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1 **Influence of dietary docosahexaenoic acid in combination with other long-chain**
2 **polyunsaturated fatty acids on expression of biosynthesis genes and phospholipid fatty acid**
3 **compositions in tissues of post-smolt Atlantic salmon (*Salmo salar*)**

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11
12 **Running title:** Effects of DHA and other LC-PUFA in Atlantic salmon post-smolts.

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23 **Abstract**

24 To investigate interactions of dietary LC-PUFA, a dose-response study with a range of
25 docosahexaenoic acid (DHA; 22:6n-3) levels (1g kg⁻¹, 5 g kg⁻¹, 10 g kg⁻¹, 15 g kg⁻¹ and 20 g kg⁻¹)
26 was performed with post-smolts (111 ± 2.6 g; mean ± S.D.) over a nine-week feeding period.
27 Additional diets included 10 g kg⁻¹ DHA in combination with 10 g kg⁻¹ of either eicosapentaenoic
28 acid (EPA; 20:5n-3) or arachidonic acid (ARA; 20:4n-6), and a diet containing 5g kg⁻¹ each of
29 DHA and EPA. Liver, brain, head kidney and gill were collected at the conclusion of the trial and
30 lipid and fatty acid compositions determined as well as expression of genes of LC-PUFA
31 biosynthesis. Total lipid content and class composition were largely unaffected by changes in
32 dietary LC-PUFA. However, phospholipid (PL) fatty acid compositions generally reflected that of
33 the diet, although the response varied between tissues. Liver most strongly reflected diet, followed
34 by head kidney. In both tissues increasing dietary DHA led to significantly increased DHA in PL
35 and inclusion of EPA or ARA led to higher levels of these fatty acids. Brain showed the most
36 conserved composition and gene expression profile, with increased dietary LC-PUFA resulting in
37 only minor changes in PL fatty acids. Dietary LC-PUFA significantly affected the expression of $\Delta 6$
38 and $\Delta 5$ desaturases, Elovl 2, 4 and 5, and SREBPs although this varied between tissues with
39 greatest effects observed in liver followed by head kidney, similar to PL fatty acid compositions.

40

41 **Key words:** Atlantic salmon, polyunsaturated fatty acid, DHA, ARA, EPA, composition, LC-PUFA
42 biosynthesis, liver, brain, head kidney, gill, muscle

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47 **1. Introduction**

48 It is now widely appreciated that fish, particularly oily species such as Atlantic salmon (*Salmo*
49 *salar*), herring (*Clupea harengus*) and mackerel (*Scomber scombrus*), represent a rich and almost
50 unique source of n-3 long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA) in the human diet
51 (Bell et al., 2001; Tocher, 2009; Monroig et al., 2010). The beneficial health effects of these fatty
52 acids are well established through the roles they play in cardiovascular disease (Calder, 2004),
53 inflammatory and autoimmune diseases (Simopoulos, 2002) and neurological disorders (Dyall &
54 Michael-Titus, 2008). At the same time, there are concerns surrounding the accumulation of
55 contaminants in fish and the perceived health risks these may pose to the human consumer,
56 although scientific evidence is lacking and risks have yet to be defined or quantified (Bell &
57 Waagbø, 2008; Tocher, 2009). However, the most urgent issue is that worldwide demand for
58 aquatic food products continues to grow beyond the sustainable limits of global capture fisheries
59 (Sargent & Tacon, 1999). This has resulted in significant growth of the aquaculture sector in recent
60 decades and, coupled with changes in public attitude towards the sustainability of the industry, the
61 continued production of high-quality, n-3 LC-PUFA-rich fish faces a number of challenges
62 (Subasinghe et al., 2009).

63 Atlantic salmon represents one of the most economically important species for aquaculture
64 worldwide but, as a carnivorous species, it also presents somewhat of a paradox. The aquafeeds
65 used to rear Atlantic salmon have traditionally relied upon high proportions of fish oils derived
66 from small, pelagic marine fish on the basis that they provide an excellent source of n-3 LC-PUFA
67 (Sargent & Tacon, 1999; Bendiksen et al., 2011). However, the majority of world stocks for these
68 forage fish are considered to be either fully or over-exploited (FAO, 2012), and the limited supply
69 of fish oil is only exacerbated by competition for inclusion in human nutritional supplements and
70 agricultural feeds (Bell et al. 2001; Naylor et al. 2009). Consequently, much of the research in
71 recent years has focussed on sustainable alternatives to fish oils, principally vegetable oils
72 (reviewed by Nasopoulou & Zabetakis, 2012). Numerous feeding trials have revealed that growth,

73 feed conversion and survival of Atlantic salmon are largely unaffected when fish oil is partially
74 replaced by vegetable oil (Tortensen et al., 2000; Bell et al., 2001; Rosenlund et al., 2001;
75 Bransden et al., 2003). However, vegetable oils are notable for their lack of LC-PUFA, indicating
76 that high replacement of fish oils cannot be accomplished without compromising product quality
77 through reduced flesh n-3 LC-PUFA content (Bell et al., 2003; Menoyo et al., 2005; Tocher, 2010).
78 Therefore, the reputation of farmed Atlantic salmon as a health promoting food seems reliant upon
79 a better understanding of the functional requirements and metabolism of LC-PUFA.

80 All vertebrates including fish require three key fatty acids for normal growth and
81 development: docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and
82 arachidonic acid (ARA; 20:4n-6) (Sargent et al., 1997; Bell, 1998; Sargent et al., 1999). These
83 biologically active fatty acids play important roles in cell membrane structure and function
84 (Sargent et al. 2002), the regulation of reproduction (Bell & Sargent, 2003) and the modulation of
85 immune responses (Waagbø, 1994). Salmonids can synthesise these LC-PUFA *de novo* from their
86 C₁₈ precursors α -linolenic acid (LNA; 18:3n-3) and linoleic acid (LOA; 18:2n-6), though their
87 capacity for this is limited (Castell et al., 1972; Bell et al., 1993; Tocher et al., 2000). The enzymes
88 involved in the bioconversion of both n-3 and n-6 PUFA to LC-PUFA are the Δ -6 and Δ -5 fatty
89 acyl desaturases (FADS2D6 and FADS2D5 respectively) and two fatty acyl elongases (ELOVL2
90 and ELOVL5). The Δ 6 desaturation of both C₁₈ and C₂₄ PUFA is likely required for the
91 biosynthesis of DHA in salmon, although there are three functional FADS2D6 in Atlantic salmon
92 that may indicate differential regulation of these desaturation steps (Monroig et al., 2010).
93 Similarly, two functional ELOVL5 (a and b) have been identified in salmon (Morais et al., 2009).
94 Functional studies suggest that ELOVL5 is mainly involved in the elongation of C₁₈→C₂₀ PUFA,
95 with residual C₂₀→C₂₂ activity, whereas ELOVL2 elongates C₂₀→C₂₂ but not C₁₈→C₂₀ (Morais et
96 al., 2009). The activity of the LC-PUFA biosynthesis pathway relies on the presence of substrates
97 but also transcription factors (TF) such as sterol regulatory element binding protein (SREBP) 1

98 and 2 or liver X receptor (LXR), which may be involved in gene regulation (Carmona-Antoñanzas
99 et al., 2014).

100 To understand performance characteristics at the level of the organism in greater detail, it is
101 necessary to evaluate the different roles that LC-PUFA play within individual tissues. Thus, liver is
102 considered an important site for LC-PUFA synthesis and lipid metabolism in Atlantic salmon
103 (Monroig et al., 2010). Neural tissues like brain and retina are characteristically rich in DHA
104 (Tocher & Harvie, 1988; Bell & Tocher, 1989), and thus, DHA-deficient diets lead to impaired
105 visual performance (Bell et al., 1995). Head kidney is of interest because it forms a key component
106 of the fish immune system (Tort et al. 2003), the functions of which are known to be influenced by
107 dietary LC-PUFA (Waagbø, 1994; Lall, 2000). Specifically, LC-PUFA are considered essential for
108 the production of eicosanoids such as leukotrienes, prostaglandins and thromboxanes, substances
109 that act as key mediators between immune cell membranes and inflammatory responses in fish
110 (Rowley et al. 1995; Martinez-Rubio et al., 2013). The gills are another tissue susceptible to
111 dietary changes in PUFA and this is of specific interest because, on top of respiration, the gills play
112 vital roles in osmoregulation and ion balance (Bell et al., 1992; 1996).

113 Phospholipids (PL), major constituents of cell lipids, tend to be fairly constant in composition
114 under normal physiological conditions, enabling functional associations to be drawn between
115 different organs/tissues (Christie, 2003a). In addition, LC-PUFA are preferentially deposited in PL
116 over triacylglycerol (TAG) (Sargent et al., 2002). It was hypothesised that the PL fatty acid
117 compositions of liver, brain, head kidney and gill tissues of Atlantic salmon would respond
118 differently to altered dietary LC-PUFA based on their individual fatty acid requirements and
119 functional roles in lipid and fatty acid metabolism. The present study therefore aimed to examine
120 the different tissue specificities for DHA, in addition to potential interactions with either EPA or
121 ARA in post-smolts fed diets containing varying levels of these essential LC-PUFA. Furthermore,
122 the study sought to evaluate the influence dietary LC-PUFA may have on total lipid contents and

123 compositions of the different tissues, in addition to understanding the molecular mechanisms
124 involved in the control and regulation of LC-PUFA metabolism.

125

126 **1. Materials and Methods**

127 *2.1. Experimental diets*

128 A single basal diet was formulated to provide protein and lipid at 460 g kg⁻¹ and 200 g kg⁻¹
129 diet at a gross energy level of 22.0 MJ kg⁻¹ (estimated digestible protein and energy of 440 g kg⁻¹
130 and 19.5 MJ kg⁻¹, respectively). A total of eight experimental diets were produced by vacuum
131 coating the dry basal extruded pellets with custom, pre-mixed oil blends as follows. To investigate
132 the effect of DHA concentration, a series of five DHA inclusion levels (1 g kg⁻¹, 5 g kg⁻¹, 10 g kg⁻¹
133 ¹, 15 g kg⁻¹ and 20 g kg⁻¹, named D1, D5, D10, D15 and D20 respectively) were created using a
134 blend of oils that included an algal DHA source derived from *Crypthecodinium* sp. (HuaTai
135 BioPharm Inc., Deyang, Sichuan, China) along with a combination of clarified butterfat and olive
136 oil as a lipid base (Table 1). To examine additional effects of EPA and ARA inclusion, three
137 further treatments were created. Two EPA diets containing either 10 g kg⁻¹ (D10E) or 5 g kg⁻¹
138 (D5E) each of EPA and DHA were formulated using anchovy oil that contained EPA and DHA in
139 equal amounts. A single ARA treatment (D10A) was formulated using fungal-derived concentrate
140 (HuaTai BioPharm Inc., Deyang, Sichuan, China) to include 10 g kg⁻¹ each of ARA and DHA. For
141 full compositional analysis of experimental diets see Table 2 and for additional information on diet
142 manufacture refer to Glencross et al. (2014).

143

144 *1.2. Fish and husbandry*

145 Prior to experimental work, Atlantic salmon smolts were sourced from Howietoun hatchery
146 (Bannockburn, Scotland) and transferred to the Marine Environmental Research Laboratory
147 (Machrihanish, Argyll, Scotland) where they were on-grown to 110.9 ± 2.61 g (mean ± S.D.) post-

148 smolts in two 10,000 L seawater tanks. All fish were anesthetized using benzocaine prior to
149 handling. The fish were weighed on an electronic toploading balance to 0.5 g accuracy and 20 fish
150 allocated to each of 24 x 500 L tanks. The experimental system comprised a flow-through,
151 ambient water temperature, 500 L x 24-tank array. Water temperature was $14.0 \pm 0.82^{\circ}\text{C}$ (mean \pm
152 S.D.) and dissolved oxygen was at $7.8 \pm 0.60 \text{ mg L}^{-1}$ (mean \pm S.D.) for the duration of the 9-week
153 experiment. All eight treatments were fed in triplicate (three tanks of 20 fish each). Experimental
154 feeds were delivered on a restricted pair-wise feeding regime to eliminate feed intake variability,
155 and feed rations were increased incrementally over the duration of the study. Further details of
156 feeding regime are provided elsewhere (Glencross et al., 2014).

157

158 *1.3. Sample collection and management*

159 At the end of the feeding trial, a total of six fish (two per tank) from each treatment were
160 randomly sampled and euthanized by benzocaine overdose. Samples of liver, brain, gill and head
161 kidney tissue were collected from each fish and immediately frozen in liquid nitrogen prior to
162 storage at -70°C . Approximately 100 mg of each individual tissue was sampled for total RNA
163 extraction, whereas paired samples from each tank were pooled to form individual replicates by
164 treatment and tissue type for total lipid extraction.

165

166 *1.4. Total lipid extraction*

167 Lipid was extracted from tissue samples using a modified method of Folch et al. (1957).
168 Briefly, liver and gill samples were homogenized in 16 ml of chloroform/methanol (2:1, v/v) using
169 an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK), while brain and head
170 kidney samples were homogenized using a glass-barrel homogenizer in the same volume of
171 solvent. Non-lipid impurities were isolated by washing with 4 ml of 0.88% aqueous KCl (w/v).
172 The upper aqueous layer was removed by aspiration and the lower solvent layer containing the

173 lipid extract dried under oxygen-free nitrogen. Total lipid content was determined gravimetrically
174 after overnight desiccation *in vacuo*.

175

176 1.5. Lipid class composition

177 Lipid classes were separated by double-development, high-performance thin-layer
178 chromatography (HPTLC) using 10 x 10 cm plates (VWR, Lutterworth, UK) according to
179 Henderson & Tocher (1992). Total lipid samples (1-2 µg) were applied as 3 mm origins and the
180 plates developed in methyl acetate/isopropanol/chloroform/methanol/0.25% aqueous KCl
181 (25:25:25:10:9, by vol.) to 5.2 cm. Excess solvent was evaporated via air drying and vacuum
182 desiccation and plates developed to 9.5 cm using a solvent mixture containing iso-hexane/diethyl
183 ether/acetic acid (80:20:1, by vol.) before termination and drying as above. Lipid classes were
184 visualized by spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid
185 and charring plates at 160 °C for 20 min. Lipid classes were quantified by densitometry using a
186 CAMAG-3 TLC scanner (version Firmware 1.14.16; CAMAG, Muttenz, Switzerland) with
187 winCATS software (Planar Chromatography Manager, version 1.2.3).

188

189 1.6. Phospholipid fatty acid composition

190 Phospholipids were isolated using thin-layer chromatography (TLC) by loading 2 mg of total
191 lipid onto 2.5 cm origins on 20 x 20 cm TLC plates (VWR, Lutterworth, UK) and running in a
192 solvent mixture comprising isohexane/diethyl ether/acetic acid (80:20:1, by vol.). Plates were
193 sprayed with 1% (w/v) 2',7'-dichlorofluorescein in 97% (v/v) methanol containing 0.05% (w/v)
194 BHT and visualized under UV light (UVGL-58 Minerallight[®] Lamp, Ultraviolet Prod. Inc., Calif.,
195 USA). Total polar lipids were scraped into test tubes and fatty acid methyl esters (FAME) were
196 prepared by acid-catalyzed transmethylation according to the method of Christie (2003b). FAME
197 were separated and quantified by gas-liquid chromatography using a Fisons GC-8160 (Thermo
198 Scientific, Milan, Italy) equipped with a 30 m × 0.32 mm i.d. µm²B -wax column

199 (Phenomenex, Cheshire, UK), on-column injector and a flame ionization detector. Hydrogen was
200 used as the carrier gas in constant flow mode at 2.5 ml min^{-1} , with an initial oven thermal gradient
201 from $50 \text{ }^{\circ}\text{C}$ to $150 \text{ }^{\circ}\text{C}$ at $40 \text{ }^{\circ}\text{C min}^{-1}$ to a final temperature of $230 \text{ }^{\circ}\text{C}$ at $2 \text{ }^{\circ}\text{C min}^{-1}$. Data were
202 collected and processed using Chromcard for Windows (version 2.01; Thermoquest Italia S.p.A.,
203 Milan, Italy). Individual FAME were identified by comparison to known standards and published
204 data (Ackman, 1980; Tocher & Harvie, 1988). Selected FAME were confirmed by gas
205 chromatography-mass spectrometry (GC-MS) using a gas chromatograph (GC8000) coupled to a
206 MD800 mass spectrometer (ThermoFisher Scientific, Hemel Hempstead, UK).

207

208 *1.7. RNA extraction and quantitative real time PCR (qPCR)*

209 Five of the eight experimental treatments were chosen for the gene expression study. These
210 diets were chosen to represent a low DHA level (D1), an “optimum” level according to previous
211 studies (D10; Glencross et al., 2014), and the three combinations of LC-PUFA (D10A, D10E and
212 D5E). Liver, brain, gill and head kidney samples from six individual fish per treatment ($n = 2$ fish
213 per tank) were homogenized in TriReagent[®] (Sigma-Aldrich, Dorset, UK) RNA extraction buffer
214 following the manufacturer’s instructions. Quantity and quality of isolated total RNA were
215 determined by spectrophotometry with an ND-1000 Nanodrop (Labtech Int., East Sussex, UK) and
216 electrophoresis using 500 ng of total RNA in a 1% agarose gel. cDNA was synthesized using 2 μg
217 of total RNA and random primers in 20 μl reactions and the High capacity reverse transcription kit
218 without RNase inhibitor according to the manufacturer’s protocol (Applied Biosystems,
219 Warrington, UK). The resulting cDNA was diluted 20-fold with milliQ water.

220 For qPCR, primers for fatty acyl desaturases and elongases, and TF involved in their
221 regulation, were used (see Table 3). The efficiency of the primers for each gene was previously
222 evaluated to ensure that it was close to 100%. In addition, two reference genes, cofilin-2 and
223 elongation factor-1 α were quantified. qPCR was performed using a Biometra TOptical
224 Thermocycler (Analytik Jena, Goettingen, Germany) in 96-well plates in duplicate 20 μl reaction

225 volumes containing 10 μ l of SYBR Green RT-PCR Master Mix (Applied Biosystems, Paisley,
226 UK), 1 μ l of the primer corresponding to the analyzed gene (10 pmol), 3 μ l of molecular biology
227 grade water and 5 μ l of cDNA, with the exception of the reference genes, which were determined
228 using 2 μ l of cDNA. In addition amplifications were carried out with a systematic negative control
229 (NTC-non template control) containing no cDNA. Standard amplification parameters contained an
230 initial activation step at 95°C for 15 min, followed by 35 cycles: 15 s at 95°C, 30 s at the
231 annealing T_m and 30 s at 72°C. A calibrator sample was included within each plate in order to
232 compare the gene expression among the different tissues/plates.

233

234 *1.8. Statistical Analysis*

235 All data are means \pm S.D. ($n = 3$) unless otherwise specified. Percentage data for total lipid
236 content, lipid class composition and polar lipid fatty acid composition were all subjected to arcsin
237 square-root transformation prior to analyses. Effects of DHA inclusion level (diets D1-D20) were
238 examined with regression analysis. Additionally, effects of EPA and ARA inclusion were
239 examined against equivalent levels of DHA. Specifically, the 10 g kg⁻¹ diets D10 and D5E were
240 examined by one-way analysis of variance (ANOVA), while the 20 g kg⁻¹ diets D20, D10A and
241 D10E were examined by one-way ANOVA followed by a Tukey-Kramer HSD multiple
242 comparison of means. All statistical analyses were performed using Minitab (version 16.1.0;
243 Minitab Inc., State college, PA).

244 Gene expression results were analyzed using the relative expression software tool (REST
245 2008; <http://www.gene-quantification.info/>), which employs a pairwise fixed reallocation
246 randomization test (10,000 randomizations) with efficiency correction (Pfaffl et al., 2002) to
247 determine the statistical significance of expression ratios (gene expression fold changes) between
248 two treatments. In addition, a supervised hierarchical clustering was applied employing the relative
249 gene expression ratio for each gene based on the PCR efficiency and Ct of sample compared to the

250 control, according to Pfaffl's mathematical model (Pfaffl, 2001). Tree View software (Page, 1996)
251 was used to generate visual representations of the classification.

252

253 **2. Results**

254 *3.1. Total lipid content*

255 In general lipid content was fairly constant across all eight dietary treatments (Tables 4-7).
256 Brain tissue had the highest mean lipid content (7.7%), followed by liver (4.8%) and head kidney
257 (4.2%), while gill tissue contained on average just 1.7% lipid (Tables 4-7). Increasing inclusion of
258 DHA in the diet (D1-D20) had no significant effect on lipid content in any of the tissues examined.
259 The lipid content of head kidney from fish fed diet D20 was lower than that of fish fed diet D10E
260 (Table 5), and brain lipid content was higher in fish fed diet D5E compared with those fed diet
261 D10 (Table 7). Other than these differences, the mixed LC-PUFA diets had no significant effects
262 on tissue lipid contents.

263

264 *2.2. Lipid class composition*

265 In general the dietary treatments had little effect on the lipid class compositions of liver, brain,
266 head kidney and gill. The proportions of phosphatidylcholine (PC) and cholesterol were slightly,
267 but significantly, increased and the proportion of phosphatidylinositol (PI) decreased, in liver with
268 increasing dietary DHA (data not shown). Increasing dietary DHA had no significant effect on the
269 lipid class composition of head kidney, gill or brain. The level of TAG in brain was highly variable
270 between fish fed the different diets although only significant in fish fed diet D10E, which had
271 higher TAG than fish fed diets D20 or D10A (data not shown). Other than this, the diets with
272 combinations of LC-PUFA had no significant effects on the lipid class compositions of salmon
273 tissues.

274

275 *2.3. Fatty acid compositions of liver and head kidney phospholipids*

276 The predominant fatty acids in liver PL were DHA, 18:1n-9 and 16:0, though overall fatty
277 acid composition was readily influenced by that of the diet (Tables 2, 4 & 5). Relative
278 concentrations of DHA, EPA and ARA in liver PL were always considerably higher than those of
279 the diet, while total saturated fatty acids (SFA) and total monounsaturated fatty acids (MUFA)
280 were consistently lower than those of the diet. Increased inclusion of DHA in the diet resulted in a
281 highly significant increase (10.4 %) in the concentration of DHA in the liver ($P < 0.001$) and
282 correspondingly increased total n-3 PUFA and LC-PUFA (Table 4). The relative contents of EPA
283 and docosapentaenoic acid (DPA; 22:5n-3) in the liver tended to decrease with increasing DHA,
284 while levels of ARA and 22:4n-6 were largely unaffected. Total SFA content of liver PL remained
285 fairly stable, but MUFA content decreased significantly across diets D1-D20, reflecting the dietary
286 levels of these fatty acid groups. The concentration of 22:5n-6 in liver PL increased significantly in
287 fish fed diets D1 through to D20 reflecting the increasing level of this fatty acid in these diets
288 (Tables 2 & 4).

289 Inclusion of other LC-PUFA (EPA and ARA) in the diet also influenced the fatty acid
290 composition of liver PL (Table 5). Inclusion of EPA (diets D5E & D10E) resulted in DHA levels
291 similar to those found in liver PL from fish fed equivalent diets containing DHA alone (D10 and
292 D20) (Tables 2 & 5). EPA inclusion also tended to increase the levels of both EPA and DPA found
293 in liver PL, with the higher inclusion of EPA resulting in highly significant increases ($P < 0.001$) in
294 these fatty acids relative to diets D20 and D10A (Table 5). Furthermore, diets D5E and D10E
295 resulted in the lowest levels of ARA in liver PL. Inclusion of ARA (D10A) resulted in significantly
296 higher levels of ARA and 22:4n-6 in liver PL compared with DHA alone (D20) (Table 5). Diet
297 D10A also resulted in the lowest level of EPA in liver PL out of all the diets. Inclusion of EPA or
298 ARA also clearly altered the EPA/ARA ratio in their favour, but there were no significant effects of
299 EPA or ARA inclusion on the relative amount of SFA or MUFA.

300 The fatty acid composition of head kidney PL differed from that of liver in that it contained
301 slightly more SFA and slightly less PUFA (Tables 4 & 5). Additionally, head kidney contained a
302 small percentage of dimethyl acetals (DMA; 2.6 % total fatty acids), derived from plasmalogen
303 PL, not observed in the liver. However, the effects of increased dietary DHA were broadly the
304 same as those described for liver PL, although the mean increase in relative DHA content in head
305 kidney was not as defined as in liver (5.6 % vs. 10.4 % total fatty acids) (Table 4). Effects of EPA
306 or ARA inclusion on the fatty acid composition in head kidney PL were also very similar to those
307 observed in liver PL (Table 5).

308 *2.4. Fatty acid compositions of brain and gill phospholipids*

309 The fatty acid composition of brain PL showed a number of general differences to that of liver
310 PL. Brain PL contained a notable proportion (> 7% total fatty acids) of DMA (Tables 6 & 7). In
311 addition, the relative content of EPA was higher, and that of ARA lower in brain PL compared to
312 liver PL. Furthermore, the relative MUFA content of brain PL (due to higher 24:1n-9, not shown)
313 was noticeably higher than that of the liver (39.6 vs. 23.3 % total fatty acids) (Tables 6 & 7).
314 Increasing DHA inclusion resulted in only a small, but significant, increase (1.7 %) in the relative
315 DHA content of brain PL (Table 6). Relative SFA contents increased slightly and relative MUFA
316 contents decreased in fish fed diets D1 through D20, reflecting dietary compositions (Tables 2 &
317 6). EPA and DPA both declined in brain PL in fish fed diets D1 through D20, although the effects
318 were subtle in comparison to the liver (Table 6). Diets D1-D20 had no effect on the relative
319 amounts of ARA and 22:4n-6 in brain PL, whereas 22:5n-6 increased in line with dietary levels
320 (Table 6). Effects of EPA and ARA inclusion on the fatty acid composition of brain PL also
321 followed the same overall patterns as seen in the liver, but the magnitude of these effects was
322 generally much lower (Table 7). It was notable that the EPA/ARA ratio was always positive, even
323 when fish were fed diet D10A with increased ARA. The fatty acid composition of gill PL
324 contained slightly higher percentages of ARA and 22:4n-6 and slightly lower percentages of EPA
325 and DPA than liver PL, and total PUFA content was consistently lower (Tables 6 & 7). Relative

326 SFA and MUFA contents were slightly higher than that of liver PL. Like brain, increasing DHA
327 inclusion did not have the same clear effect on relative DHA content in gill PL as observed in liver,
328 and the relationship was non-significant ($P = 0.057$) (Table 6). Effects of EPA or ARA inclusion
329 were similar to those for liver but less pronounced (Table 7).

330 2.5. Tissue expression profile of lipid metabolism genes

331 Differing dietary LC-PUFA contents affected the expression of the studied genes, although the
332 effects varied between the tissues. Therefore, brain showed the more stable expression pattern
333 whereas, in contrast, liver showed the highest number of altered genes, followed by head kidney
334 (Fig. 1). Increased dietary DHA content from 1 to 10 g kg⁻¹ significantly reduced the mRNA
335 abundance of desaturases *fads2d6b*, *fads2d6c* and *fads2d5*, as well as *elovl2* and *elovl4* in liver
336 (Fig. 2), but no dietary effect was observed in brain (Fig. 3). Reduced expression of both *fads2d6b*
337 and *fads2d5* was also observed in head kidney and gill, respectively, although statistically non-
338 significant (Figs. 4 & 5). Generally, fish fed diet D10A displayed the lowest expression of all the
339 fatty acyl desaturases and elongases genes studied in head kidney and liver, though this tendency
340 was not observed in brain and gill. Both *elovl5a* and *5b* showed variable expression in all tissues
341 with the only statistically significant difference found in brain where fish fed diet D10A showed
342 highest expression of *elovl5b*.

343 Regarding TF, *lxr* gene expression did not differ among fish fed any of the diets in any of the
344 tissues. In contrast, *srebp1* expression was up-regulated in liver (Fig. 2) and gill (Fig. 5) of fish fed
345 diet D1, head kidney of fish fed D10E (Fig. 3), and brain of fish fed D10A (Fig. 4). Gene
346 expression of *srebp2* was only significantly regulated only in liver, with lower expression in fish
347 fed D10A (Fig. 2).

348

349 3. Discussion

350 Increasing dietary DHA and additional EPA and ARA to Atlantic salmon had clear effects on
351 PL fatty acid compositions of all tissues although the precise nature and magnitude of the effects
352 varied markedly between the tissues. Liver, brain, head kidney and gill were chosen in the present
353 study based on the different roles they play in LC-PUFA metabolism and vice versa, the varied role
354 LC-PUFA play in the functions of the tissues. In this context, the liver was of interest because of its
355 important roles in LC-PUFA biosynthesis and overall body lipid homeostasis in Atlantic salmon
356 (Tocher et al., 2003; Monroig et al., 2010; Martinez-Rubio et al., 2013). In contrast, in common
357 with other higher vertebrates, brain of fish is characteristically enriched in DHA (Tocher & Harvie,
358 1988; Bell & Tocher, 1989) and so was of particular interest in the present study where dietary
359 DHA was the primary variable. Head kidney in fish forms an integral component of the immune
360 system (Tort et al., 2003; Gjøen et al., 2007), and so was of interest because dietary lipid and PUFA
361 content are known to influence immune function and thus health status of fish (Waagbø, 1994; Lall,
362 2000; Martinez-Rubio et al., 2013). Finally, previous work had shown that gill phospholipid fatty
363 acid compositions significantly alter during the smoltification process and that these changes can be
364 influenced by diet (Bell et al., 1997; Tocher et al., 2000).

365 The results of the present study showed that lipid content of the brain was the highest of all the
366 tissues examined (~ 7.7% wet weight), which was comparable with the value of 7.1% reported for
367 Atlantic salmon by Stoknes et al. (2004). There was a clear trend for lipid content in brain to
368 increase when EPA or ARA was added to the diets although this was only significant in fish fed
369 D5E. The increased lipid content was driven by increased TAG content but the reason for this was
370 unclear. It may be that the relative level of DHA was lower when EPA and ARA was present and so
371 not all uptake was able to be processed for PL synthesis/turnover. Liver and head kidney lipid
372 content did not vary significantly, perhaps because the lipid and energy content was fairly stable
373 across all dietary treatments (Martinez-Rubio et al., 2013), while analysis of gill revealed the lowest
374 lipid content of any of the tissues examined, on average just 1.65% wet tissue weight, which is

375 similar to the low gill lipid content (range 0.6 – 1.4%) found for turbot, *Scophthalmus maximus*
376 (Castell et al., 1994).

377 The fatty acid profiles of the four tissues analyzed showed different patterns likely related to
378 their physiological functions. The fatty acid composition of liver PL largely reflected that of the
379 diet, which is consistent with other studies on Atlantic salmon (Brodtkorb et al., 1997; Bell et al.,
380 2003; Bransden et al., 2003). The relative DHA content of liver PL was always considerably higher
381 than that of the diet, indicating the important role of this fatty acid in cell membranes (Sargent et al.,
382 2002). In contrast, relative levels of SFA and MUFA in liver PL were noticeably lower than those
383 of the feed, suggesting selective discrimination and/or preferential β -oxidation of these fatty acids
384 (Henderson & Sargent, 1985; Turchini & Francis, 2009). The fatty acid composition of brain PL
385 was much more conserved and less affected by diet than the liver, consistent with other studies
386 examining these tissues in Atlantic salmon (Bell et al., 1990; Brodtkorb et al., 1997) and turbot
387 (Bell et al., 1999). This implied a slower turnover of lipids and fatty acids in the brain, although the
388 relatively short duration of the experiment may have limited the ability to detect greater differences
389 between dietary groups.

390 Ruyter et al. (2000) showed that increasing inclusion of DHA and EPA in the diets of Atlantic
391 salmon fry led to increased percentages of these fatty acids in liver PL. Similarly, in the present
392 study, increasing dietary DHA through diets D1-D20 led to a highly significant increase in the
393 percentage of DHA in liver PL. However, this contrasted with the results of Bell et al. (1989) who
394 found no significant differences in the relative amount of DHA in liver PL when Atlantic salmon
395 post-smolts were fed diets containing either fish oil or a combination of corn oil and lard, despite
396 the former diet containing three times more DHA. Regression analysis showed that increasing
397 dietary DHA led to only a small increase in the relative DHA content of brain PL, probably
398 reflecting the fact that brain PL fatty acid composition is much more tightly controlled rather than
399 any particular control on DHA uptake. Indeed, work on juvenile Atlantic salmon by Brodtkorb et al.

400 (1997) found no effects of increasing dietary DHA content on the fatty acid composition or on DHA
401 levels within individual lipid classes of the brain. However, the diets used in the previous study
402 contained much higher levels of DHA (range 6.3 – 17.9 % total fatty acids) representing dietary
403 contents far above any reported requirement levels compared with the present study that included
404 levels of DHA that may be more limiting (range 0.5 – 7.6 % total fatty acids). Interestingly, the
405 significance of the response in brain in the present study was removed when the lowest DHA
406 treatment (D1) was excluded from analysis ($P = 0.454$), supporting the view that only very low
407 DHA affected brain PL fatty acid compositions, and not dietary DHA above requirement levels.

408 Previous studies on the replacement of fish oil with soybean oil in the diets of Atlantic salmon
409 showed a reduction in the percentage of DHA in head kidney total lipid, consistent with the reduced
410 DHA content of vegetable oil-based diets (Gjøen et al., 2004). Similarly, the present study showed
411 that increasing inclusion of DHA in the diet was reflected in head kidney PL with increased
412 percentages of DHA, though the magnitude of effect was only half that observed in liver. The head
413 kidney contains high numbers of immune cells and so changes in dietary fatty acid composition has
414 the potential to alter cell membrane physiology and immune function of these cells (Mourente et al.,
415 2007). In contrast, increasing dietary DHA did not significantly affect the percentage of DHA in gill
416 PL, although variation observed between replicates in D10-fed fish may have influenced this.
417 However, there was still no obvious trend and so the effect, if any, was subtle in comparison to the
418 liver. Overall, this may suggest more selective uptake of DHA in gill PL, or more conserved
419 composition, as observed in brain PL.

420 Given that the impact of dietary DHA was most clearly observed in the liver, it is likely that
421 liver in Atlantic salmon plays an important role in the initial selectivity for DHA, as reported in
422 other vertebrates (Polozova & Salem, 2007). Despite very low levels of DPA in the diet, its higher
423 content in liver PL may suggest that endogenous synthesis of DHA from LNA and/or EPA may
424 have occurred in at least the lower inclusion of DHA (D1). Consistent with this, *fads2d6b*, *fadsd26c*

425 and *fads2d5* expression were higher in fish fed 1 g kg⁻¹ of DHA denoting an upregulation of the LC-
426 PUFA biosynthesis pathway as previously described (Moya-Falcón et al., 2005; Thomassen et al.,
427 2012). The elongation of EPA to DPA appears to be very active in Atlantic salmon liver
428 (Thomassen et al., 2012), which is in agreement with the present study, where high expression of
429 both *elovl2* and *elovl5* was observed together with high levels of DPA. However, only statistical
430 differences were found in *elovl2*, suggesting a greater role of this enzyme compared to *elovl5* in
431 elongation of C₂₀ PUFA. Similarly, heterologous expression in yeast showed that salmon *elovl5*
432 elongated C₁₈ and C₂₀ PUFA, with low activity towards C₂₂, whereas *elovl2* elongated C₂₀ and C₂₂
433 PUFA with lower activity towards C₁₈ (Morais et al., 2009). Interestingly, *elovl4* expression was
434 also upregulated in liver of fish fed 1 g kg⁻¹ of DHA. ELOVL4 is involved in the synthesis of very
435 long-chain PUFA (> C₂₄) in mammals, but it was recently shown that Atlantic salmon *elovl4* open
436 reading frame (ORF) was able to elongate both EPA and ARA (15.4 % and 11.5% conversion,
437 respectively) indicating that it was also involved in LC-PUFA biosynthesis (Carmona-Antoñanzas
438 et al., 2011). These data are consistent with the results in the present study, where high
439 concentrations of DPA could be due to combined activity of the different fatty acid elongases. In
440 contrast, the highest inclusion of DHA (D20) was at the upper end of the documented requirement
441 for LC-PUFA in salmonids (Ruyter et al., 2000) and this probably suppressed further endogenous
442 synthesis of DHA in the liver, gill and head kidney (Bell & Sargent, 2003; Zheng et al., 2004).

443 Dietary inclusion of EPA or ARA typically resulted in increased levels of these fatty acids in
444 the PL of all studied tissues, reflecting the preferential incorporation of LC-PUFA into cell
445 membranes (Sargent et al., 2002). These two fatty acids had inverse reciprocal effects on their
446 respective levels in tissue PL such that inclusion of one reduced the relative amount of the other,
447 highlighting their strong biological link in fatty acid metabolism (Bell et al., 1989). In addition, the
448 tissue proportion of DHA was increased in fish fed both diets containing EPA, regardless of total
449 LC-PUFA content, whereas inclusion of ARA appeared to have the opposite effect in that it
450 lowered the relative amount of DHA and also EPA present in liver, head kidney and gill PL. In

451 contrast, DHA levels in the brain were not affected by dietary EPA or ARA denoting once again the
452 importance of this fatty acid for neural functions. It was noteworthy that EPA did not appear to
453 influence the activity of fatty acyl desaturases, as the EPA level in diet D1 was similar to the other
454 diets, but fish fed diet D1 displayed an up-regulation in these enzymes, especially in liver, head
455 kidney and gill. This may indicate a key role for DHA in the regulation of desaturase expression as
456 has been suggested previously (Thomassen et al., 2012). However, results obtained from a previous
457 *in vitro* study showed inhibition of desaturation and elongation of 18:3n-3 when EPA or DHA were
458 added to the cell medium (Zheng et al., 2009). This earlier study was performed on an established
459 cell line where cells reflect the fatty acid composition of the foetal bovine serum present in the
460 medium, thus the response to LC-PUFA may vary when compared to an *in vivo* model (Tocher et
461 al., 1988).

462 Interestingly, brain PL consistently maintained a positive EPA/ARA ratio, even when fish were
463 fed diet D10A, which contained much more ARA than EPA (5.1% vs 0.6% total fatty acids). A
464 similar effect was seen on a whole-body mass basis in a study with Asian seabass (*Lates calcarifer*)
465 when they were fed diets similar to the D10A and D10E used in the present study (Glencross et al.,
466 2011). Both EPA and ARA are well known to compete as substrates for eicosanoid synthesis in
467 vertebrates (Bell et al., 1994; Calder, 2006). This suggests a preferential incorporation of EPA over
468 ARA in brain PL. Inclusion of EPA and ARA in the diet was of particular relevance to the head
469 kidney because of the key role of eicosanoids in immune/inflammatory responses (Martinez-Rubio
470 et al., 2013). In this sense, the addition of ARA to the diet resulted in significantly higher ARA in
471 head kidney PL, which may have increased inflammatory potential in these fish. This may have in
472 part contributed to the lower survival of this dietary group reported previously (Glencross et al.,
473 2014). On the other hand, inclusion of EPA in the diet resulted in an increased percentage of EPA in
474 head kidney PL, and this would presumably increase the availability of anti-inflammatory
475 eicosanoids. Indeed, recent trials using functional feeds containing both reduced lipid content and
476 increased EPA have been shown to reduce the intensity of inflammatory responses associated with

477 Atlantic salmon reovirus-induced HSMI (heart and skeletal muscle inflammatory disease)
478 (Martinez-Rubio et al., 2012). Another trial modulating the inclusion of EPA and DHA in the diet
479 of Asian seabass found acutely contrasting effects on a range of both clinical and sub-clinical
480 inflammation markers (Glencross et al., 2011). Interestingly, the inclusion of EPA in the present
481 study still only resulted in an EPA/ARA ratio of 1.1 in gill PL, reaffirming the bias toward ARA
482 over EPA in this tissue. Extreme dietary alterations in these fatty acids might therefore compromise
483 osmoregulatory function and overall health of the fish.

484 Regulation of lipid metabolism is complex and controlled by several TF including SREBPs and
485 LXR. In mammals, SREBP1 is involved in activation of genes that participate in fatty acid
486 metabolism and *de novo* lipogenesis whereas SREBP2 is more selective for genes involved in
487 cholesterol homeostasis (Horton et al., 2004). Furthermore, n-3 and n-6 fatty acids can induce
488 transcription of *lxr* through DR1 elements (Tobin et al., 2002) and regulate the expression of *srebp1*
489 (Joseph et al., 2002), which is a major regulator of lipogenesis in mammals (Davidson, 2006). In the
490 present study the diet with highest LC-PUFA content (D10A; 13.5 %) was found to down-regulate
491 the expression of *srebp1* in liver, whereas fish fed diet D1, with only 1% LC-PUFA, showed the
492 highest expression. This is in agreement with previous studies in Atlantic salmon both *in vitro*
493 (Minghetti et al., 2011) and *in vivo* (Morais et al., 2011) where *srebp1* expression was reduced by
494 LC-PUFA supplementation, denoting a similar nutritional regulation to mammals (Davidson et al.,
495 2006; Caputo et al., 2010). Similarly, this lower expression profile was reflected in lower
496 expression levels of some SREBP1 target genes such as *fads2d6c* and *fads2d5*. However, this
497 pattern of expression was not observed in all tissues and so, in contrast, *srebp1* expression in brain
498 was highest in D10A-fed fish. Conversely, *lxr* gene expression was not affected by dietary PUFA
499 content. The explanation to this could be that LXR is activated by a variety of sterols, including
500 intermediates in the synthesis of cholesterol, and adequate levels of cholesterol were present in all
501 of the diets, which could also explain why *srebp2* expression was unaffected.

502 In summary, the present study demonstrated that manipulation of dietary LC-PUFA directly
503 affected the fatty acid profile of tissue PL and gene expression of key metabolic tissues in post-
504 smolt Atlantic salmon. Liver displayed the greatest response to dietary DHA, accumulating this
505 fatty acid in higher amounts than any other tissue, with increased expression of key enzymes
506 involved in LC-PUFA synthesis in fish fed the lowest DHA diet. A qualitatively similar but
507 quantitatively lower effect was observed in head kidney. In contrast, PL fatty acid profile and gene
508 expression was more conserved in brain and less affected by dietary treatment, and a similar
509 response to diet was observed in gill. The tissue variation observed most likely reflected the unique
510 functions of each tissue.

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799 **Legends**

800 **Figure 1. Heat map of the eleven target genes analyzed based on qPCR gene data.**

801 Columns represent mean data values of the five different dietary treatments analyzed in the four
802 tissues and rows represent single genes. Expression level of each gene was squared-root
803 normalized in relation to a single sample, so that comparisons could be made in any sense. Means
804 are depicted by a colour scale, green indicating low (green), neutral (black) or high (red) relative
805 expression levels, as indicated by the colour bar on the left. *fads2d6a*, delta-6 fatty acyl desaturase
806 isoform a; *fads2d6b*, delta-6 fatty acyl desaturase isoform b; *fads26c*, delta-6 fatty acyl desaturase
807 isoform c; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*, fatty acyl elongase 2; *elovl5a*, fatty acyl
808 elongase 5 isoform a; *elovl5b*, fatty acyl elongase isoform b; *elovl4*, fatty acyl elongase 4; *lxr*, liver
809 X receptor; *srebp*, sterol regulatory element binding protein.

810 **Figure 2. Expression of transcription factors and LC-PUFA biosynthesis pathway genes**

811 **in Atlantic salmon liver after nine weeks of feeding.** Results are normalized expression ratios
812 (average +SE, n = 6) of the expression of these genes in fish fed the different diets in relation to
813 fish fed D5 diet. Diets contain either 5 g kg⁻¹ DHA (D5), 10 g kg⁻¹ DHA (D10) and DHA+EPA
814 (D5E) or 20 g kg⁻¹ of DHA+ARA (D10A) and DHA+EPA (D10E). *fads2d6a*, delta-6 fatty acyl
815 desaturase isoform a; *fads2d6b*, delta-6 fatty acyl desaturase isoform b; *fads26c*, delta-6 fatty acyl
816 desaturase isoform c; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*, fatty acyl elongase 2; *elovl5a*,
817 fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase isoform b; *elovl4*, fatty acyl elongase
818 4; *lxr*, liver X receptor; *srebp*, sterol regulatory element binding protein.

819 **Figure 3. Expression, measured by qPCR, of transcription factors and LC-PUFA**
820 **biosynthesis pathway genes in Atlantic salmon brain after nine weeks of feeding.** Results are

821 normalized expression ratios (average +SE, n = 6) of the expression of these genes in fish fed the
822 different diets in relation to fish fed D5 diet. Diets contain either 5 g kg⁻¹ DHA (D5), 10 g kg⁻¹
823 DHA (D10) and DHA+EPA (D5E) or 20 g kg⁻¹ of DHA+ARA (D10A) and DHA+EPA (D10E).
824 *fads2d6a*, delta-6 fatty acyl desaturase isoform a; *fads2d6b*, delta-6 fatty acyl desaturase isoform
825 b; *fads26c*, delta-6 fatty acyl desaturase isoform c; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*,
826 fatty acyl elongase 2; *elovl5a*, fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase isoform
827 b; *elovl4*, fatty acyl elongase 4; *lxr*, liver X receptor; *srebp*, sterol regulatory element binding
828 protein.

829 **Figure 4. Expression, measured by qPCR, of transcription factors and LC-PUFA**
830 **biosynthesis pathway genes in Atlantic salmon head kidney after nine weeks of feeding.**

831 Results are normalized expression ratios (average +SE, n = 6) of the expression of these genes in
832 fish fed the different diets in relation to fish fed D5 diet. Diets contain either 5 g kg⁻¹ DHA (D5),
833 10 g kg⁻¹ DHA (D10) and DHA+EPA (D5E) or 20 g kg⁻¹ of DHA+ARA (D10A) and DHA+EPA
834 (D10E). *fads2d6a*, delta-6 fatty acyl desaturase isoform a; *fads2d6b*, delta-6 fatty acyl desaturase
835 isoform b; *fads26c*, delta-6 fatty acyl desaturase isoform c; *fads2d5*, delta-5 fatty acyl desaturase;
836 *elovl2*, fatty acyl elongase 2; *elovl5a*, fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase
837 isoform b; *elovl4*, fatty acyl elongase 4; *lxr*, liver X receptor; *srebp*, sterol regulatory element
838 binding protein.

839 **Figure 5. Expression, measured by qPCR, of transcription factors and LC-PUFA**
840 **biosynthesis pathway genes in Atlantic salmon gill after nine weeks of feeding.**

841 Results are normalized expression ratios (average +SE, n = 6) of the expression of these genes in fish fed the
842 different diets in relation to fish fed D5 diet. Diets contain either 5 g kg⁻¹ DHA (D5), 10 g kg⁻¹
843 DHA (D10) and DHA+EPA (D5E) or 20 g kg⁻¹ of DHA+ARA (D10A) and DHA+EPA (D10E).
844 *fads2d6a*, delta-6 fatty acyl desaturase isoform a; *fads2d6b*, delta-6 fatty acyl desaturase isoform
845 b; *fads26c*, delta-6 fatty acyl desaturase isoform c; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*,

846 fatty acyl elongase 2; *elovl5a*, fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase isoform
847 b; *elovl4*, fatty acyl elongase 4; *lxr*, liver X receptor; *srebp*, sterol regulatory element binding
848 protein.

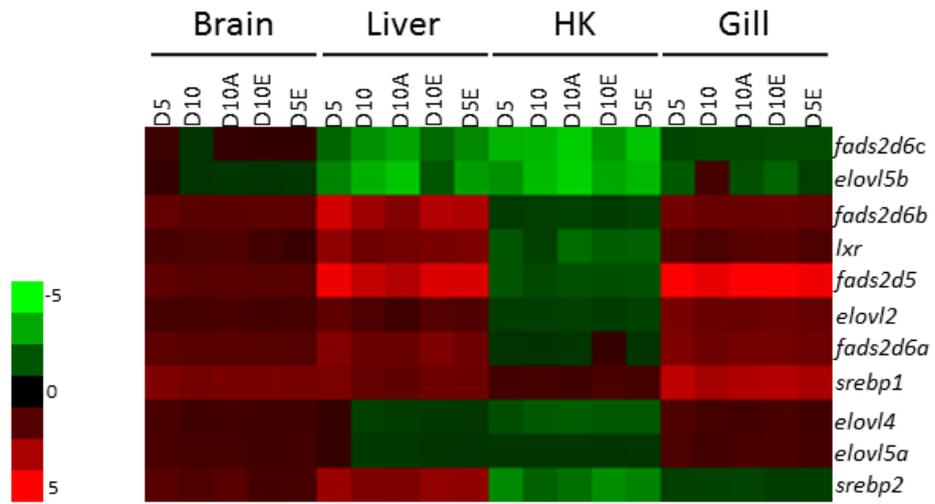


Figure 1.

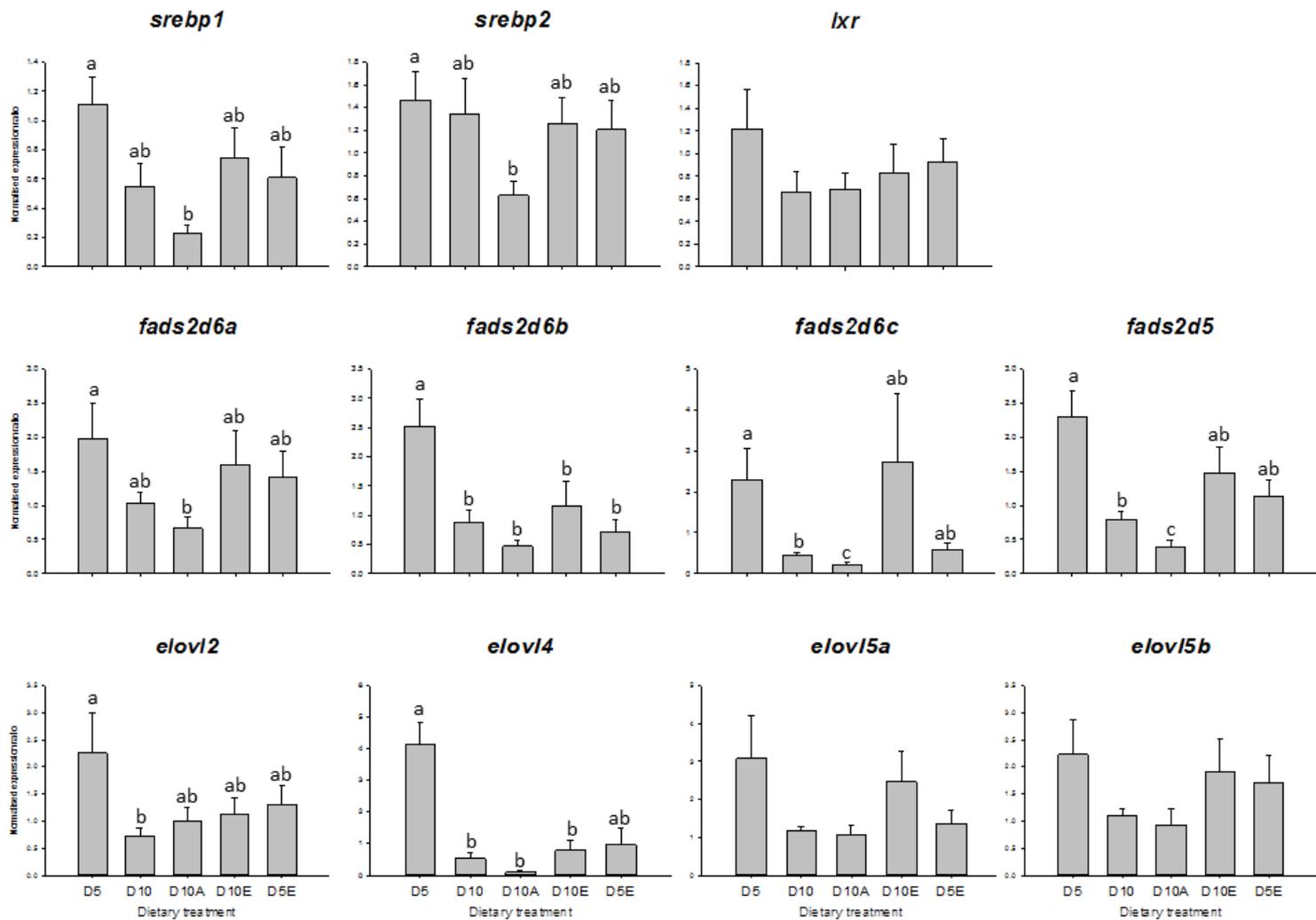


Figure 2.

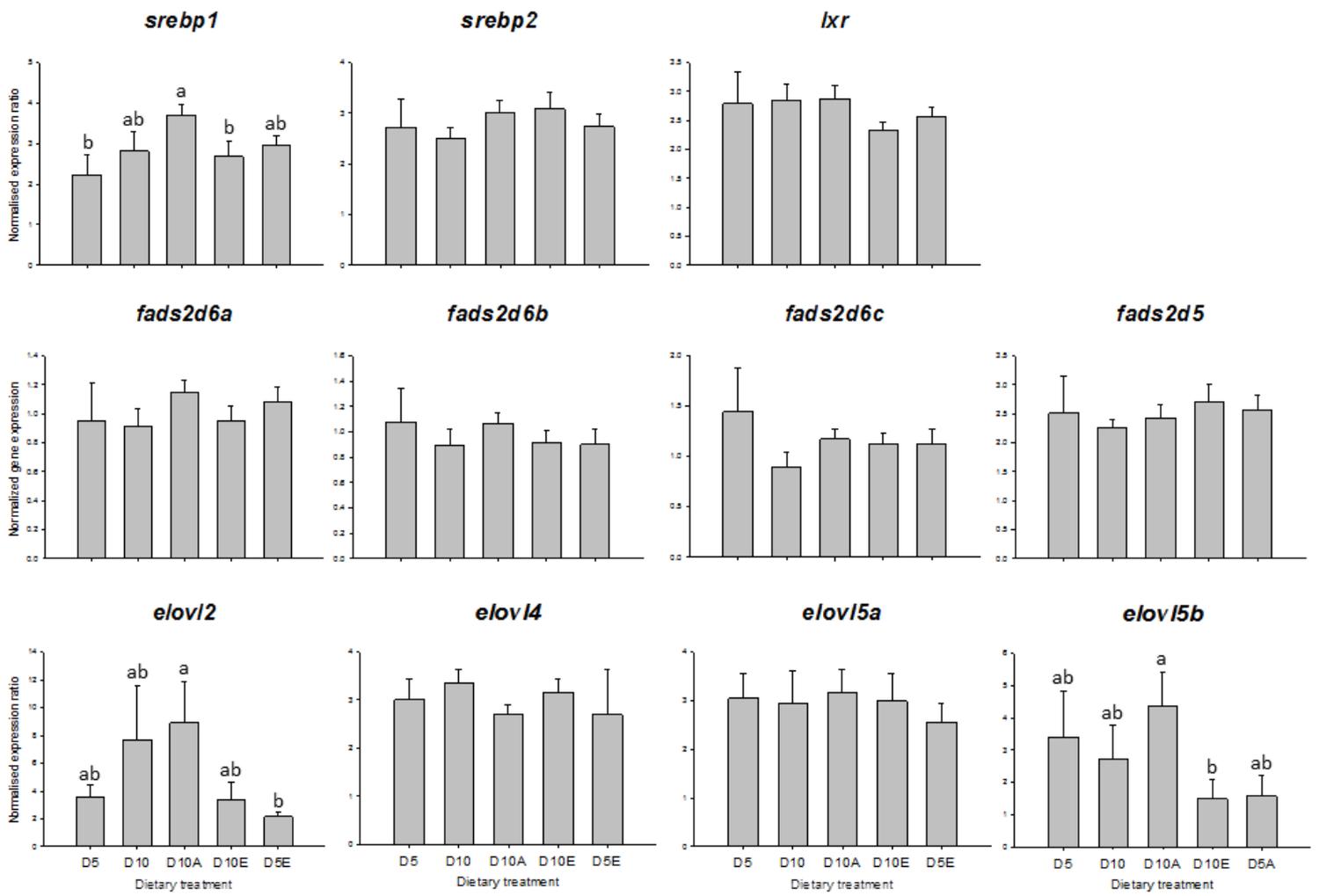


Figure 3.

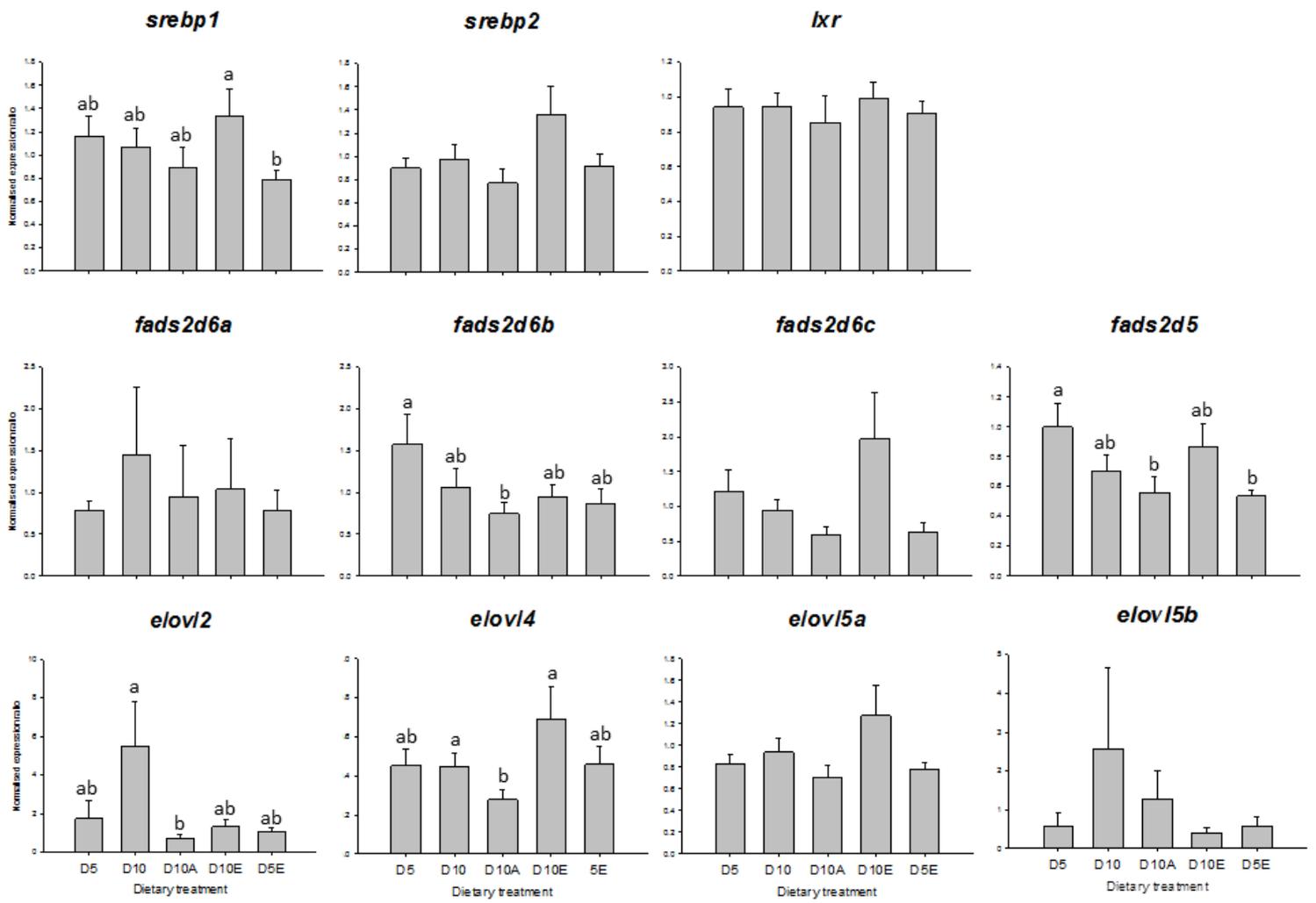


Figure 4.

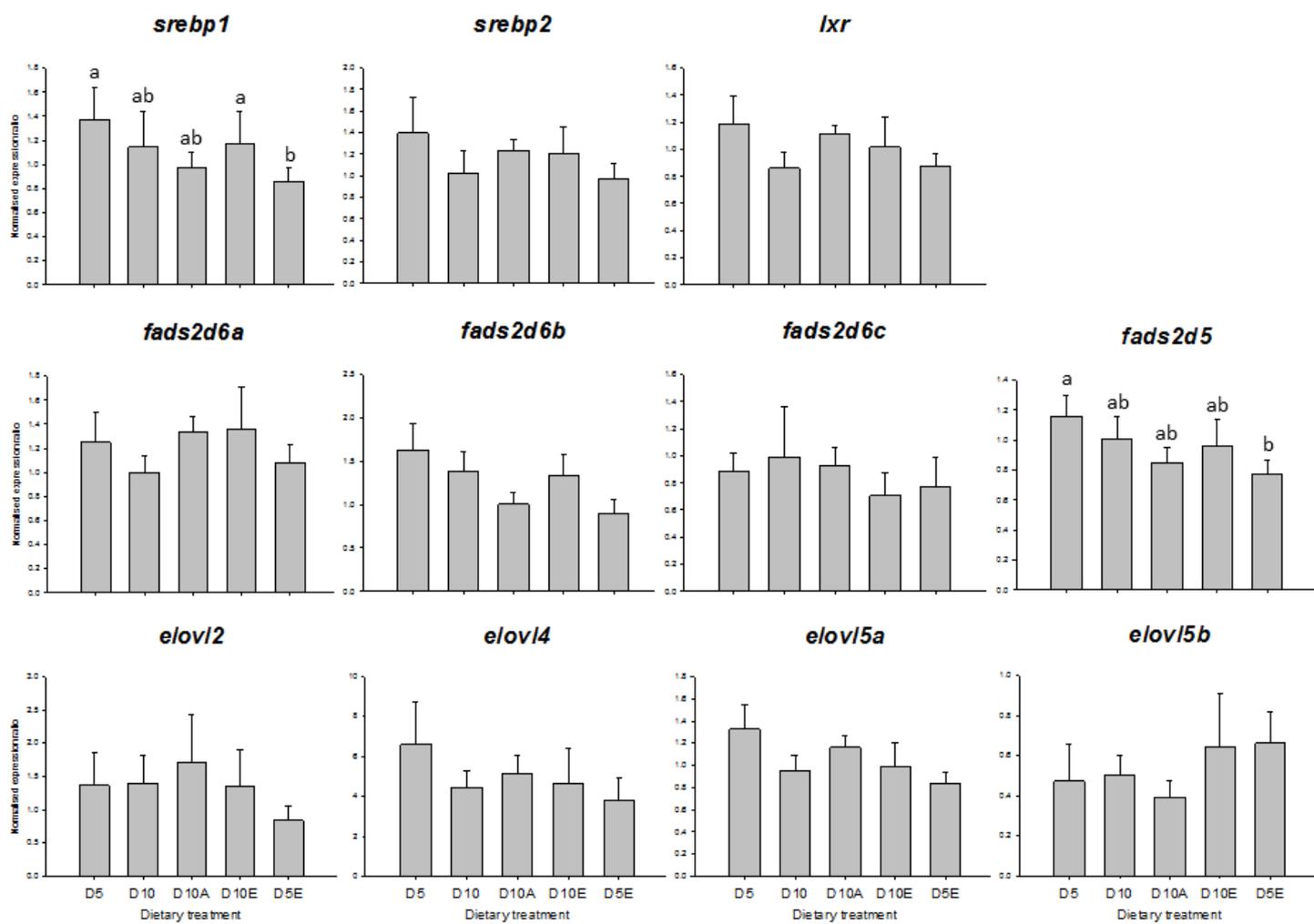


Figure 5.

Table 1. Formulations of experimental diets (all values are g kg⁻¹).

Ingredient	D1	D5	D10	D15	D20	D10A	D10E	D5E
Defatted fish meal ^a	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0
Pregelised starch ^b	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Wheat gluten ^b	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Wheat flour ^b	155.0	155.0	155.0	155.0	155.0	155.0	155.0	155.0
Soy protein isolate ^c	221.0	221.0	221.0	221.0	221.0	221.0	221.0	221.0
Fish oil ^a	0.0	0.0	0.0	0.0	0.0	0.0	75.0	30.0
Olive oil ^d	92.5	88.3	82.0	77.8	71.5	68.3	55.0	77.5
DHASCO ^e	0.0	8.4	21.0	29.4	42.0	21.0	0.0	0.0
ARASCO ^e	0.0	0.0	0.0	0.0	0.0	27.5	0.0	0.0
Butter fat ^f	92.5	88.3	82.0	77.8	71.5	68.3	55.0	77.5
L-Histidine ^g	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
L-Lysine ^g	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DL-Methionine ^g	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
L-Threonine ^g	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Yttrium oxide ^h	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CaPO ₄ ^g	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamins/minerals ⁱ	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

^a Fish meal (prior to being defatted): Chilean anchovy meal and oil, Skretting Australia, Cambridge, TAS, Australia. ^b Wheat gluten, wheat flour and pregelatinised starch: Manildra, Auburn, NSW, Australia. ^c Soy protein isolate: ADM, Decatur, IL, USA. ^d Refined olive oil: Conga Foods, Coburg North, VIC, Australia. ^e DHASCO and ARASCO oils: HuaTai BioPharm Inc, Deyang, Sichuan, China. ^f Butterfat: Woolworths Dairies, Bella Vista, NSW, Australia. ^g Amino acids and monocalcium phosphate: BEC Feed Solutions, Carole Park, QLD, Australia. ^h Yttrium oxide: Stanford Materials, Aliso Viejo, California, United States. ⁱ* Vitamin and mineral premix includes (IU kg⁻¹ or g kg⁻¹ of premix): Vitamin A, 2.5MIU; Vitamin D3, 0.25 MIU; Vitamin E, 16.7 g; Vitamin K₃, 1.7 g; Vitamin B1, 2.5 g; Vitamin B2, 4.2 g; Vitamin B3, 25 g; Vitamin B5, 8.3; Vitamin B6, 2.0 g; Vitamin B9, 0.8; Vitamin B12, 0.005 g; Biotin, 0.17 g; Vitamin C, 75 g; Choline, 166.7 g; Inositol, 58.3 g; Ethoxyquin, 20.8 g; Copper, 2.5 g; Ferrous iron, 10.0 g; Magnesium, 16.6 g; Manganese, 15.0 g; Zinc, 25.0 g.

Table 2. Nutrient composition of experimental diets (adapted from Glencross et al. 2014).

Ingredient	D1	D5	D10	D15	D20	D10A	D10E	D5E
Dry matter (g/kg)	958	967	952	961	943	921	946	944
Protein (g/kg DM)	525	526	511	513	521	519	517	518
Fat (g/kg DM)	181	176	204	205	204	178	186	182
Carbohydrate (g/kg DM)	186	239	230	253	206	214	194	213
Ash (g/kg DM)	82	72	68	69	71	86	82	74
Gross energy (kJ/g)	22.3	22.4	23.1	22.7	22.1	22.7	22.5	23.0
Protein:Energy (g/MJ)	23.5	23.5	22.1	22.6	23.6	22.9	23.0	22.5
<i>All fatty acid data are %TFA</i>								
14:0	6.2	6.0	6.7	6.8	7.4	5.8	7.3	6.8
16:0	21.5	20.6	21.5	22.9	23.7	20.7	21.9	22.5
18:0	8.4	7.7	7.3	7.7	7.2	7.4	6.9	7.8
∑ saturated	36.7	34.4	35.9	37.3	38.7	34.9	36.6	37.4
16:1n-7	1.4	1.3	1.8	1.9	2.1	1.4	5.1	3.2
18:1n-9	49.7	48.2	44.3	42.5	39.2	38.8	35.5	43.5
18:1n-7	4.0	3.9	3.7	3.7	3.7	3.1	3.8	4.0
∑ monounsaturated	56.0	54.1	51.1	49.1	46.0	44.1	45.8	51.7
18:2n-6	5.8	6.7	5.9	5.3	4.9	6.5	5.6	6.1
20:4n-6	0.1	0.1	0.1	0.1	0.1	5.1	0.4	0.1
22:5n-6	0.0	0.6	1.3	1.5	1.6	1.3	0.0	0.0
∑ n-6	5.9	7.7	7.7	6.9	6.8	13.8	6.3	6.2
18:3n-3	0.5	0.8	0.6	0.5	0.5	0.6	0.7	0.6
20:5n-3	0.4	0.6	0.5	0.4	0.4	0.6	4.8	2.0
22:5n-3	0.0	0.1	0.2	0.0	0.0	1.9	0.6	0.0
22:6n-3	0.5	2.0	3.6	5.7	7.6	4.1	3.9	1.7
∑ n-3	1.4	3.8	5.3	6.6	8.5	7.1	11.3	4.7
∑ LC-PUFA	1.0	3.4	5.8	7.8	9.8	13.5	10.0	3.8
n-3/n-6	0.24	0.49	0.69	0.96	1.25	0.52	1.78	0.76

%TFA = percentage of total fatty acids. LC-PUFA = long chain polyunsaturated fatty acids.

Table 3. Details of PCR primers used in the present study for real-time quantitative PCR (qPCR), The data include sequences and annealing temperatures (Ta) for primer pairs, amplicon sizes and accession numbers.

Transcript	Primer sequence (5'-3')	Amplicon (bp)	Ta (°C)	Accession No.
<i>Fads2d6a</i>	F: CCCAGACGTTTGTGTCAG R: CCTGGATTGTTGCTTTGGAT	180	56	AY458652 ^a
<i>Fads2d6b</i>	F: ATAGAGGGTTTATATAGTAGGGCC R: GGTGGGACGCTAGAAAGTTAA	204	58	NM_001172281.1 ^a
<i>Fads2d6c</i>	F: CCCACCCCATCTTAAACT R: CTGGGGTCCAAACAAGGTTA	171	60	NM_001171780.1 ^a
<i>Fads2d5</i>	F: GTGAATGGGGATCCATAGCA R: AAACGAACGGACAACCAGA	192	56	AF478472 ^a
<i>Elovl2</i>	F: CGGGTACAAAATGTGCTGGT R: TCTGTTTCCGATAGCCATT	145	60	TC91192 ^b
<i>Elovl5a</i>	F: ACAAGACAGGAATCTCTTTCAGATTAA R: TCTGGGGTTACTGTGCTATAGTGATAC	137	60	AY170327 ^a
<i>Elovl5b</i>	F: ACAAAAAGCCATGTTTATCTGAAAGA R: AAGTGGGTCTCTCTGGGGCTGTG	141	60	DW546112 ^a
<i>Elovl4</i>	F: TTGTCAAATTGGTCCTGTGC R: TTTAAAAGCCCTTTGGGATGA	191	61	HM208347 ^a
<i>Srebp1</i>	F: GCCATGCGCAGGTTGTTTCTTCA R: TCTGGCCAGGACGCATCTCACACT	151	63	TC148424 ^b
<i>Srebp2</i>	F: GACAGGCACAACACAAGGTG R: CAGCAGGGGTAAGGGTAGGT	215	60	DY733476 ^a
<i>Lxr</i>	F: GCCGCCGCTATCTGAAATCTG R: CAATCCGGCAACCAATCTGTAGG	210	58	FJ470290 ^a
<i>Cofilin-2</i>	F: AGCCTATGACCAACCCACTG R: TGTTACAGCTCGTTTACCG	224	60	TC63899 ^b
<i>elf-1a</i>	F: CTGCCCTCCAGGACGTTTACAA R: CACCGGGCATAGCCGATTCC	175	60	AF321836 ^a

^aGenBank (<http://www.ncbi.nlm.nih.gov/>)

^bAtlantic salmon Gene Index (<http://compbio.dfci.harvard.edu/tgi/>)

Table 4. Fatty acid compositions (percentage of total fatty acids) of liver and head kidney polar lipids of Atlantic salmon post-smolts fed diets containing increasing levels of DHA.

Fatty acid	D1	D5	D10	D15	D20	R ²	P-value
Liver							
Lipids % (wet wt.)	4.9 ± 1.2	4.9 ± 1.2	5.1 ± 0.9	5.1 ± 0.4	5.0 ± 0.5	0.001	0.761
∑ saturated	26.8 ± 0.8	28.7 ± 0.9	28.2 ± 0.8	28.1 ± 0.4	26.7 ± 0.2	0.019	0.625
∑ MUFA	28.7 ± 0.9	24.5 ± 1.9	23.2 ± 1.5	22.7 ± 1.3	20.5 ± 0.5	0.780	0.000
18:2n-6	3.8 ± 0.2	3.6 ± 0.2	3.2 ± 0.6	3.0 ± 0.2	2.7 ± 0.1	0.728	0.000
20:2n-6 ¹	1.4 ± 0.1	1.1 ± 0.2	1.0 ± 0.0	0.9 ± 0.2	0.8 ± 0.0	0.708	0.000
20:3n-6	4.5 ± 0.6	3.1 ± 0.9	2.3 ± 0.6	1.5 ± 0.4	1.1 ± 0.1	0.869	0.000
20:4n-6	5.2 ± 0.7	4.1 ± 0.4	4.0 ± 0.3	4.2 ± 0.6	4.8 ± 0.4	0.037	0.495
22:4n-6	0.4 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.179	0.116
22:5n-6	1.1 ± 0.2	3.7 ± 0.6	5.1 ± 1.8	6.7 ± 1.1	7.9 ± 0.4	0.848	0.000
∑ n-6 PUFA ²	16.6 ± 0.3	16.0 ± 1.9	15.9 ± 1.9	16.6 ± 1.7	17.5 ± 0.5	0.057	0.393
18:3n-3	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.061	0.374
20:5n-3	3.1 ± 0.1	3.0 ± 1.5	3.1 ± 1.4	2.2 ± 0.9	1.7 ± 0.2	0.304	0.033
22:5n-3	1.5 ± 0.2	1.0 ± 0.4	0.9 ± 0.4	0.7 ± 0.3	0.5 ± 0.0	0.601	0.001
22:6n-3	22.3 ± 0.7	26.2 ± 2.7	28.0 ± 2.6	29.4 ± 1.3	32.7 ± 0.5	0.816	0.000
∑ n-3 PUFA ³	27.3 ± 0.6	30.4 ± 4.7	32.4 ± 4.1	32.6 ± 2.5	35.2 ± 0.7	0.502	0.003
DMA	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
∑ n-3 LC-PUFA ⁴	27.0 ± 0.6	30.3 ± 4.6	32.2 ± 4.0	32.4 ± 2.5	35.0 ± 0.6	0.514	0.003
EPA/ARA	0.6 ± 0.1	0.7 ± 0.4	0.8 ± 0.4	0.5 ± 0.2	0.4 ± 0.1	0.164	0.135
Head kidney							
Lipids % (wet wt.)	5.3 ± 2.8	4.3 ± 1.2	3.7 ± 0.9	3.8 ± 0.7	3.4 ± 1.1	0.182	0.105
∑ saturated	30.4 ± 0.2	29.8 ± 0.6	30.5 ± 0.3	30.8 ± 0.7	30.6 ± 0.6	0.123	0.199
∑ MUFA	26.5 ± 0.5	24.8 ± 0.6	24.7 ± 0.8	22.4 ± 0.4	21.0 ± 1.1	0.868	0.000
18:2n-6	3.6 ± 0.2	3.2 ± 0.2	2.9 ± 0.1	2.5 ± 0.1	2.4 ± 0.0	0.883	0.000
20:2n-6 ¹	1.0 ± 0.0	0.8 ± 0.1	0.7 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.935	0.000
20:3n-6	2.7 ± 0.2	2.0 ± 0.3	1.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.0	0.940	0.000
20:4n-6	5.3 ± 0.1	4.2 ± 0.4	4.4 ± 0.1	5.0 ± 0.3	5.1 ± 0.7	0.009	0.741
22:4n-6	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.576	0.001
22:5n-6	0.9 ± 0.1	3.3 ± 0.3	5.4 ± 0.1	6.1 ± 0.1	7.2 ± 0.1	0.885	0.000
∑ n-6 PUFA ²	13.8 ± 0.5	13.6 ± 0.5	14.7 ± 0.0	15.1 ± 0.3	16.0 ± 0.6	0.774	0.000
18:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.049	0.427
20:5n-3	3.6 ± 0.2	3.5 ± 0.5	2.2 ± 0.3	2.4 ± 0.3	2.1 ± 0.4	0.641	0.000
22:5n-3	0.9 ± 0.0	0.7 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.879	0.000
22:6n-3	21.5 ± 0.5	24.4 ± 0.6	24.5 ± 0.5	25.8 ± 0.8	27.1 ± 1.0	0.830	0.000
∑ n-3 PUFA ³	26.4 ± 0.7	29.1 ± 1.0	27.4 ± 0.7	29.0 ± 1.1	29.9 ± 1.3	0.429	0.008
DMA	2.7 ± 0.1	2.5 ± 0.4	2.5 ± 0.1	2.6 ± 0.4	2.5 ± 0.3	0.024	0.578
∑ n-3 LC-PUFA ⁴	26.1 ± 0.7	28.9 ± 1.0	27.2 ± 0.6	28.8 ± 1.1	29.7 ± 1.3	0.444	0.007
EPA/ARA	0.7 ± 0.0	0.8 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.633	0.000

Data expressed as means ± S.D. (*n* = 3). Diets D1-D20 represent feeds with increasing levels of DHA as described in the Materials and Methods section. Statistical differences were determined by regression analysis (*P* < 0.05).

DHA, docosahexaenoic acid; DMA, dimethyl acetal; EPA, eicosapentaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids. ¹ Includes trace amounts of 20:3n-9; ² Totals include 18:3n-6; ³ Totals include 18:4n-3, 20:3n-3 and 20:4n-3; ⁴ Totals include 20:3n-3 and 20:4n-3.

Table 5. Fatty acid compositions (percentage of total fatty acids) of liver and head kidney polar lipids of Atlantic salmon post-smolts fed diets containing various combinations of DHA, ARA and EPA.

Fatty acid	10 g kg ⁻¹ diets		20 g kg ⁻¹ diets		
	D10	D5E	D20	D10A	D10E
Liver					
Lipids % (wet wt.)	5.1 ± 0.9	4.9 ± 0.8	5.0 ± 0.5	4.4 ± 1.0	4.1 ± 0.0
∑ saturated	28.2 ± 0.8	26.7 ± 1.6	26.7 ± 0.2	27.9 ± 1.4	28.0 ± 1.1
∑MUFA	23.2 ± 1.5	25.7 ± 0.7	20.5 ± 0.5	20.1 ± 0.9	21.3 ± 1.4
18:2n-6	3.2 ± 0.6	3.2 ± 0.1	2.7 ± 0.1	1.9 ± 0.6	2.4 ± 0.1
20:2n-6 ¹	1.0 ± 0.0	1.1 ± 0.1	0.8 ± 0.0 ^a	0.6 ± 0.1 ^b	0.7 ± 0.1 ^{ab}
20:3n-6	2.3 ± 0.6	2.8 ± 0.7	1.1 ± 0.1	1.4 ± 0.1	1.3 ± 0.3
20:4n-6	4.0 ± 0.3	3.3 ± 0.6	4.8 ± 0.4 ^b	13.8 ± 0.6 ^a	2.9 ± 0.1 ^c
22:4n-6	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0 ^b	1.5 ± 0.2 ^a	0.1 ± 0.0 ^c
22:5n-6	5.1 ± 1.8	0.6 ± 0.0*	7.9 ± 0.4 ^a	6.9 ± 0.7 ^a	0.5 ± 0.1 ^b
∑ n-6 PUFA ²	15.9 ± 1.9	11.2±0.3*	17.5 ± 0.5 ^b	26.1 ± 1.0 ^a	8.0 ± 0.3 ^c
18:3n-3	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
20:5n-3	3.1 ± 1.4	4.7 ± 0.6	1.7 ± 0.2 ^b	1.1 ± 0.2 ^b	6.6 ± 0.8 ^a
22:5n-3	0.9 ± 0.4	1.5 ± 0.1	0.5 ± 0.0 ^b	0.7 ± 0.1 ^b	2.1 ± 0.2 ^a
22:6n-3	28.0 ± 2.6	29.6 ± 2.0	32.7 ± 0.5 ^a	23.9 ± 0.9 ^b	33.4 ± 0.4 ^a
∑ n-3 PUFA ³	32.4 ± 4.1	36.2 ± 2.6	35.2 ± 0.7 ^b	25.8 ± 0.9 ^c	42.6 ± 1.0 ^a
∑ DMA	n.d.	n.d.	n.d.	n.d.	n.d.
∑ n-3 LC-PUFA ⁴	32.2 ± 4.0	36.0 ± 2.5	35.0 ± 0.6 ^b	25.7 ± 0.9 ^c	42.3 ± 1.0 ^a
EPA/ARA	0.8 ± 0.4	1.5 ± 0.1	0.4 ± 0.1 ^b	0.1 ± 0.0 ^c	2.3 ± 0.2 ^a
Head kidney					
Lipids % (wet wt.)	3.7 ± 0.9	4.2 ± 0.7	3.4 ± 1.1	3.7 ± 0.1	5.1 ± 0.2
∑ saturated	30.5 ± 0.3	31.5±0.2*	30.6 ± 0.6	31.5 ± 1.3	32.3 ± 0.5
∑MUFA	24.7 ± 0.8	25.6 ± 0.5	21.0 ± 1.1	22.1 ± 0.9	22.4 ± 1.3
18:2n-6	2.9 ± 0.1	2.8 ± 0.2	2.4 ± 0.0 ^a	2.3 ± 0.0 ^b	2.2 ± 0.0 ^c
20:2n-6 ¹	0.7 ± 0.0	0.6 ± 0.0*	0.5 ± 0.0 ^a	0.3 ± 0.0 ^b	0.4 ± 0.0 ^b
20:3n-6	1.2 ± 0.1	1.6 ± 0.1*	0.7 ± 0.0	0.9 ± 0.1	0.7 ± 0.1
20:4n-6	4.4 ± 0.1	3.4 ± 0.3*	5.1 ± 0.7 ^b	14.6 ± 0.5 ^a	3.7 ± 0.3 ^c
22:4n-6	0.1 ± 0.0	0.1 ± 0.0*	0.1 ± 0.0 ^b	0.8 ± 0.1 ^a	0.1 ± 0.0 ^b
22:5n-6	5.4 ± 0.1	0.4 ± 0.0*	7.2 ± 0.1 ^a	4.0 ± 0.3 ^b	0.4 ± 0.0 ^c
∑ n-6 PUFA ²	14.7 ± 0.0	8.9 ± 0.1*	16.0 ± 0.6 ^b	22.9 ± 1.1 ^a	7.5 ± 0.1 ^c
18:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:5n-3	2.2 ± 0.3	5.3 ± 0.5*	2.1 ± 0.4 ^b	1.1 ± 0.2 ^b	6.9 ± 1.8 ^a
22:5n-3	0.5 ± 0.0	1.3 ± 0.2*	0.4 ± 0.0 ^b	0.5 ± 0.0 ^b	1.5 ± 0.1 ^a
22:6n-3	24.5 ± 0.5	24.0 ± 0.7	27.1 ± 1.0 ^a	19.1 ± 1.0 ^b	26.3 ± 0.6 ^a
∑ n-3 PUFA ³	27.4 ± 0.7	31.1±0.4*	29.9 ± 1.3 ^b	20.9 ± 1.2 ^c	35.1 ± 2.1 ^a
∑ DMA	2.5 ± 0.1	2.7 ± 0.1	2.5 ± 0.3	2.7 ± 0.1	2.6 ± 0.4
∑ n-3 LC-PUFA ⁴	27.2 ± 0.6	30.9±0.5*	29.7 ± 1.3 ^b	20.7 ± 1.2 ^c	35.0 ± 2.1 ^a
EPA/ARA	0.5 ± 0.1	1.6 ± 0.1*	0.4 ± 0.0 ^b	0.1 ± 0.0 ^c	1.8 ± 0.3 ^a

Data expressed as means ± S.D. (*n* = 3). Diets represent feeds containing 10 g kg⁻¹ DHA (D10) or DHA+EPA (D5E), and feeds containing 20 g kg⁻¹ DHA (D20), DHA+ARA (D10A) or DHA+EPA (D10E). Asterisks denote statistical differences between the 10 g kg⁻¹ diets as determined by one-way ANOVA (*P*<0.05). Different superscript letters within a row represent significant differences between the 20 g kg⁻¹ diets as determined by one-way ANOVA with Tukey's comparison test (*P*<0.05). DHA, docosahexaenoic acid; DMA, dimethyl acetal; EPA, eicosapentaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids. ¹ Includes trace amounts of 20:3n-9; ² Totals include 18:3n-6; ³ Totals include 18:4n-3, 20:3n-3 and 20:4n-3; ⁴ Totals include 20:3n-3 and 20:4n-3.

Table 6. Fatty acid compositions (percentage of total fatty acids) of head kidney polar lipids of Atlantic salmon post-smolts fed diets containing increasing levels of DHA.

Fatty acid	D1	D5	D10	D15	D20	R ²	P-value
Brain							
Lipids % (wet wt.)	7.5 ± 0.4	7.6 ± 0.7	7.4 ± 0.7	7.4 ± 0.3	7.0 ± 0.2	0.15	0.149
∑ saturated	23.6 ± 0.6	24.5 ± 0.6	24.1 ± 0.6	24.9 ± 0.4	25.0 ± 0.4	0.457	0.006
∑ MUFA	40.8 ± 0.8	39.1 ± 0.9	40.1 ± 1.5	38.8 ± 0.7	38.5 ± 1.1	0.329	0.025
18:2n-6	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.737	0.000
20:2n-6 ¹	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
20:3n-6	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
20:4n-6	1.3 ± 0.1	1.3 ± 0.0	1.3 ± 0.1	1.4 ± 0.0	1.4 ± 0.0	0.494	0.003
22:4n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.054	0.405
22:5n-6	0.1 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.836	0.000
∑ n-6 PUFA ²	2.3 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	0.782	0.000
18:3n-3	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
20:5n-3	4.4 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.0 ± 0.0	4.0 ± 0.0	0.839	0.000
22:5n-3	1.8 ± 0.2	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.0	1.6 ± 0.0	0.412	0.010
22:6n-3	19.0 ± 0.6	20.3 ± 0.6	19.7 ± 1.0	20.3 ± 0.7	20.7 ± 0.7	0.304	0.033
∑ n-3 PUFA ³	25.3 ± 0.5	26.4 ± 0.6	25.6 ± 1.0	26.1 ± 0.6	26.4 ± 0.8	0.127	0.192
DMA	7.8 ± 0.3	7.4 ± 0.3	7.4 ± 0.2	7.3 ± 0.3	7.2 ± 0.2	0.411	0.010
∑ n-3 LC-PUFA ⁴	25.3 ± 0.5	26.4 ± 0.6	25.6 ± 1.0	26.1 ± 0.6	26.4 ± 0.8	0.127	0.192
EPA/ARA	3.4 ± 0.2	3.3 ± 0.1	3.1 ± 0.2	2.9 ± 0.1	2.8 ± 0.0	0.736	0.000
Gill							
Lipids % (wet wt.)	1.6 ± 0.4	2.2 ± 0.5	1.5 ± 0.1	1.6 ± 0.3	1.8 ± 0.5	0.001	0.787
∑ saturated	31.6 ± 0.3	31.6 ± 0.2	32.6 ± 2.1	33.4 ± 0.6	32.4 ± 0.2	0.195	0.100
∑ MUFA	29.0 ± 0.6	28.2 ± 1.3	28.7 ± 1.4	27.2 ± 1.3	25.7 ± 0.4	0.535	0.002
18:2n-6	3.0 ± 0.1	2.5 ± 0.3	2.3 ± 0.1	2.1 ± 0.2	2.0 ± 0.1	0.818	0.000
20:2n-6 ¹	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.789	0.000
20:3n-6	2.2 ± 0.1	1.5 ± 0.2	1.0 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.934	0.000
20:4n-6	5.8 ± 0.6	4.6 ± 0.1	4.9 ± 0.3	5.0 ± 0.2	5.2 ± 0.2	0.034	0.509
22:4n-6	0.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.043	0.458
22:5n-6	0.9 ± 0.1	3.1 ± 0.3	4.7 ± 0.5	5.3 ± 0.2	6.2 ± 0.2	0.863	0.000
∑ n-6 PUFA ²	13.1 ± 0.8	12.7 ± 0.7	13.8 ± 0.8	13.9 ± 0.5	14.7 ± 0.2	0.530	0.002
18:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.001	0.907
20:5n-3	2.3 ± 0.3	2.0 ± 0.2	1.6 ± 0.3	1.6 ± 0.2	1.7 ± 0.1	0.521	0.002
22:5n-3	0.7 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.797	0.000
22:6n-3	18.8 ± 0.3	20.6 ± 1.3	19.0 ± 2.2	19.9 ± 0.8	21.8 ± 0.5	0.251	0.057
∑ n-3 PUFA ³	22.1 ± 0.5	23.4 ± 1.3	21.2 ± 2.6	22.0 ± 0.6	23.9 ± 0.4	0.047	0.440
DMA	4.0 ± 0.2	4.1 ± 0.3	3.6 ± 0.1	3.4 ± 0.2	3.2 ± 0.1	0.711	0.000
∑ n-3 LC-PUFA ⁴	21.9 ± 0.5	23.2 ± 1.3	21.0 ± 2.5	21.9 ± 0.6	23.8 ± 0.4	0.054	0.403
EPA/ARA	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.438	0.007

Data expressed as means ± S.D. (*n* = 3). Diets D1-D20 represent feeds with increasing levels of DHA as described in the Materials and Methods section. Statistical differences were determined by regression analysis (*P* < 0.05). DHA, docosahexaenoic acid; DMA, dimethyl acetal; EPA, eicosapentaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids.¹ Includes trace amounts of 20:3n-9; ² Totals include 18:3n-6; ³ Totals include 18:4n-3, 20:3n-3 and 20:4n-3; ⁴ Totals include 20:3n-3 and 20:4n-3.

Table 7. Fatty acid compositions (percentage of total fatty acids) of brain and gill polar lipids of Atlantic salmon post-smolts fed diets containing various combinations of DHA, ARA and EPA.

Fatty acid	10 g kg ⁻¹ diets		20 g kg ⁻¹ diets		
	D10	D5E	D20	D10A	D10E
Brain					
Lipids % (wet wt.)	7.4 ± 0.7 ^b	8.3 ± 0.6 ^a	7.0 ± 0.2	7.8 ± 0.2	8.5 ± 1.5
∑ saturated	24.1 ± 0.6	23.9 ± 0.8	25.0 ± 0.4 ^a	23.9 ± 0.1 ^b	24.9 ± 0.2 ^a
∑MUFA	40.1 ± 1.5	40.5 ± 1.4	38.5 ± 1.1	40.2 ± 0.5	39 ± 0.7
18:2n-6	0.2 ± 0.0	0.5 ± 0.4	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:2n-6 ¹	n.d.	n.d.	n.d.	n.d.	n.d.
20:3n-6	n.d.	n.d.	n.d.	n.d.	n.d.
20:4n-6	1.3 ± 0.1	1.0 ± 0.1*	1.4 ± 0.0 ^b	2.6 ± 0.2 ^a	1.0 ± 0.0 ^c
22:4n-6	0.1 ± 0.0	0.1 ± 0.0*	0.1 ± 0.0 ^b	0.2 ± 0.0 ^a	0.1 ± 0.0 ^c
22:5n-6	0.7 ± 0.0	0.1 ± 0.0*	0.8 ± 0.0 ^a	0.6 ± 0.0 ^a	0.0 ± 0.0 ^b
∑ n-6 PUFA ²	2.6 ± 0.1	2.0 ± 0.3*	2.8 ± 0.1 ^b	3.8 ± 0.2 ^a	1.5 ± 0.1 ^c
18:3n-3	n.d.	n.d.	n.d.	n.d.	n.d.
20:5n-3	4.1 ± 0.1	4.6 ± 0.2*	4.0 ± 0.0 ^b	3.8 ± 0.0 ^c	4.8 ± 0.1 ^a
22:5n-3	1.6 ± 0.1	1.9 ± 0.0*	1.6 ± 0.0 ^b	1.5 ± 0.0 ^b	2.0 ± 0.1 ^a
22:6n-3	19.7 ± 1.0	18.9 ± 1.2	20.7 ± 0.7 ^a	18.7 ± 0.2 ^b	20.0 ± 0.3 ^a
∑ n-3 PUFA ³	25.6 ± 1.0	25.7 ± 1.3	26.4 ± 0.8 ^a	24.2 ± 0.1 ^b	27.0 ± 0.6 ^a
∑ DMA	7.4 ± 0.2	7.9 ± 0.8	7.2 ± 0.2 ^b	7.8 ± 0.1 ^a	7.5 ± 0.2 ^{ab}
∑ n-3 LC-PUFA ⁴	25.6 ± 1.0	25.7 ± 1.3	26.4 ± 0.8 ^a	24.2 ± 0.1 ^b	27.0 ± 0.6 ^a
EPA/ARA	3.1 ± 0.2	4.6 ± 0.2*	2.8 ± 0.0 ^b	1.5 ± 0.1 ^c	5.0 ± 0.1 ^a
Gill					
Lipids % (wet wt.)	1.5 ± 0.1	1.5 ± 0.0	1.8 ± 0.5	1.5 ± 0.1	1.6 ± 0.1
∑ saturated	32.6 ± 2.1	31.8 ± 0.9	32.4 ± 0.2	32.8 ± 1.1	34.0 ± 1.7
∑MUFA	28.7 ± 1.4	28.9 ± 0.8	25.7 ± 0.4	25.2 ± 0.3	26.9 ± 1.4
18:2n-6	2.3 ± 0.1	2.5 ± 0.1*	2.0 ± 0.1 ^a	1.7 ± 0.1 ^b	1.9 ± 0.1 ^{ab}
20:2n-6 ¹	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0 ^a	0.4 ± 0.0 ^b	0.4 ± 0.0 ^b
20:3n-6	1.0 ± 0.0	1.4 ± 0.1*	0.6 ± 0.0	0.6 ± 0.1	0.7 ± 0.1
20:4n-6	4.9 ± 0.3	4.2 ± 0.1*	5.2 ± 0.2 ^b	11.6 ± 1.0 ^a	4.1 ± 0.3 ^c
22:4n-6	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0 ^b	1.5 ± 0.2 ^a	0.3 ± 0.0 ^b
22:5n-6	4.7 ± 0.5	0.5 ± 0.0*	6.2 ± 0.2 ^a	3.8 ± 0.1 ^b	0.5 ± 0.0 ^c
∑ n-6 PUFA ²	13.8 ± 0.8	9.5 ± 0.1*	14.7 ± 0.2 ^b	19.6 ± 1.4 ^a	7.8 ± 0.3 ^c
18:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:5n-3	1.6 ± 0.3	4.0 ± 0.6*	1.7 ± 0.1 ^b	1.0 ± 0.0 ^c	4.7 ± 0.2 ^a
22:5n-3	0.4 ± 0.1	1.1 ± 0.2*	0.4 ± 0.0 ^b	0.4 ± 0.0 ^b	1.2 ± 0.1 ^a
22:6n-3	19.0 ± 2.2	20.9 ± 1.1	21.8 ± 0.5 ^a	17.1 ± 0.5 ^b	21.7 ± 2.6 ^a
∑ n-3 PUFA ³	21.2 ± 2.6	26.2 ± 0.4*	23.9 ± 0.4 ^a	18.6 ± 0.5 ^b	27.9 ± 2.7 ^a
∑ DMA	3.6 ± 0.1	3.5 ± 0.3	3.2 ± 0.1 ^b	3.8 ± 0.1 ^a	3.4 ± 0.1 ^b
∑ n-3 LC-PUFA ⁴	21.0 ± 2.5	26.0 ± 0.4*	23.8 ± 0.4 ^a	18.4 ± 0.5 ^b	27.7 ± 2.7 ^a
EPA/ARA	0.3 ± 0.1	0.9 ± 0.1*	0.3 ± 0.0 ^b	0.1 ± 0.0 ^c	1.1 ± 0.1 ^a

Data expressed as means ± S.D. (*n* = 3). Diets represent feeds containing 10 g kg⁻¹ DHA (D10) or DHA+EPA (D5E), and feeds containing 20 g kg⁻¹ DHA (D20), DHA+ARA (D10A) or DHA+EPA (D10E). Asterisks denote statistical differences between the 10 g kg⁻¹ diets as determined by one-way ANOVA (*P*<0.05). Different superscript letters within a row represent significant differences between the 20 g kg⁻¹ diets as determined by one-way ANOVA with Tukey's comparison test (*P*<0.05). DHA, docosahexaenoic acid; DMA, dimethyl acetal; EPA, eicosapentaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids.¹ Includes trace amounts of 20:3n-9; ² Totals include 18:3n-6; ³ Totals include 18:4n-3, 20:3n-3 and 20:4n-3; ⁴ Totals include 20:3n-3 and 20:4n-3.