β-oxidation in Atlantic
385 salmon hepatocytes. The relative expression of PPARα in rainbow trout liver and Atlantic
386 salmon hepatocytes, was also found previously to be effected by sesamin, although in
387 these previous cases, this expression was downregulated. The highest levels of DPA and
388 DHA were found in PL and TAG of the MO 0.58 group, which also had increased
389 relative expression of HSL3. HSL activate intracellular hydrolysis of TAG, which can
390 then be used for β-oxidation (Watt et al. 2003). The increase in DHA could also indicate
391 increased β-oxidation as DHA is produced through β-oxidation of longer n-3 fatty acids
392 (Voss et al. 1991).
393
394 The average values for each group in the EROD analysis indicate that the activity
395 decreases with increased levels of sesamin and for the CYP analysis the content is lower
396 at intermediate doses than at low and high dose of sesamin (Figure 2). However, due to
397 the large variation within groups, it is difficult to draw any conclusions. It would be
398 interesting to study the dose dependent response in vitro conditions. The large individual
399 response of enzymes involved in the defence against xenobiotic compounds also support
400 the above mentioned suggestion that individual fish react differently to bioactive
401 compounds in the diet, also in terms of lipid metabolism.
402
403 To improve lipid metabolism in farmed fish may be a useful tool in the future to meet the
404 demands for production of farmed fish with a healthy FA composition produced on less
405 amounts of fish based raw materials. This study indicates that the response of Atlantic
406 salmon (Swedish Baltic origin) to sesamin is less than in rainbow trout in vivo (Trattner
407 et al 2008a) and in Atlantic salmon in vitro (Trattner et al. 2008b). This difference
408 deserves further evaluation and may be utilised for selection to improve desaturation
409 capacity in farmed fish fed vegetable oils.

410 Table 1. Basic feed ingredients in the experimental diets.

Ingredient	$g100g^{-1}$
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

411 * The oil used was a mixture of linseed and sunflower oil (6:4) in the MO diets, in the

412 SesO diet sesame oil : linseed oil (1:1) was used and in the FO diet fish oil was used.

413 Sesamin / episesamin was added at a level of 0.29, 0.58 and 1.16 g $100g^{-1}$ diet to the MO

414 0.29, MO 0.58 and MO 1.16 diet.

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417 Tabel 2. Fatty acid composition of the diets, (% of total FA), and tocopherols (µg / g

418 lipid), duplicate analyses

	MO	FO	SesO	MO 0.29	MO 0.58	MO1.16
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

419 Abbreviations: SAFA = saturated fatty acids (20:0, 20:2, 22:0, 24:0), MUFA =

420 monounsaturated fatty acids (14:1, 18:1 n-5, 24:1), PUFA = polyunsaturated fatty acids.

421 MO = mixed oil, FO= fish oil, SesO = sesame oil : linseed oil, MO+0.29 = mixed oil with

422 sesamin addition 0.29 g 100g^{-1}diet, MO+0.58 = mixed oil with sesamin addition 0.58 g
423 100g^{-1}diet, MO+1.16 = mixed oil with sesamin addition 1.16 g 100g^{-1}diet.
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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

428 Abbreviations see Table 2
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435
436 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
Δ-5	GAGAGCTGGCACCGACAGAG	GAGCTGCATTTTTCCCATGG	1.77
Δ6	AGAGCGTAGCTGACACAGCG	TCCTCGGTTCTCTCTGCTCC	1.90

437 Abbreviations: RPL2 = RNA polymerase II polypeptide, EF1A = Elongation factor 1α, PPAR =
438 peroxisome proliferator-activated receptor, SRB-I = scavenger receptor type B, CD 36 = cluster of
439 differentiation 36, HSL3 = hormone sensitive lipase, Δ5 = Δ5 desaturase, Δ6 = Δ6 desaturase.
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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$^{z\,y}$	15.8±1.15$^{z\,y}$
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
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.

$^{u-z}$ 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.
$^{u-z}$ Mean values across the row not sharing a common superscript are significantly different by $P < 0.05$

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

Reference	18:3n3 (%) diet	Oil sourse	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			

Abbreviations: 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).

Figure 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.

Figure 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).

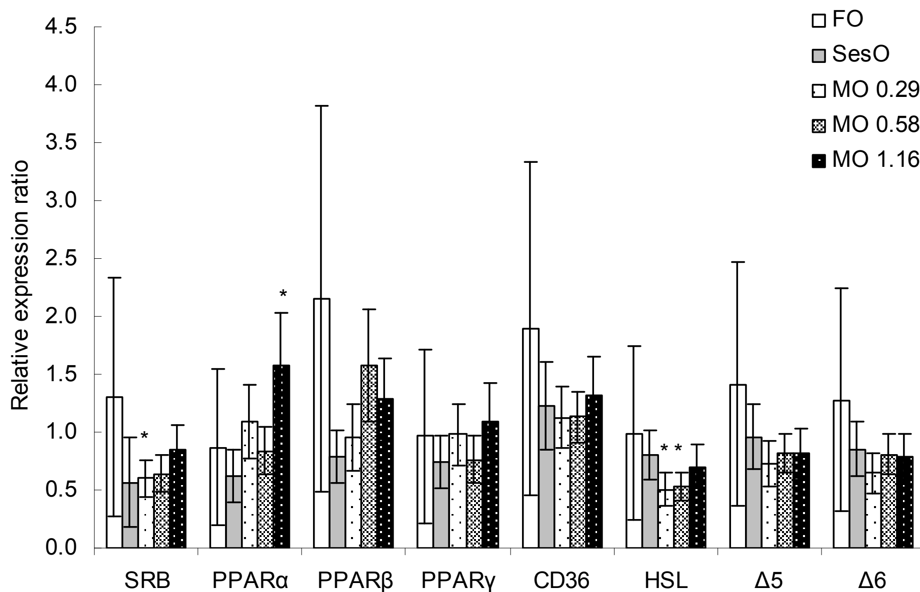

Figure 1.

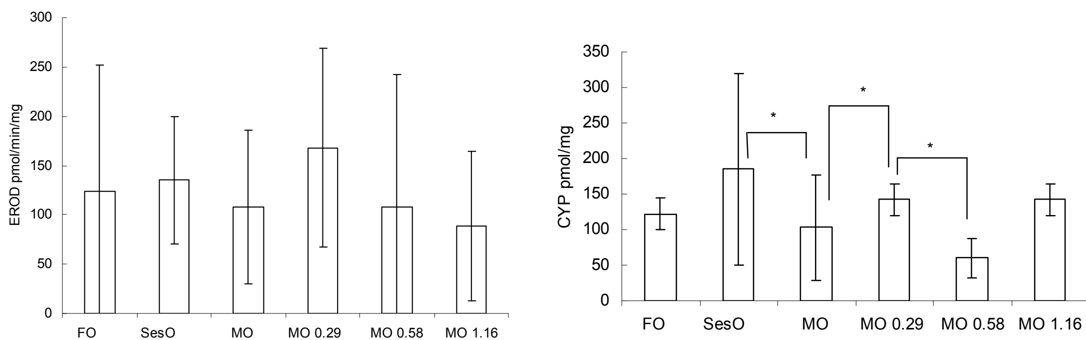

Figure 2.

References

Appelqvist, L. 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. Royal Swedish academy of Science (Kungliga Svenska Vetenskapsakademien) 28: 551-570.

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

Bell, J. G., J. McEvoy, D. R. Tocher, F. McGhee, P. J. Campbell and J. R. Sargent. 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (Salmo salar) affects tissue lipid compositions and hepatocyte fatty acid metabolism. Journal of Nutrition 131(5): 1535-1543.

Berge, K., K. J. Tronstad, E. N. Flindt, T. H. Rasmussen, L. Madsen, K. Kristiansen and R. K. Berge. 2001. Tetradecylthioacetic acid inhibits growth of rat glioma cells ex vivo and in vivo via PPAR-dependent and PPAR-independent pathways. Carcinogenesis 22(11): 1747-1755.

FAO. 2007. FAOSTAT, faostat.fao.org/. (8 November 2007).

Frank, J., A. Kamal-Eldin and M. G. Traber (2004). Consumption of sesame oil muffins decreases the urinary excretion of gamma-tocopherol metabolites in humans. Vitamin E and Health. 1031**:** 365-367.

Hara, A. and N. S. Radin. 1978. Lipid extraction of tissue with low toxicity solvent. Analytic Biochemistry 90: 420-426.

Havelkova, M., T. Randak, V. Zlabek, J. Krijt, H. Kroupova, J. Pulkrabova and Z. Svobodova. 2007. Biochemical markers for assessing aquatic contamination. Sensors 7: 2599-2611.

Huong, D. T. T. and T. Ide. 2008. Dietary lipoic acid-dependent changes in the activity and mRNA levels of hepatic lipogenic enzymes in rats. British Journal of Nutrition 100(1): 79-87.

Ide, T., D. D. Hong, P. Ranasinghe, Y. Takahashi, M. Kushiro and M. Sugano. 2004. Interaction of dietary fat types and sesamin on hepatic fatty acid oxidation in rats. Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids 1682(1-3): 80-91.

Jeng, K. C. G. and R. C. W. Hou. 2005. Sesamin and sesamolin: Nature's Therapeutic Lignans. Current enzyme Inhibition 1: 11-20.

Jönsson, M., A. Abrahamson, B. Brunström and I. Brandt. 2006. Cytochrome P4501A induction in rainbow trout gills and liver following exposure to waterborne indigo, benzo(a)pyrene and 3,3´,4´,5-pentachlorobiphenyl

Aquatic Toxicology 79: 226-232.

Kamal-Eldin, A., J. Frank, A. Razdan, S. Tengblad, S. Basu and B. Vessby. 2000. Effects of dietary phenolic compounds on tocopherol, cholesterol, and fatty acids in rats. Lipids 35(4): 427-435.

Kamaleldin, A., D. Pettersson and L. A. Appelqvist. 1995. Sesamin (a Compound from Sesame Oil) Increases Tocopherol Levels in Rats Fed Ad-Libitum. Lipids 30(6): 499-505.

Kiso, Y., N. Tsuruoka, A. Kidokoro, I. Matsumoto and K. Abe. 2005. Sesamin ingestion regulates the transcription levels of hepatic metabolizing enzymes for alcohol and lipids in rats. Alcoholism-Clinical and Experimental Research 29(11): 116S-120S.

Kleveland, E. J., B. Ruyter, A. Vegusdal, H. Sundvold, R. K. Berge and T. Gjoen. 2006. Effects of 3-thia fatty acids on expression of some lipid related genes in Atlantic salmon (Salmo salar L.). Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 145(2): 239-248.

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

Leaver, M. J., L. A. N. Villeneuve, A. Obach, L. Jensen, J. E. Bron, D. R. Tocher and J. B. Taggart. 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.

Moazzami, A. and A. Kamal-Eldin. 2006. Sesame seed is a rich source of dietary lignans. Journal of the American Oil Chemists Society 83(8): 719-723.

Morais, S., O. Monroig, X. Z. Zheng, M. J. Leaver and D. R. Tocher. 2009. Highly Unsaturated Fatty Acid Synthesis in Atlantic Salmon: Characterization of ELOVL5-and ELOVL2-like Elongases. Marine Biotechnology 11(5): 627-639.

Moya-Falcon, C., E. Hvattum, T. N. Tran, M. S. Thomassen, J. Skorve and B. Ruyter. 2006. Phospholipid molecular species, beta-oxidation, desaturation and elongation of fatty acids in Atlantic salmon hepatocytes: Effects of temperature and 3-thia fatty acids. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 145(1): 68-80.

Mozaffarian, D. and E. B. Rimm. 2006. Fish intake, contaminants, and human health - Evaluating the risks and the benefits. Jama-Journal of the American Medical Association 296(15): 1885-1899.

Murray, M. 2000. Mechanisms of inhibitory and regulatory effects of methylenedioxyphenyl compounds on cytochrome P450-dependent drug oxidation. . Current Drug Metabolism 1: 67-84.

Nelson, D. R., L. Koymans, T. Kamataki, J. J. Stegeman, R. Feyereisen, D. J. Waxman, M. R. Waterman, O. Gotoh, M. J. Coon, R. W. Estabrook, I. C. Gunsalus and D. W. Nebert. 1996. P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics 6(1): 1-42.

Omura, T. and R. Sato. 1964. The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. J Biol Chem 239: 2379-2385.

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

Pfaffl, M. W., G. W. Horgan and L. Dempfle. 2002. Relative expression software tool (REST (c)) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Research 30(9).

Schlechtriem, C., J. E. Bron and D. R. Tocher. 2007. Inter-individual variation in total fatty acid compositions of flesh of Atlantic salmon smolts-fed diets containing fish oil or vegetable oil. Aquaculture Research 38(10): 1045-1055.

Shimizu, S., K. Akimoto, Y. Shinmen, H. Kawashima, M. Sugano and H. Yamada. 1991. Sesamin Is a Potent and Specific Inhibitor of Delta-5 Desaturase in Polyunsaturated Fatty-Acid Biosynthesis. Lipids 26(7): 512-516.

Smith, P. K., R. I. Krohn, G. T. Hermansson, A. K. Malilia, Gartner, F.H., M. D. Provenzano, E. K. Fujimito, N. M. Goeke, B. J. Olson and D. C. Klenk. 1985. Measurement of protein using bicinchoninic acid. Analytical Biochemistry 150: 76-85.

Tacon, A. G. J. (2005). Global trends in aquaculture and compound aquafeed production. International Aquafeed Directory and Buyers' Guide. A. G. J. Tacon, R. A. I., Turret Uxbridge, Middlesex (United Kingdom)**:** 8-23.

Tocher, D. R., J. Fonseca-Madrigal, J. G. Bell, J. R. Dick, R. J. Henderson and J. R. Sargent. 2002. Effects of diets containing linseed oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes in Atlantic salmon (Salmo salar). Fish Physiology and Biochemistry 26(2): 157-170.

Torstensen, B. E., J. G. Bell, G. Rosenlund, R. J. Henderson, I. E. Graff, D. R. Tocher, O. Lie and J. R. Sargent. 2005. Tailoring of Atlantic salmon (Salmo salar L.) flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend. Journal of Agricultural and Food Chemistry 53(26): 10166-10178.

Trattner, S., A. Kamal-Eldin, E. Brannas, A. Moazzami, V. Zlabek, P. Larsson, B. Ruyter, T. Gjoen and J. Pickova. 2008. 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., J. Pickova, K. H. Park, J. Rinchard and K. Dabrowski. 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., B. Ruyter, T. K. Ostbye, T. Gjoen, V. Zlabek, A. Kamal-Eldin and J. Pickova. 2008. 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.

Umeda-Sawada, R., M. Ogawa and O. Igarashi. 1998. The Metabolism and n-6/n-3 Ratio of Essential Fatty Acids in Rats: Effect of Dietary Arachidonic Acid and Mixture of Sesame Lignans (sesamin and episesamin). Lipids 33(6): 567-527.

Umeda-Sawada, R., M. Ogawa and O. Igarashi. 1999. The metabolism and distribution of sesame lignans (sesamin and episesamin) in rats. Lipids 34(6): 633-637.

Watt, M. J., G. R. Steinberg, G. J. F. Heigenhauser, L. L. Spriet and D. J. Dyck. 2003. Hormone-sensitive lipase activity and triacylglycerol hydrolysis are decreased in rat soleus muscle by cyclopiazonic acid. American Journal of Physiology-Endocrinology and Metabolism 285(2): E412-E419.
Williams, D. E., J. J. Lech and D. R. Buhler. 1998. Xenobiotics and xenoestrogens in fish: modulation of cytochrome P450 and carcinogenesis. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis 399(2): 179-192.
Voss, A., M. Reinhart, S. Sankarappa and H. Sprecher. 1991. The Metabolism of 7,10,13,16,19-Docosapentaenoic Acid to 4,7,10,13,16,19-Docosahexaenoic Acid in Rat-Liver Is Independent of a 4-Desaturase. Journal of Biological Chemistry 266(30): 19995-20000.
Zheng, X. Z., B. E. Torstensen, D. R. Tocher, J. R. Dick, R. J. Henderson and J. G. Bell. 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). Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids 1734(1): 13-24.