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

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Abstract

To investigate interactions of dietary LC-PUFA, a dose-response study with a range of 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}) was performed with post-smolts (111 ± 2.6 g; mean ± S.D.) over a nine-week feeding period. Additional diets included 10 g kg^{-1} DHA in combination with 10 g kg^{-1} of either eicosapentaenoic acid (EPA; 20:5n-3) or arachidonic acid (ARA; 20:4n-6), and a diet containing 5g kg^{-1} each of DHA and EPA. Liver, brain, head kidney and gill were collected at the conclusion of the trial and lipid and fatty acid compositions determined as well as expression of genes of LC-PUFA biosynthesis. Total lipid content and class composition were largely unaffected by changes in dietary LC-PUFA. However, phospholipid (PL) fatty acid compositions generally reflected that of the diet, although the response varied between tissues. Liver most strongly reflected diet, followed by head kidney. In both tissues increasing dietary DHA led to significantly increased DHA in PL and inclusion of EPA or ARA led to higher levels of these fatty acids. Brain showed the most conserved composition and gene expression profile, with increased dietary LC-PUFA resulting in only minor changes in PL fatty acids. Dietary LC-PUFA significantly affected the expression of Δ6 and Δ5 desaturases, Elovl 2, 4 and 5, and SREBPs although this varied between tissues with greatest effects observed in liver followed by head kidney, similar to PL fatty acid compositions.

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

1. Introduction

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

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

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

All vertebrates including fish require three key fatty acids for normal growth and development: docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (ARA; 20:4n-6) (Sargent et al., 1997; Bell, 1998; Sargent et al., 1999). These biologically active fatty acids play important roles in cell membrane structure and function (Sargent et al. 2002), the regulation of reproduction (Bell & Sargent, 2003) and the modulation of immune responses (Waagbø, 1994). Salmonids can synthesise these LC-PUFA *de novo* from their C_{18} precursors α-linolenic acid (LNA; 18:3n-3) and linoleic acid (LOA; 18:2n-6), though their capacity for this is limited (Castell et al., 1972; Bell et al., 1993; Tocher et al., 2000). The enzymes involved in the bioconversion of both n-3 and n-6 PUFA to LC-PUFA are the Δ-6 and Δ-5 fatty acyl desaturases (FADS2D6 and FADS2D5 respectively) and two fatty acyl elongases (ELOVL2 and ELOVL5). The Δ6 desaturation of both C_{18} and C_{24} PUFA is likely required for the biosynthesis of DHA in salmon, although there are three functional FADS2D6 in Atlantic salmon that may indicate differential regulation of these desaturation steps (Monroig et al., 2010). Similarly, two functional ELOVL5 (a and b) have been identified in salmon (Morais et al., 2009). Functional studies suggest that ELOVL5 is mainly involved in the elongation of $C_{18}\rightarrow C_{20}$ PUFA, with residual $C_{20}\rightarrow C_{22}$ activity, whereas ELOVL2 elongates $C_{20}\rightarrow C_{22}$ but not $C_{18}\rightarrow C_{20}$ (Morais et al., 2009). The activity of the LC-PUFA biosynthesis pathway relies on the presence of substrates 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

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

1. Materials and Methods

2.1. Experimental diets

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

1.2. Fish and husbandry

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

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

1.3. Sample collection and management

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

1.4. Total lipid extraction

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

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

1.5. Lipid class composition

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

1.6. Phospholipid fatty acid composition

Phospholipids were isolated using thin-layer chromatography (TLC) by loading 2 mg of total lipid onto 2.5 cm origins on 20 x 20 cm TLC plates (VWR, Lutterworth, UK) and running in a solvent mixture comprising isohexane/diethyl ether/acetic acid (80:20:1, by vol.). Plates were sprayed with 1% (w/v) 2',7'-dichlorofluorescein in 97% (v/v) methanol containing 0.05% (w/v) BHT and visualized under UV light (UVGL-58 Mineralight® Lamp, Ultraviolet Prod. Inc., Calif., USA). Total polar lipids were scraped into test tubes and fatty acid methyl esters (FAME) were prepared by acid-catalyzed transmethylation according to the method of Christie (2003b). FAME were separated and quantified by gas-liquid chromatography using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30 m × 0.32 mm i.d. × 0.25 µm ZB-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 °C to 150 °C at 40 °C min^{-1} to a final temperature of 230 °C at 2 °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 inhibiter 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-1α were quantified. qPCR was performed using a Biometra TOptical
224 Thermocycler (Analytik Jena, Goettingen, Germany) in 96-well plates in duplicate 20 μl reaction

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

1.8. Statistical Analysis

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

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

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

2. Results

3.1. Total lipid content

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

2.2. Lipid class composition

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

2.3. Fatty acid compositions of liver and head kidney phospholipids

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

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

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

2.4. Fatty acid compositions of brain and gill phospholipids

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

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

2.5. Tissue expression profile of lipid metabolism genes

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

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

3. Discussion

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

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

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

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

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

and *fads2d5* expression were higher in fish fed 1 g kg^{-1} of DHA denoting an upregulation of the LC-PUFA biosynthesis pathway as previously described (Moya-Falcón et al., 2005; Thomassen et al., 2012). The elongation of EPA to DPA appears to be very active in Atlantic salmon liver (Thomassen et al., 2012), which is in agreement with the present study, where high expression of both *elovl2* and *elovl5* was observed together with high levels of DPA. However, only statistical differences were found in *elovl2*, suggesting a greater role of this enzyme compared to *elovl5* in elongation of C_{20} PUFA. Similarly, heterologous expression in yeast showed that salmon *elovl5* elongated C_{18} and C_{20} PUFA, with low activity towards C_{22}, whereas *elovl2* elongated C_{20} and C_{22} PUFA with lower activity towards C_{18} (Morais et al., 2009). Interestingly, *elovl4* expression was also upregulated in liver of fish fed 1 g kg^{-1} of DHA. ELOVL4 is involved in the synthesis of very long-chain PUFA (> C24) in mammals, but it was recently shown that Atlantic salmon *elovl4* open reading frame (ORF) was able to elongate both EPA and ARA (15.4 % and 11.5% conversion, respectively) indicating that it was also involved in LC-PUFA biosynthesis (Carmona-Antoñanzas et al., 2011). These data are consistent with the results in the present study, where high concentrations of DPA could be due to combined activity of the different fatty acid elongases. In contrast, the highest inclusion of DHA (D20) was at the upper end of the documented requirement for LC-PUFA in salmonids (Ruyter et al., 2000) and this probably suppressed further endogenous synthesis of DHA in the liver, gill and head kidney (Bell & Sargent, 2003; Zheng et al., 2004).

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

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

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

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

Regulation of lipid metabolism is complex and controlled by several TF including SREBPs and LXR. In mammals, SREBP1 is involved in activation of genes that participate in fatty acid metabolism and *de novo* lipogenesis whereas SREBP2 is more selective for genes involved in cholesterol homeostasis (Horton et al., 2004). Furthermore, n-3 and n-6 fatty acids can induce transcription of *lxr* through DR1 elements (Tobin et al., 2002) and regulate the expression of *srebp1* (Joseph et al., 2002), which is a major regulator of lipogenesis in mammals (Davidson, 2006). In the present study the diet with highest LC-PUFA content (D10A; 13.5 %) was found to down-regulate the expression of *srebp1* in liver, whereas fish fed diet D1, with only 1% LC-PUFA, showed the highest expression. This is in agreement with previous studies in Atlantic salmon both *in vitro* (Minghetti et al., 2011) and *in vivo* (Morais et al., 2011) where *srebp1* expression was reduced by LC-PUFA supplementation, denoting a similar nutritional regulation to mammals (Davidson et al., 2006; Caputo et al., 2010). Similarly, this lower expression profile was reflected in lower expression levels of some SREBP1 target genes such as *fads2d6c* and *fads2d5*. However, this pattern of expression was not observed in all tissues and so, in contrast, *srebp1* expression in brain was highest in D10A-fed fish. Conversely, *lxr* gene expression was not affected by dietary PUFA content. The explanation to this could be that LXR is activated by a variety of sterols, including intermediates in the synthesis of cholesterol, and adequate levels of cholesterol were present in all of the diets, which could also explain why *srebp*2 expression was unaffected.

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

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797 L.) Δ6 fatty acyl desaturase gene promoters. Comp. Biochem. Physiol. B 154, 255-263.

798

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; *fads2d6*b, 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.

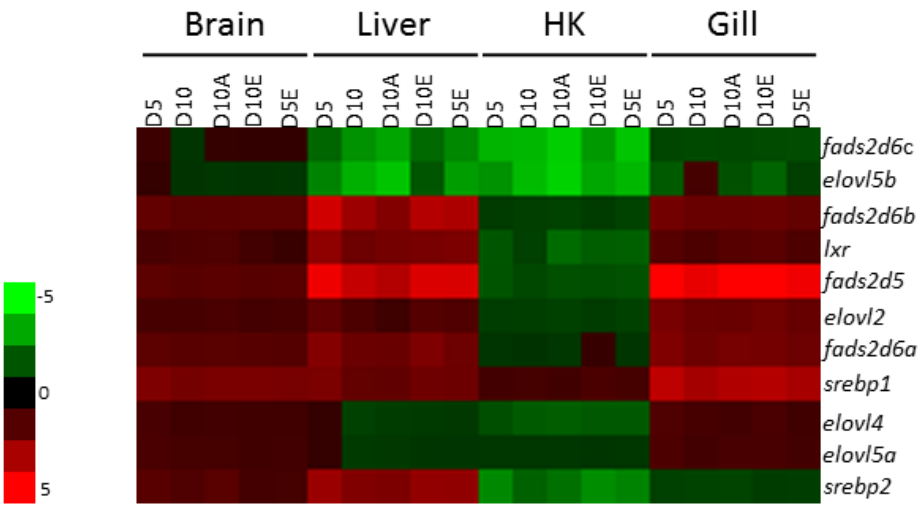
819 **Figure 3. Expression, measured by qPCR, of transcription factors and LC-PUFA**
820 **biosynthesis pathway genes in Atlantic salmon brain after nine weeks of feeding.** Results are

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

Figure 4. Expression, measured by qPCR, of transcription factors and LC-PUFA biosynthesis pathway genes in Atlantic salmon head kidney after nine weeks of feeding. Results are normalized expression ratios (average +SE, n = 6) of the expression of these genes in fish fed the different diets in relation to fish fed D5 diet. Diets contain either 5 g kg^{-1} DHA (D5), 10 g kg^{-1} DHA (D10) and DHA+EPA (D5E) or 20 g kg^{-1} of DHA+ARA (D10A) and DHA+EPA (D10E). *fads2d6a*, delta-6 fatty acyl desaturase isoform a; *fads2d6b*, delta-6 fatty acyl desaturase isoform b; *fads26c*, delta-6 fatty acyl desaturase isoform c; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*, fatty acyl elongase 2; *elovl5a*, fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase isoform b; *elovl4*, fatty acyl elongase 4; *lxr*, liver X receptor; *srebp*, sterol regulatory element binding protein.

Figure 5. Expression, measured by qPCR, of transcription factors and LC-PUFA biosynthesis pathway genes in Atlantic salmon gill after nine weeks of feeding. Results are normalized expression ratios (average +SE, n = 6) of the expression of these genes in fish fed the different diets in relation to fish fed D5 diet. Diets contain either 5 g kg^{-1} DHA (D5), 10 g kg^{-1} DHA (D10) and DHA+EPA (D5E) or 20 g kg^{-1} of DHA+ARA (D10A) and DHA+EPA (D10E). *fads2d6a*, delta-6 fatty acyl desaturase isoform a; *fads2d6b*, delta-6 fatty acyl desaturase isoform b; *fads26c*, delta-6 fatty acyl desaturase isoform c; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*,

fatty acyl elongase 2; *elovl5a*, fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase isoform b; *elovl4*, fatty acyl elongase 4; *lxr*, liver X receptor; *srebp*, sterol regulatory element binding protein.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Liver
HK
Gill
D5
D10
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D10E
D5E
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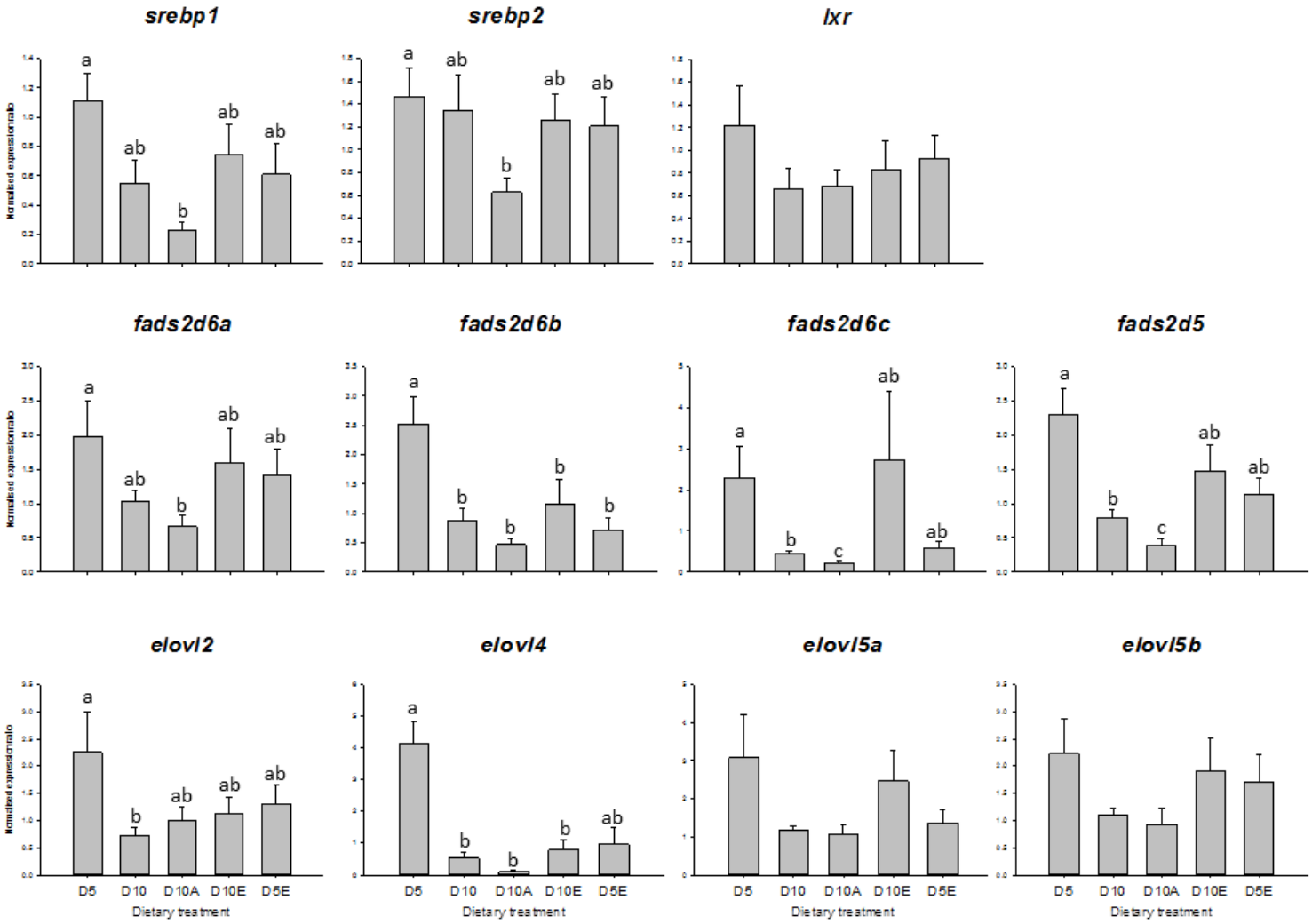
Figure 1.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fads2d6b
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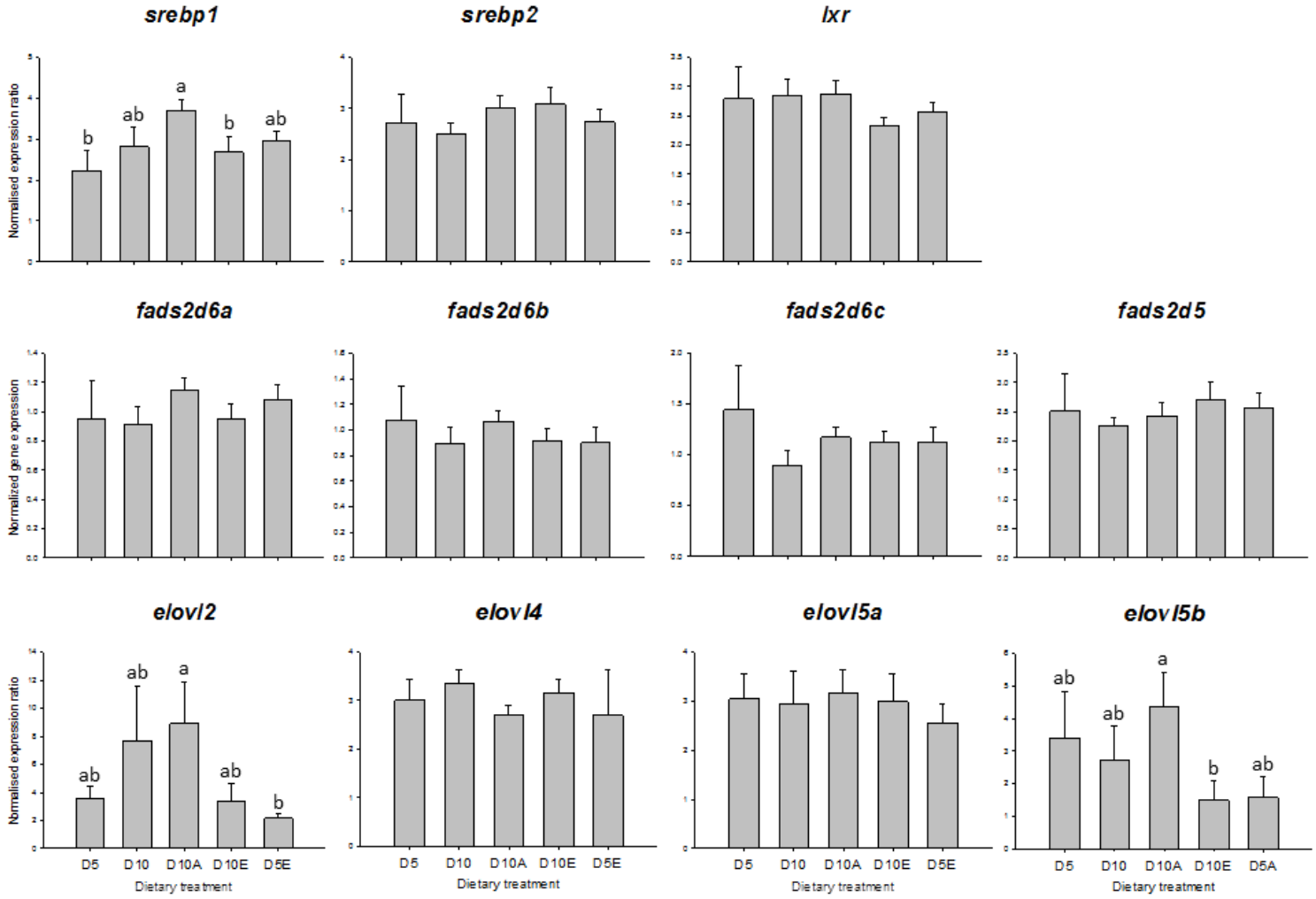
elovl2
elovl4
elovl5a
elovl5b
Normalized expression ratio
D5
D10
D10A
D10E
D5E
Dietary treatment

Figure 2.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fads2d5
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elovl4
elovl5a
elovl5b
Normalised expression ratio
Normalized gene expression
Dietary treatment
D5
D10
D10A
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D5E
D5A
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ab
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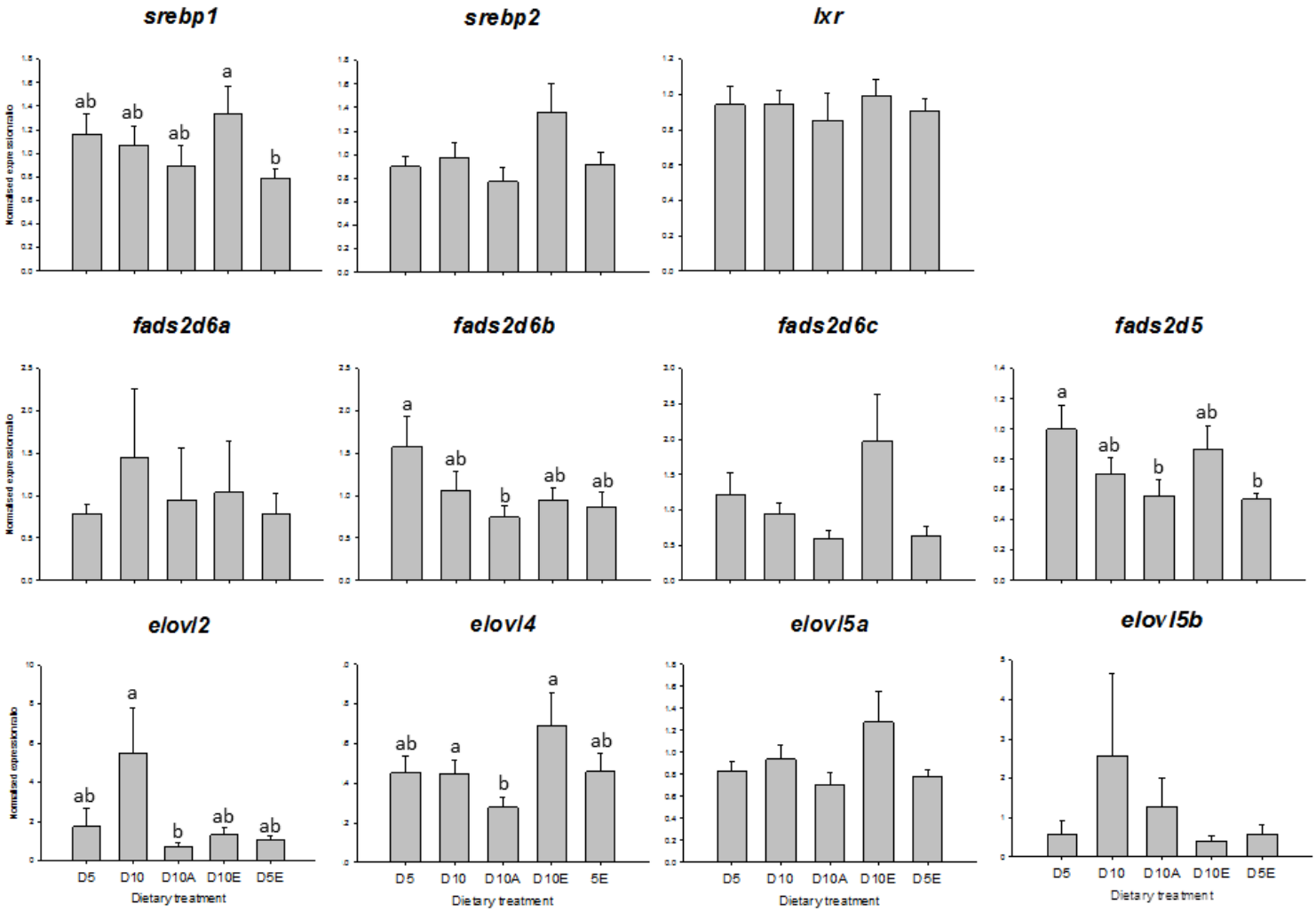
Figure 3.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lxr
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fads2d5
elovl2
elovl4
elovl5a
elovl5b
Normalised expression ratio
D5
D10
D10A
D10E
D5E
5E
Dietary treatment

Figure 4.

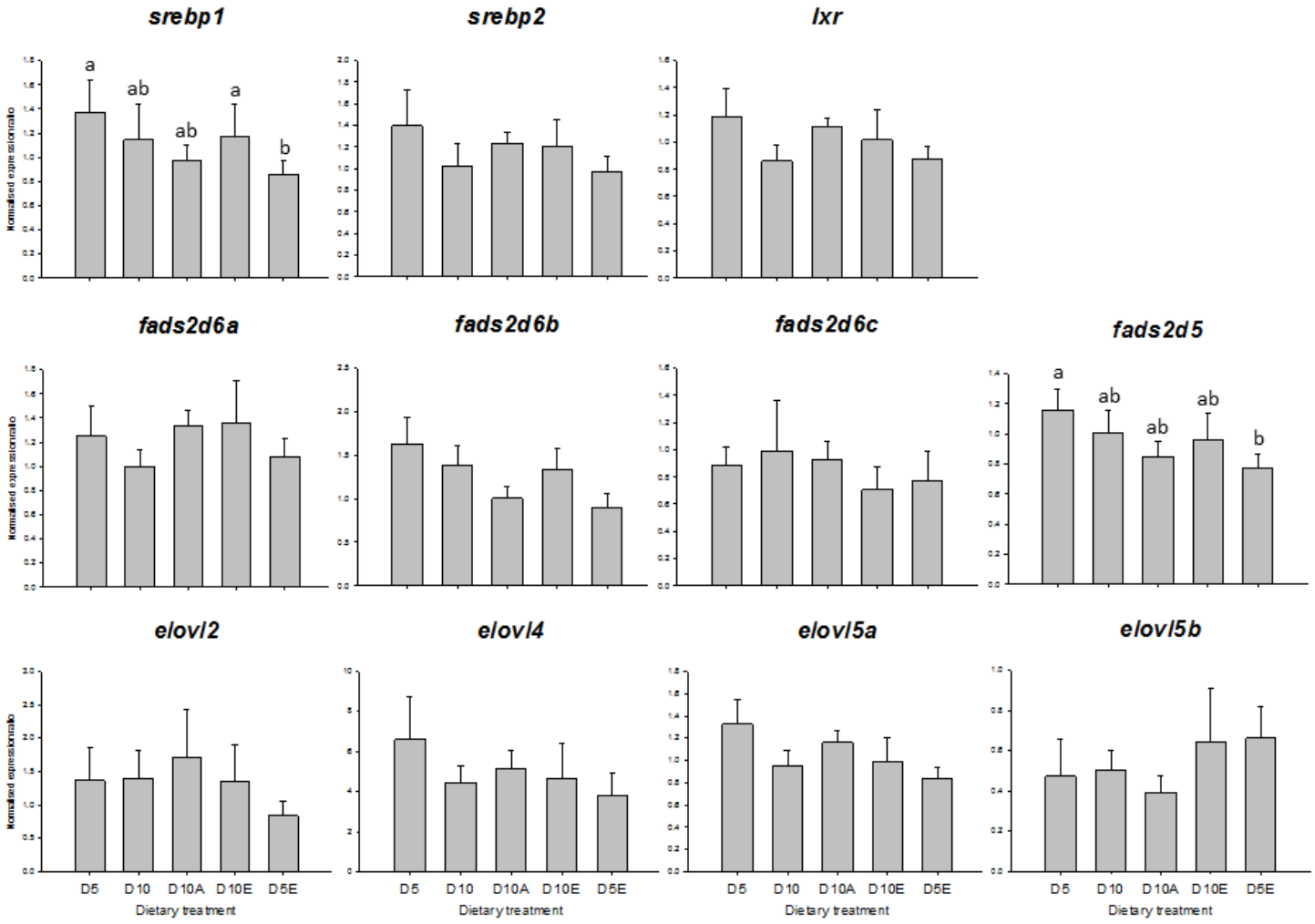
srebp1
srebp2
lxr
fads2d6a
fads2d6b
fads2d6c
fads2d5
elovl2
elovl4
elovl5a
elovl5b
a
ab
ab
a
b
Normalised expressionratio
D5
D10
D10A
D10E
D5E
Dietary treatment

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
Pregelled 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 K,3, 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
All fatty acid data are %TFA								
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.
Fads2d6a	F: CCCCAGACGTTTGTGTCAG R: CCTGGATTGTTGCTTTGGAT	180	56	AY458652[a]
Fads2d6b	F:ATAGAGGGTTTATATAGTAGGGCC R: GGTGGGACGCTAGAAGTTAA	204	58	NM_001172281.1[a]
Fads2d6c	F: CCCACCCCCATCTTAAAACT R: CTGGGGTCCAAACAAGGTTA	171	60	NM_001171780.1[a]
Fads2d5	F: GTGAATGGGGATCCATAGCA R: AAACGAACGGACAACCAGA	192	56	AF478472[a]
Elovl2	F: CGGGTACAAAATGTGCTGGT R: TCTGTTTGCCGATAGCCATT	145	60	TC91192[b]
Elovl5a	F:ACAAGACAGGAATCTCTTTCAGATTAA R:TCTGGGGTTACTGTGCTATAGTGTAC	137	60	AY170327[a]
Elovl5b	F: ACAAAAAGCCATGTTTATCTGAAAGA R: AAGTGGGTCTCTCTGGGGCTGTG	141	60	DW546112[a]
Elovl4	F: TTGTCAAATTGGTCCTGTGC R: TTAAAAGCCCTTTGGGATGA	191	61	HM208347[a]
Srebp1	F: GCCATGCGCAGGTTGTTTCTTCA R: TCTGGCCAGGACGCATCTCACACT	151	63	TC148424[b]
Srebp2	F: GACAGGCACAACACAAGGTG R: CAGCAGGGGTAAGGGTAGGT	215	60	DY733476[a]
Lxr	F: GCCGCCGCTATCTGAAATCTG R: CAATCCGGCAACCAATCTGTAGG	210	58	FJ470290[a]
Cofilin-2	F: AGCCTATGACCAACCCACTG R: TGTTCACAGCTCGTTTACCG	224	60	TC63899[b]
elf-1α	F: CTGCCCCTCCAGGACGTTTACAA R: CACCGGGCATAGCCGATTCC	175	60	AF321836[a]

[a]GenBank (http://www.ncbi.nlm.nih.gov/)

[b]Atlantic 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.

	10 g kg^{-1} diets		20 g kg^{-1} diets		
Fatty acid	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.

	10 g kg^{-1} diets		20 g kg^{-1} diets		
Fatty acid	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 ± 0.1a
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.