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3 **Transcriptomic analyses of intestinal gene expression of juvenile Atlantic cod (*Gadus***
4 ***morhua*) fed diets with Camelina oil as replacement for fish oil**

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19
20 **Abstract**

21 For aquaculture of marine species to continue to expand, dietary fish oil (FO) must be replaced
22 with more sustainable vegetable oil (VO) alternatives. Most VO are rich in n-6 polyunsaturated
23 fatty acids (PUFA) and few are rich in n-3 PUFA but Camelina oil (CO) is unique in that, besides
24 high 18:3n-3 and n-3/n-6 PUFA ratio, it also contains substantial long-chain monoenes,
25 commonly found in FO. Cod (initial weight ~1.4 g) were fed for 12 weeks diets in which FO was
26 replaced with CO. Growth performance, feed efficiency and biometric indices were not affected
27 but lipid levels in liver and intestine tended to increase and those of flesh, decrease, with
28 increasing dietary CO although only significantly for intestine. Reflecting diet, tissue n-3 long-
29 chain PUFA levels decreased whereas 18:3n-3 and 18:2n-6 increased with inclusion of dietary
30 CO. Dietary replacement of FO by CO did not induce major metabolic changes in intestine, but

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31 affected genes with potential to alter cellular proliferation and death as well as change structural
32 properties of intestinal muscle. Although the biological effects of these changes are unclear,
33 given the important role of intestine in nutrient absorption and health, further attention should be
34 given to this organ in future.

35
36 Keywords: Alternative diets; *Camelina sativa*; fish oil replacement; *Gadus morhua*; intestine;
37 microarrays

38 39 **1. Introduction**

40 Sustainable development of aquaculture of carnivorous marine finfish species requires feed
41 formulations to be based more on terrestrial plant products, plant meals and vegetable oils (VO),
42 rather than the traditional marine resources, fishmeal and fish oil (FO) (Naylor et al., 2009;
43 Hardy, 2010). Recent research has demonstrated that marine fish can be grown on feeds
44 containing high levels of replacement (up to 70%) of FO with VO without compromising growth
45 performance (Turchini et al., 2010). However, VO lack long-chain polyunsaturated fatty acids
46 ($\geq C_{20}$; LC-PUFA) and most are rich in linoleic acid (18:2n-6; LOA), and their inclusion in feeds
47 reduces the n-3/n-6 PUFA ratio of the fish flesh potentially compromising its nutritional quality
48 to human consumers (Turchini et al., 2010). In contrast there are few oils rich in n-3 PUFA and
49 they are only produced in relatively small volumes (Gunstone, 2010).

50 A potentially new source of n-3 PUFA is the (re)emerging Brassicaceae species *Camelina*
51 *sativa* (commonly known as gold-of-pleasure, or false flax). *Camelina* used to be a major
52 European oilseed crop prior to the early 20th century, and has recently been re-established as a
53 potential bioenergy crop. This is on account of *Camelina*'s modest input requirements and also its
54 ability to thrive in semi-arid conditions (Tocher et al., 2010) and, in the USA, it is being actively
55 grown as a biofuels crop (Murphy, 2011). In addition to these desirable agronomic traits,
56 *Camelina* also accumulates high levels of α -linolenic acid (18:3n-3; ALA). Thus, *Camelina* oil
57 (CO) can contain up to 45 % ALA with an ALA/LOA ratio generally around 2.5. However, CO
58 also has a high antioxidant content with approximately 800 mg total tocopherol per kg,
59 predominantly the γ -isomer and, as a result, the crude oil exhibits strong oxidative stability
60 despite the high ALA content (Tocher et al., 2010). *Camelina* oil is relatively low in saturated
61 fatty acids and, almost uniquely among VO, it contains high levels of long-chain C₂₀ and C₂₂
62 monoenes (20:1 and 22:1) that are regarded as good energy sources in fish (Tocher, 2003), giving

63 CO a potential nutritional advantage over other ALA-rich oils such as linseed and perilla (Tocher
64 et al., 2010). Furthermore, CO has already been shown to be suitable for inclusion in feeds for
65 Atlantic salmon and appears to be low in anti-nutritional factors that could be detrimental to fish
66 growth (Petropoulos et al., 2009).

67 Until depletion of the commercial stocks in the 1990's, Atlantic cod (*Gadus morhua* L.) was
68 the most valued food fish obtained from the North Atlantic (Kurlansky, 1998). Significant
69 progress has been made in the culture of cod and the life cycle was closed some years ago,
70 allowing production independent of wild fisheries (Brown et al., 2003). The large and highly
71 developed market for cod, high market price and quotas set to preserve wild stocks, have
72 stimulated considerable interest in cod culture over the years (Morais et al., 2001; Hemre et al.,
73 2004; Hansen et al., 2011; Kortner et al., 2011). However, the establishment of large-scale,
74 sustainable cod culture will require solutions to several nutritional issues including broodstock
75 and larval nutrition and replacement of dietary FO (Lall and Nanton, 2002; Salze et al., 2005).
76 Recently the genome of Atlantic cod was released, providing an important resource for further
77 studies and development of cod as an aquaculture species (Star et al., 2011).

78 In addition to traditional measures of the effects of dietary formulations on fish performance
79 (growth and feed efficiency), tissue composition and nutritional quality, recent advances in
80 functional genomics, essentially the study of gene expression, has opened up new possibilities for
81 understanding the basic mechanisms involved in the adaptation to new diets or feeds (Leaver et
82 al., 2008a). Determining patterns of gene expression through study of tissue transcriptomes
83 (mRNA expression) or proteomes (protein expression) can provide large amounts of information
84 on individual molecular responses that, with detailed bioinformatic analyses, can provide great
85 insights into the biochemical and physiological responses underpinning nutritional adaptations
86 (Panserat et al., 2009; Taggart et al., 2008). In recent years, we have specifically determined the
87 effects of replacement of FO with VO on tissue gene expression in Atlantic salmon using both
88 quantitative real-time PCR (candidate genes) and transcriptomic/microarray (global gene
89 expression) approaches (Leaver et al., 2008b; Torstensen and Tocher, 2010; Morais et al.,
90 2011a,b).

91 Our overall objective is to determine the utility of CO as a replacement for dietary FO in feeds
92 for Atlantic cod and, to this aim, cod were fed CO in a nutritional trial with a regression design
93 and the effects on growth performance, feed efficiency, basic biometry and tissue lipid and fatty
94 acid composition determined. However, the primary focus of the present study was to determine

95 the effects of dietary CO on gene expression in cod in order to elucidate metabolic pathways of
96 adaptation and provide an underpinning fundamental science base for similar nutritional studies.
97 Therefore, transcriptome analysis of intestinal tissue was performed using a recently developed
98 Atlantic cod 16k cDNA microarray (Edvardsen et al., 2011).

99

100 **2. Materials and methods**

101 *2.1. Diets and animals*

102 Four diets containing approximately 55 % crude protein and 15 % crude lipid were formulated to
103 satisfy the nutritional requirements of marine fish (National Research Council, 2011). The control
104 diet (C0) was formulated with 100% FO (capelin oil) and three other diets contained an
105 increasing proportion of crude cold-pressed *Camelina sativa* oil (CO) replacing 33 % (C33), 66
106 % (C66) and 100 % (C100) of the added fish oil (Table 1). The dry ingredients were combined
107 and mixed for 5 min using a Hobart commercial mixer (Model 200A, Hobart, Glasgow, UK), and
108 the oils, including antioxidant, then added and mixed for 5 min before adding water (10% w/w)
109 and mixing for a further 5 min. Pellets of 1 mm diameter were formed by extrusion through an
110 appropriate die using a California pellet mill (model CL3, California Pellet Mill Inc., San
111 Francisco, CA). After pelleting the feeds were dried overnight in a heated cabinet at 25°C. The
112 fatty acid compositions of the diets are shown in Table 2.

113 Four hundred and twenty juveniles of Atlantic cod (*Gadus morhua* L.), obtained from Marine
114 Farms Ltd, Machrihanish, UK, of initial mean weight 1.4 ± 0.1 g were randomly distributed
115 among 12 circular fibreglass tanks of 0.4 m³ with 35 fish/tank and supplied with UV treated,
116 filtered flow-through seawater at a rate of 1 L/min at the Marine Environmental Research
117 Laboratory, Machrihanish, Scotland. Triplicate tanks of fish were fed one of the four feeds for 12
118 weeks between February and May when ambient water temperature was 9.0 ± 1.2 °C (range, 7 –
119 11). Fish were fed a fixed ration of tank biomass ranging from 5.6 – 2.6 % as the experiment
120 progressed with fish in each tank bulk weighed every 14 days and ration adjusted accordingly. At
121 the end of the trial, 6 fish per tank (18 fish per dietary treatment) were anaesthetized with
122 metacaine sulphonate (MS222; 50 mg/L) and killed by a blow to the head. Samples of liver and
123 intestine (midgut) for analysis of gene expression were frozen in liquid nitrogen and subsequently
124 stored at – 80 °C prior to RNA extraction. Flesh (muscle), liver and intestine for lipid analyses
125 were also frozen in liquid nitrogen and stored at -20 °C prior to analysis. Fish were not starved
126 prior to sampling and so any gut contents were gently extruded prior to freezing.

127 *2.2. Proximate composition*

128 Diets were ground prior to determination of proximate composition according to standard
129 procedures (AOAC, 2000). Moisture contents were obtained after drying in an oven at 110 °C for
130 24 h and ash content determined after incineration at 600 °C for 16 h. Crude protein content was
131 measured by determining nitrogen content ($N \times 6.25$) using automated Kjeldahl analysis (Tecator
132 Kjeltex Auto 1030 analyzer, Foss, Warrington, U.K), and crude lipid content determined after
133 acid hydrolysis followed by Soxhlet lipid extraction (Tecator Soxtec system 2050 Auto
134 Extraction apparatus, Foss, Warrington, U.K).

135 *2.3. Lipid content and fatty acid analysis*

136 Samples of liver, intestine and skinned and deboned flesh (white muscle) from three fish per tank
137 (9 per treatment) were utilized for lipid analysis, with samples from the three fish per tank
138 prepared as pooled tissue homogenates and so there were three replicate pools of three fish per
139 treatment ($n = 3$). Total lipid was extracted according to the method of Folch et al. (1957).
140 Approximately 1 g samples of pooled tissues were homogenized in 20 ml of ice-cold
141 chloroform/methanol (2:1, by vol) using an Ultra-Turrax tissue disrupter (Fisher Scientific,
142 Loughborough, U.K.). The non-lipid and lipid layers were separated by addition of 5 ml of 0.88
143 % (w/v) KCl and allowed to separate on ice for 1 h. The upper non-lipid layer was aspirated and
144 the lower lipid layer dried under oxygen-free nitrogen. The lipid content was determined
145 gravimetrically after drying overnight in a vacuum desiccator. Fatty acid methyl esters (FAME)
146 were prepared from total lipid by acid-catalyzed transesterification at 50 °C for 16 h according to
147 the method of Christie (1993). Extraction and purification of FAME was carried out as described
148 by Tocher and Harvie (1988). The FAME were separated and quantified by gas-liquid
149 chromatography (Carlo Erba Vega 8160, ThermoFisher Scientific, Hemel Hempstead, UK) using
150 a 30m x 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, U.K.) and on-
151 column injection at 50 °C. Hydrogen was used as carrier gas and temperature programming was
152 from 50 °C to 150 °C at 40 °C min⁻¹ and then to 230 °C at 2.0 °C min⁻¹. Individual methyl esters
153 were identified by comparison with known standards and by reference to published data
154 (Ackman, 1980; Tocher and Harvie, 1988). Data were collected and processed using Chromcard
155 for Windows (version 1.19).

156 *2.4. RNA extraction*

157 Liver and intestinal tissue (0.2 g) from six individuals per experimental group (2 per tank)
158 were homogenized in 2mL of TRI Reagent (Ambion, Applied Biosystems, Warrington, U.K.)
159 using an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, U.K.). The individuals
160 were selected from the group of randomly sampled fish to represent the global average weight of
161 fish in the experiment. Total RNA was isolated following manufacturer's instructions, and RNA
162 quality (integrity and purity) and quantity was assessed by gel electrophoresis and
163 spectrophotometry (NanoDrop ND-1000, Thermo Scientific, Wilmington, U.S.A.).

164 *2.5. Transcriptome analysis*

165 A 16 k cDNA microarray from Atlantic cod was used in this experiment. Details of the
166 microarray construction are given in Edvardsen et al. (2011). Only intestinal samples from the
167 control (C0) and one of the CO dietary treatments (C66) were hybridized. The C66 diet was
168 chosen rather than C100, as this would be the more likely commercial formulation. The low yield
169 and quality of RNA obtained from liver, possibly due to high co-precipitation with glycogen,
170 precluded the use of liver samples for the microarray experiment. Total RNA was purified using
171 the Invitrogen iPrep Trizol® Plus RNA Kit on the iPrepT Purification Instrument following the
172 manufacturer's recommendations. Samples were randomly labelled in a single batch and
173 hybridized in 2 batches of 6 slides each, containing 3 samples from each treatment in each batch.
174 Briefly, 10 µg total RNA was used for cDNA synthesis and labelling using Fair Play®
175 Microarray Labeling Kit (Stratagene, USA) according to manufacturer's instructions. Samples
176 were labelled with Cy5 and a common reference standard (obtained by pooling 40% intestinal
177 RNA from all samples in the microarray experiment plus 60% RNA from a mixture of different
178 tissues) was labelled with Cy3. Labelling efficiency and quantity of labelled cDNA were
179 determined using the NanoDrop spectrophotometer. Slides were pre-hybridized in 20× SSC
180 (Calbiochem, USA), 10% SDS (Sigma-Aldrich Chemie, Steinheim, Germany) and 1% BSA
181 (Sigma-Aldrich Chemie) for 45 min at 65 °C followed by washing twice in water and once in
182 isopropanol, and then drying by centrifugation. Sample and reference labelled cDNA were
183 pooled and diluted in Tris buffer pH 8.0. After sample denaturation (100 °C, 2 min),
184 hybridization was performed at 60 °C overnight with rotation using Agilent 2× hybridization
185 buffer (250 µL) in Agilent hybridization chambers. The slides were put in 2× SSC/0.1% SDS at
186 65 °C to remove gasket slide and then washed for 5 min in 1×SSC at 65 °C, for 5 min in 0.2×
187 SSC at RT, for 45 s in 0.05× SSC at RT, and centrifuged dry. Slides were immediately scanned

188 using an Agilent scanner (G2505 B Microarray Scanner, Agilent Technologies, Santa Clara,
189 USA) at a resolution of 10 μm with default settings.

190 The scanned microarray images were analyzed using the GenePix Pro 6.0 software package
191 and exported as image quantitation files (gpr- and jpg-files). The data files were quality
192 controlled using R (R Development Core Team, 2005, <http://www.r-project.org>), and analyzed
193 using J-Express Pro v.2.7 (Dysvik and Jonassen, 2001, <http://www.molmine.com>). Control
194 probes, empty spots and probes marked as bad quality were removed from the analysis. Genes
195 with more than 30% missing values were removed from the analysis and the remaining missing
196 values were estimated using LSimpute Adaptive (Bø et al., 2004). Each array was normalized by
197 Lowess (Cleveland and Devlin, 1988). Log 2 transformed ratios of foreground signals were used
198 in the final gene expression matrix. MIAME-compliant (Brazma et al., 2001) descriptions of the
199 microarray study and results are available in the EBI ArrayExpress database
200 (<http://www.ebi.ac.uk/arrayexpress>) under accession number E-TABM-1178.

201 Statistical analysis of the microarray hybridization data was performed in GeneSpring GX
202 version 11 (Agilent Technologies, Wokingham, Berkshire, U.K.) using a Welch (unpaired
203 unequal variance) t-test, at 0.05 significance and 1.2 fold change cut-off level. No multiple test
204 correction was employed as previous analyses, confirmed by RT-qPCR, indicate that such
205 corrections are over-conservative for this type of nutritional data (Leaver et al., 2008b; Morais et
206 al., 2011a). Gene Ontology (GO) enrichment analysis was performed using the same software, at
207 $p < 0.05$.

208 2.6. Reverse transcription real-time quantitative PCR (RT-qPCR)

209 Expression of candidate genes of interest (fatty acyl elongase, *elovl5*, and desaturase, *$\Delta 6fad$*)
210 in intestine and liver from fish fed all four treatments, as well as genes for microarray validation
211 in intestinal samples of fish fed C0 and C66, was determined by reverse transcription quantitative
212 real time PCR (RT-qPCR). Details on the target qPCR primer sequences and on the reference
213 sequences used to design them are given in Table 3. Primers were designed using Primer3
214 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi; Rozen and Skaletsky, 2000). Results
215 were normalized by amplification of three reference genes, β -actin (*bact*), ubiquitin (*ubq*) and
216 glyceraldehyde 3-phosphate dehydrogenase (*gapdh*), which in our experimental conditions
217 presented M values, generated by the gene stability analysis software package geNorm
218 (Vandesompele et al., 2002), of 0.292, 0.268 and 0.326, respectively.

219 For RT-qPCR, 1 µg of total RNA per sample was reverse transcribed into cDNA using the
220 Verso™ cDNA kit (ABgene, Surrey, U.K.), following manufacturer's instructions, using a
221 mixture of random hexamers (400ng/µL) and anchored oligo-dT (500ng/µL) at 3:1 (v/v).
222 Negative controls (containing no enzyme) were performed to check for genomic DNA
223 contamination. A similar amount of cDNA was pooled from all samples and the remaining
224 cDNA was then diluted 20-fold with water. RT-qPCR analysis used relative quantification with
225 the amplification efficiency of the primer pairs being assessed by serial dilutions of the cDNA
226 pool. qPCR amplifications were carried out in duplicate (Quanta, Techne, Cambridge, U.K.) in
227 a final volume of 20 µL containing either 5 µL or 2 µL diluted (1/20) cDNA, 0.5 µM of each
228 primer and 10 µL Absolute™ QPCR SYBR® Green mix (ABgene). Amplifications were
229 carried out with a systematic negative control (NTC-non template control). The qPCR profiles
230 contained an initial activation step at 95 °C for 15 min, followed by 30 to 35 cycles: 15 s at 95
231 °C, 15 s at the specific primer pair annealing temperature (Ta; Table 3) and 15 s at 72 °C. After
232 the amplification phase, a melt curve of 0.5 °C increments from 75 °C to 90 °C was performed,
233 enabling confirmation of the amplification of a single product in each reaction. RT-qPCR product
234 sizes were checked by agarose gel electrophoresis and the identity of amplicons was confirmed
235 by sequencing. PCR efficiency was above 90% for all primer pairs.

236 Gene expression results assessed by RT-qPCR were analyzed by the $\Delta\Delta C_t$ method using the
237 relative expression software tool (REST 2008, <http://www.gene-quantification.info/>), employing
238 a pair wise fixed reallocation randomization test (10,000 randomisations) with efficiency
239 correction (Pfaffl et al., 2002), to determine the statistical significance of expression ratios
240 between two treatments.

241 2.7. Statistical analysis

242 All data are presented as means \pm SD (n value as stated). The effects of dietary treatment on
243 growth performance were analyzed by one-way analysis of variance (ANOVA) followed, where
244 appropriate, by Tukey's post hoc test. The relationship between dietary treatment and chemical
245 composition was analyzed by regression analysis. Percentage data and data identified as non-
246 homogeneous (Levene's test) or non-normality (Shapiro-Wilks's test) were subjected to arcsine
247 transformation before analysis. ANOVA and regression analysis were performed using a SPSS
248 Statistical Software System version 14 (SPSS inc, Chicago IL, USA). Differences were regarded
249 as significant when $P < 0.05$ (Zar, 1999).

250

251 **3. Results**

252 *3.1. Growth and biometry*

253 The juvenile cod showed a 5-fold increase in weight over the course of the feeding experiment
254 (Table 4). There were no significant differences between treatments for any of the growth and
255 feeding performance parameters although there was a trend for growth performance to increase
256 with CO inclusion up to 66% with fish fed diet C66 showing highest final weight, SGR and TGC
257 and lowest FCR. In general, very high and variable lipid content was observed in liver, as is
258 typical of farmed cod which normally present enlarged fatty livers (Morais et al., 2001). There
259 were also trends for increased HSI and VSI, as well as liver lipid contents with CO inclusion but
260 only intestinal lipid content was significantly higher in fish fed diet C66 compared to fish fed diet
261 C0 (Table 4).

262 *3.2. Diet and cod tissue fatty acid compositions*

263 The fatty acid compositions of the feeds reflected the increasing content of CO, with decreasing
264 proportions of total saturated fatty acids, mainly 14:0 and 16:0, and total monoenes, specifically
265 16:1 and 22:1, although 18:0, and 18:1n-9 increased (Table 2). It was noteworthy that the level of
266 20:1 was constant across the feeds indicating that the 20:1 content of the FO (a northern
267 hemisphere oil) was balanced by the 20:1 content of CO. In contrast, LOA (18:2n-6) and ALA
268 (18:3n-3) and their immediate elongation products, 20:2n-6 and 20:3n-3 respectively, increased
269 with dietary CO inclusion whereas the LC-PUFA, including arachidonic acid (20:4n-6; ARA),
270 eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) all decreased.
271 The overall effect on dietary PUFA levels was increased total n-6 PUFA, n-3 PUFA and total
272 PUFA, but decreasing n-3/n-6 ratio (Table 2). The fatty acid compositions of the cod tissues
273 reflected those of the feeds with liver (Table 5), intestine (Table 6) and muscle (Table 7) all
274 characterized by generally decreasing proportions of saturated and monounsaturated fatty acids,
275 and increasing proportions of PUFA as dietary CO inclusion increased. These effects were due to
276 decreasing proportions of 16:0, 16:1n-7, 18:1n-7, 22:1, ARA, EPA and DHA, and increased
277 proportions of 18:1n-9, LOA, ALA, 20:2n-6 and 20:3n-3, which together resulted in decreased
278 tissue n-3/n-6 PUFA ratios as CO inclusion increased (Tables 5-7).

279 *3.3. Expression of fatty acyl elongase and desaturase in liver and intestine*

280 The expression of a fatty acyl elongase (*elovl5*) and desaturase (*Δ6des*) in the liver and intestine
281 of Atlantic cod fed increasing levels of CO was assessed by RT-qPCR (Fig. 1). Changes in the

282 relative expression of both genes in fish fed the three diets containing CO, in relation to the C0
283 diet, were not significantly different. In the case of *elov15*, expression ratios were around 1 in
284 both intestine and liver in fish fed all diets, denoting no change in transcript levels. In liver, in
285 spite of a high biological variability, a trend for higher expression of *Δ6des* in fish fed C66,
286 followed by C100, in comparison to fish fed C0 (2.4- and 1.8-fold up-regulated, respectively),
287 was observed, whereas such a trend was not evident (only 1.2-fold up-regulated in C66) in the
288 intestine.

289 3.4. Transcriptomic analysis of intestinal tissue

290 Statistical analysis of the microarray data returned a list of 289 features differentially
291 expressed in the intestine between cod fed C0 and C66. Genes in this list were categorized
292 according to their biological function, by determining the main biological processes in which
293 they are involved in mammalian counterparts. Distribution of the EST's, after removing non-
294 annotated genes (36%) and features representing the same gene product (Fig. 2), revealed that the
295 most affected biological processes or categories were translation (18% of all genes), cell
296 proliferation, differentiation and apoptosis (14%) and genes with a structural molecule activity
297 (12%), followed by transporter activity (9%) and immune response (7%). In contrast, metabolism
298 appeared to be less affected, with 6% of the genes involved in proteolysis, 5% involved in energy
299 metabolism or generation of precursor metabolites and 4% in lipid metabolism. Other minor
300 categories represented were regulation of transcription (4%), signalling (4%) and protein folding
301 (3%). A more detailed analysis of the list was restricted to the top 100 most significant hits,
302 which presented a broadly similar distribution of genes by biological categories, with translation
303 (25%) and cell proliferation, differentiation and apoptosis (20%) predominating, followed by
304 structural molecules (9%) and immune response (9%) (Table 8).

305 Gene ontology (GO) enrichment analysis was performed on the entire significant dataset. This
306 enabled identification of GO terms significantly enriched in the input entity list compared to the
307 whole array dataset, providing evidence for which biological processes may be particularly
308 altered in the experimental conditions being compared. The analysis returned 208 significant GO
309 terms at $p < 0.05$. However, most of the GO terms were interrelated and many of them were
310 significantly enriched largely due to the repetition of multiple features for the same gene, either
311 apolipoprotein A-IV (apoA-IV) or creatine kinase (CK) (Table 8). Nonetheless, some GO terms
312 could be considered as significantly enriched by the presence of multiple genes. With respect to
313 molecular function, the term "actin binding" was significantly enriched (adjusted p-value:

314 1.825E-4), containing features corresponding to myosin heavy chain, tropomyosin alpha-1 chain,
315 actin alpha cardiac muscle 1, cofilin 1 (non-muscle), ectodermal-neural cortex (with BTB-like
316 domain) and transgelin (Table 9). On the other hand, a few related categories of biological
317 process, namely “multicellular organismal development” (adjusted p-value: 0.002), “anatomical
318 structural development” (adjusted p-value: 0.003), “developmental process” (adjusted p-value:
319 0.006), “epidermal cell differentiation” (adjusted p-value: 0.031), and “regulation of cytoskeleton
320 organization” (adjusted p-value: 0.01), were significantly enriched due to the presence of the
321 following genes: CK, zinc finger protein 313, P-cadherin, collagen type II alpha 1, caspase 3,
322 myosin light polypeptide 6B, thioredoxin interacting protein, phytanoyl-CoA 2-hydroxylase,
323 cofilin 1 (non-muscle), NAD(P) dependent steroid dehydrogenase-like, ectodermal-neural cortex
324 (with BTB-like domain), dihydrolipoamide dehydrogenase, ribosomal protein L35, thioredoxin
325 interacting protein, peripheral myelin protein 22, tropomyosin 1 (alpha), intraflagellar transport
326 172 homolog, tumor susceptibility gene 101, transgelin, heat shock protein 90kDa alpha
327 (cytosolic) class B member 4, stress-associated endoplasmic reticulum protein 2 and guanine
328 nucleotide binding protein (G protein) (changes in expression between treatments can be found in
329 supplementary material).

330 3.5. RT-qPCR validation of microarray results

331 The expression of selected genes was measured by RT-qPCR in order to validate the
332 microarray results (Table 10). These genes were selected from within the categories that were
333 more highly represented in the top100 most-significant list. In general, changes in expression
334 levels were subtle, as in the microarray analysis, which explains why differences in transcript
335 levels between fish fed diets C0 and C66 determined by RT-qPCR were not always statistically
336 significant. However, a good match between the fold changes measured by microarray and RT-
337 qPCR results was found for most genes related to cell proliferation and apoptosis, such as tumor
338 protein p63 regulated 1-like (*tprg1l*), retinoic acid receptor responder protein 3 (*rarres3*),
339 translationally-controlled tumour protein homolog (*tctp*) and caspase 3 (*casp3*), even if
340 differences assessed by RT-qPCR were only significant for *rarres3* and *casp3*, these being 7.8-
341 and 1.9-fold up-regulated, respectively, in the intestine of fish fed diet C66. In contrast, no
342 agreement was found between the fold changes measured by microarray and RT-qPCR in genes
343 related to translation, namely, elongation factor 1-alpha (*elf1a*), 60S ribosomal protein L13
344 (*rpl13*) and eukaryotic translation initiation factor 3 subunit F (*etf3f*). However, good agreement
345 was found for the gene coding for the structural protein tropomyosin alpha-1 chain (*tpm*), which

346 was 1.5-fold up-regulated in diet C66 in both the microarray and RT-qPCR analysis, although
347 RT-qPCR did not confirm the microarray results for other structural genes, *tektin-4* (*tekt4*) and
348 cadherin 3 type 1 (*cdh3*).

349

350 **4. Discussion**

351 There was no detrimental effect, and even a tendency for positive effects, of dietary CO on
352 growth of cod up to 66 % inclusion in the fishmeal-based feeds used in the present trial. As a
353 result the microarray gene expression data compared fish fed diet C0 (control) with fish fed diet
354 C66, which showed good adaptation to the alternative formulation. The effects on growth were
355 similar to those previously reported for replacement of dietary FO with the most common ALA-
356 rich oil, linseed, in marine fish including gilthead sea bream (*Sparus aurata*), sharpsnout sea
357 bream (*Diplodus puntazzo*), European sea bass (*Dicentrarchus labrax*) and turbot (*Psetta*
358 *maxima*) (Tocher et al., 2010). In these studies, up to 60 % of dietary FO could be replaced by
359 linseed oil without affecting growth, but higher levels of replacement could reduce growth
360 dependent upon species, fish size and duration of feeding (Izquierdo et al., 2003; Regost et al.,
361 2003; Menoyo et al., 2004; Mourente et al., 2005; Piedecausa et al., 2007). There are no previous
362 studies reporting the effects of CO in marine fish but, in the only study reported to date, growth
363 of Atlantic salmon smolts was not affected by feeding a diet with 100% of FO replaced with a
364 VO blend containing rapeseed, palm and Camelina oils in a 5:3:2 ratio (Bell et al., 2010).

365 The effects of dietary CO on tissue fatty acid composition in cod were as expected
366 considering the extensive data in the literature describing the effects of VO in fish, with increased
367 C18 fatty acids, including 18:0, 18:1n-9, LOA and ALA, and decreased levels of EPA and DHA
368 (Turchini et al., 2010). In the only trials to date utilizing CO, the fatty acid composition of liver in
369 Atlantic salmon fed a VO blend containing CO was characterized by increased levels of ALA
370 and its elongation product 20:3n-3, but decreased levels of all other n-3 fatty acids (Petropoulos
371 et al., 2009; Bell et al., 2010). The data in the present study with cod and CO are comparable with
372 the data obtained in other marine fish species fed ALA-rich oils (Tocher et al., 2010). Studies
373 feeding linseed oil to sea bass, turbot, gilthead and sharpsnout sea bream all showed increased
374 percentages of ALA, LOA and their elongation products, 20:3n-3 and 20:2n-6, and decreased
375 proportions of EPA, DHA and ARA and no increased percentages of any desaturated
376 intermediates such as 18:4n-3, 20:4n-3, 18:3n-6 or 20:3n-6 in tissue lipids (Izquierdo et al., 2003;
377 Regost et al., 2003; Menoyo et al., 2004; Mourente et al., 2005; Piedecausa et al., 2007).

378 Therefore, provision of ALA substrate was not able to compensate for the lack of LC-PUFA in
379 the diet of marine fish and there was little evidence of any significant desaturation of dietary
380 ALA. Although feeding high levels of CO did not prevent reductions in EPA and DHA compared
381 to fish fed FO, the increased levels of ALA in flesh are useful as it is also recognized as an
382 essential fatty acid in human nutrition (Burdge, 2006; Brenna et al., 2009). The human diet is
383 imbalanced with overly high n-6/n-3 ratios, particularly in the developed countries (Simopoulos,
384 2006) and, although production of fish containing high levels of EPA and DHA should be a
385 major goal in aquaculture, a product with a high n-3/n-6 ratio is still desirable even if some of the
386 n-3 PUFA is ALA rather than EPA and DHA. In comparison to EPA and DHA, ALA is inferior
387 as a component of fish flesh, but it is preferable to LOA (Whelan, 2008).

388 In order to directly address the question of whether cod have the capacity to up-regulate the
389 LC-PUFA biosynthesis pathway in response to increasing levels of CO, the expression of fatty
390 acyl desaturase (*Δ6fad*) and elongase (*elovl5*) genes, which have been shown to be
391 transcriptionally regulated, and correlated with enzymatic activity, in Atlantic salmon (Zheng et
392 al., 2004, 2005), was measured by RT-qPCR. Results in both liver and intestine showed no
393 significant nutritional regulation of *elovl5* in cod fed CO. In liver, a trend for higher *Δ6fad*
394 expression in the two treatments with higher levels of FO replacement, particularly C66, in
395 comparison to the C0 diet was noticeable, but biological variability of the data was high and the
396 results were not significant. A previous study on the nutritional regulation of these genes in cod
397 showed that dietary FO replacement by VO tended to increase the expression of both *Δ6fad* and
398 *elovl5* in hepatocytes and enterocytes, but there was high biological variability and so only *elovl5*
399 in enterocytes was significant, although there was no significant effect on LC-PUFA biosynthesis
400 enzyme activities in either tissue (Tocher et al., 2006). In the case of *Δ6fad*, this is likely due to
401 lower basal expression of this gene, compared to salmon, perhaps associated with lower activity
402 of the cod *Δ6fad* promoter (Zheng et al., 2009).

403 Recent studies have begun to investigate the molecular effects of alternative diet formulations,
404 and hepatic responses to feeds containing high levels of replacement of FO by VOs are being
405 characterized (Panserat et al., 2009; Morais et al., 2011a,b). However, studies on the intestinal
406 transcriptome in fish have mainly focused on replacement of fishmeal by plant proteins, in
407 particular soybean meal, given its potential to cause enteritis in some species (Murray et al.,
408 2010; Skugor et al., 2011). Furthermore, the majority of these transcriptomic studies have been
409 conducted on salmonid species, which can tolerate high levels of fishmeal and FO replacement

410 (Turchini et al., 2010; Hardy, 2010), whereas few data exist for marine species. Here we
411 examined the effects of CO on the intestinal transcriptome of cod. This knowledge is important
412 as it is well established that the intestine is more than simply the site of nutrient uptake, and that
413 enterocytes are also sites of significant lipid metabolism (Tocher et al., 2002; Bell et al., 2003).
414 For example, despite LC-PUFA biosynthesis ability being low in cod, the activity of this pathway
415 was around 7-fold higher in enterocytes than in hepatocytes (Tocher et al., 2006). In addition, the
416 intestine has a vital role in protecting against the entry of pathogens and is one of the largest
417 immune organs in the body. Hence, its proper function is essential for maintaining optimal
418 balance and health and can be significantly affected by replacing dietary FO by VO,
419 accompanied by reductions in n-3/n-6 LC-PUFA ratios (Teitelbaum and Walker, 2001).

420 The microarray analysis identified translation, cell proliferation or differentiation and
421 apoptosis, as well as structural molecules, as the biological categories in cod intestine most
422 highly affected by dietary CO. Surprisingly, lipid metabolism and metabolism in general were
423 little affected by changes in diet formulation, changes that were exclusively in lipid composition.
424 The main observed effect was up-regulation of apoA-IV in the intestine of cod fed the C66 diet.
425 In humans and rodents this protein is mainly found free in plasma but is also a major component
426 of chylomicrons and very low density lipoproteins (VLDL) and is synthesized by enterocytes in
427 the small intestine (Green and Glickman, 1981). In mammals, apoA-IV is the only apolipoprotein
428 that showed a marked response to intestinal absorption and transport of lipid, through increased
429 expression, synthesis and secretion (Kalogeris et al., 1997; Tso et al., 2001). A commonly
430 reported effect of the inclusion of VO in the diet of carnivorous fish is an accumulation of lipid
431 droplets in the enterocytes, which might be explained by a reduction in LC-PUFA levels leading
432 to changes in re-acylation mechanisms and phospholipid synthesis rates and consequent lower
433 lipoprotein assembly and export from intestinal cells (Caballero et al., 2003; Olsen et al., 2003).
434 However, apoA-IV is one of the least well characterized apolipoproteins in fish species and there
435 is no information regarding its physiological function. Furthermore, even in mammals, apoA-IV
436 has diverse roles, including the regulation of appetite and gastrointestinal function (enzyme
437 secretion and gastric emptying), and displays anti-oxidant and anti-atherogenic properties in
438 rodents (Kalogeris et al., 1997; Tso et al., 2001; Stan et al., 2003). The biochemical results
439 showed a trend for higher lipid content in fish fed the diets containing CO, and intestinal lipid
440 was significantly higher in fish fed C66 compared to C0. Hence, although other factors might

441 explain the changes in apoA-IV expression, they may be related to effects of dietary lipid
442 composition on lipid absorption and transport.

443 A large number of ribosomal proteins were slightly, but consistently, down-regulated in the
444 intestine of cod fed the CO diet. Ribosomal proteins typically show a highly coordinated
445 response in terms of expression changes in salmon microarrays and Skugor et al. (2011) reported
446 increased expression in a number of these in response to cellular stressors. However, RT-qPCR
447 did not confirm the microarray data, preventing firm conclusions on potential effects of diet on
448 translation. In contrast, good agreement was obtained between the microarray and RT-qPCR
449 results for some genes involved in cell proliferation or differentiation and apoptosis with *rarres3*
450 and *casp* being 7.8- and 1.9-fold up-regulated, respectively, in the intestine of cod fed the C66
451 diet. Retinoic acid receptor responder protein 3 (*rarres3*), also known as retinoid-inducible gene
452 1 protein or tazarotene-induced gene 3, is a nuclear receptor and transcriptional regulator that is
453 thought to act as a tumour suppressor, regulating growth and differentiation of many cell types,
454 mediated via inhibition of cellular growth (G0/G1 arrest) or induction of apoptosis (DiSepio et
455 al., 1998; Huang et al., 2000). The strong up-regulation of this transcript in the intestine of cod
456 fed CO is thus likely reflected in lower cellular proliferation. Furthermore, up-regulation of *casp*
457 in cod fed CO indicates increased apoptosis and hence possibly also lower intestinal tissue
458 growth.

459 Other transcripts that were up-regulated (in the top100 most significant list) in the CO
460 treatment were tumor protein p63 regulated 1 (*tprg1l*), 40S ribosomal protein SA (*rpsa*), origin
461 recognition complex subunit 2-like and voltage-dependent anion-selective channel protein 1. The
462 p63 tumor protein is a transcription factor that is highly expressed in basal cells of epithelial
463 layers in mammals, and at least some of its variants have effects on induction of apoptosis and
464 arresting growth (Yang et al., 1998; Wu et al., 2003). The origin recognition complex is essential
465 for the initiation of DNA replication and heterochromatin assembly in eukaryotic cells, ensuring
466 tight regulation of the cell cycle and preventing re-replication of DNA during a single cell
467 division cycle. This may suggest tighter control of replication in the intestine of cod fed the CO
468 diet, although this complex has other non-replication roles (Chesnokov, 2007; Sasaki and Gilbert,
469 2007). Voltage-dependent anion-selective channel protein 1 is also associated with regulation of
470 cell growth and death, interacting with several pre- or anti-apoptotic proteins to allow the
471 formation of pores in outer mitochondrial and plasma membranes, affecting their permeability.
472 When the channel adopts an open conformation it enables cytochrome c release into the cytosol,

473 which triggers caspase activation and apoptosis (Shoshan-Barmatz et al., 2006). In addition,
474 down-regulation of proline-rich protein BCA3-like and translationally-controlled tumor protein
475 homolog (*tctp*) might indicate lower cell proliferation in intestine of cod fed CO. Proline-rich
476 protein BCA3-like is over-expressed tumor cell lines while normal tissues have low levels of
477 expression (Kitching et al., 2003). Several features coding for *tctp* were down-regulated in the
478 microarray results, confirmed by RT-qPCR. This protein is important for microtubule
479 stabilization and was up-regulated in several tumours (Hsu et al., 2007), and reduction of its level
480 can induce tumour reversion, and spatial distribution of *tctp* expression in *Hydra* correlated with
481 regions of active cell proliferation (Yan et al., 2000), all indicating an important role in cell
482 growth and proliferation (Tuynder et al., 2004).

483 The data therefore suggest a hypothesis of lower cell proliferation and/or higher apoptosis in
484 intestine of cod fed diet C66. Gastrointestinal cell proliferation and apoptosis were previously
485 found to be significantly reduced in Atlantic salmon when VO replaced dietary FO (Olsvik et al.,
486 2007). Although the cell's normal renewal capacity could have been impaired after a period of
487 potentially adverse intestinal conditions when feeding VO, given that lower transcription was
488 also measured for genes related to cellular and oxidative stress, the authors attributed their results
489 to reduced oxidative stress associated with lower levels of LC-PUFA in intestinal membranes of
490 fish fed VO (Olsvik et al., 2007). This would result in lower potential for lipid peroxidation and
491 production of reactive oxygen species (ROS), which are important signalling molecules in
492 apoptotic processes (Ramachandran et al., 2000). In the present study the evidence, albeit
493 circumstantial, collectively supports the concept of reduced cellular proliferation and increased
494 apoptosis in the intestine of fish fed CO, compared to those fed the FO diet. Furthermore, results
495 from GO analysis identified several categories of biological processes broadly related to
496 development, cell differentiation and cytoskeleton organization, which indicate potential effects
497 of VO on intestinal regenerative processes or repair, but this requires further investigation.

498 In addition to a high preponderance of structural proteins in the list of genes showing
499 significant effects of diet, GO analysis identified actin-binding as one of the molecular function
500 categories that was over-represented. Genes in this functional category included tropomyosin
501 alpha-1 chain, myosin heavy chain, actin alpha cardiac muscle 1, cofilin 1, ectodermal-neural
502 cortex and transgelin, which were 1.4- to 1.8-fold up-regulated (except actin, which was 1.2-fold
503 down-regulated) in cod fed diet C66. Changes in the expression of these genes indicate likely
504 modifications in the structural composition of smooth muscle, possibly affecting contractile

505 activity or motility of the intestine. Genes with similar biological function, including tropomyosin
506 and myosin, were down-regulated in Atlantic halibut intestine in response to partial soybean meal
507 replacement of fishmeal (Murray et al., 2010). In that study, genes coding for structural proteins
508 were regulated in a coordinated matter with others involved in muscle physiology, such as
509 creatine kinase (CK), which was also the case here, even if opposite changes were observed,
510 indicating potentially conflicting effects of fishmeal and FO replacement in these two species.
511 Creatine kinase has a key role in muscle energetic metabolism and CK activity relates to the
512 oxidative capacity as well as contractile characteristics of muscle (Clark, 1994). Changes in CK
513 activity and expression level are diagnostic markers for conditions involving muscle damage in
514 mammals, including intestinal infarction or ischemia (Fried et al., 1991; Block et al., 2011).
515 Furthermore, skeletal muscle atrophy has been associated with variations in gene expression
516 profiles similar to those observed here, namely up-regulation of M-type CK as well as of fast-
517 type isoforms of myosin heavy chains (Cros et al., 1999). These effects could not have been
518 anticipated and, unfortunately, no samples were collected for histological analysis preventing
519 gene expression to be related to intestinal morphology.

520 In summary, CO may be a useful more sustainable alternative for the replacement of FO in the
521 diets of Atlantic cod. Replacement of FO by CO did not induce major metabolic changes in
522 intestinal tissue, as might have been expected, but rather potentially affected rates of cellular
523 proliferation and death, and changes in the structural properties of the intestinal muscle, most
524 likely leading to different rates of tissue regeneration and/or repair, as well as potential changes
525 in contractile activity or mechanical characteristics. The underlying molecular mechanisms
526 explaining these effects on gene expression cannot be determined conclusively but, considering
527 the important role of the intestine in nutrient absorption and whole body balance and health,
528 further attention should be given to this organ in future studies examining effects of FO
529 replacement by VO.

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537 **Supplementary data:** Features present in the following categories of biological process, all
538 found to be significantly enriched by GO analysis: “multicellular organismal development”
539 (adjusted p-value: 0.002), “anatomical structural development” (adjusted p-value: 0.003),
540 “developmental process” (adjusted p-value: 0.006), “epidermal cell differentiation” (adjusted p-
541 value: 0.031), and “regulation of cytoskeleton organization” (adjusted p-value: 0.01). Shown are
542 fold changes in cod intestine between diets C66 and C0.

543 **References**

- 544 Ackman, R.G., 1980. Fish lipids, in: Connell, J.J. (Ed.), *Advances in Fish Science and*
545 *Technology*. Fishing News Books, Farnham, pp. 83-103.
- 546 AOAC, 2000. *Official methods of analysis*. Association of Official Analytical Chemists,
547 Gaithersburg, Maryland, USA.
- 548 Bell, M.V., Dick, J.R., Porter, A.E.A., 2003. Pyloric caeca are a major site of newly synthesised
549 22:6n-3 in rainbow trout (*Oncorhynchus mykiss*). *Lipids* 38, 39-44.
- 550 Bell, J.G., Pratoomyot, J., Strachan, F., Henderson, R.J., Fontanillas, R., Hebard, A., Guy, D.R.,
551 Hunter, D., Tocher, D.R., 2010. Growth, flesh adiposity and fatty acid composition of Atlantic
552 salmon (*Salmo salar*) families with contrasting flesh adiposity: Effects of replacement of
553 dietary fish oil with vegetable oils. *Aquaculture* 306, 225-232.
- 554 Block, T., Isaksson, H.S., Acosta, S., Björck, M., Brodin, D., Nilsson, T.K., 2011. Altered
555 mRNA expression due to acute mesenteric ischaemia in a porcine model. *Eur. J. Vasc.*
556 *Endovasc. Surg.* 41, 281-287.
- 557 Bø, T.H., Dysvik, J., Jonassen, I., 2004. LSImpute: accurate estimation of missing values in
558 microarray data with least squares methods. *Nucleic Acids Res.* 32, e34.
- 559 Brazma, A., Hingamp, P., Quackenbush, J., Sherlock, G., Spellman, P., Stoeckert, C., Aach, J.,
560 Ansorge, W., Ball, C.A., Causton, H.C., Gaasterland, T., Glenisson, P., Holstege, F.C., Kim,
561 I.F., Markowitz, V., Matese, J.C., Parkinson, H., Robinson, A., Sarkans, U., Schulze-Kremer,
562 S., Stewart, J., Taylor, R., Vilo, J., Vingron, M., 2001. Minimum information about a
563 microarray experiment (MIAME)-toward standards for microarray data. *Nat. Genet.* 29, 365-
564 371.
- 565 Brenna, J.T., Salem, N., Sinclair, A.J., Cunnane, S.C., 2009. α -Linolenic acid supplementation
566 and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins*
567 *Leukotr. Essent. Fatty Acids* 80, 85-91.

568 Brown, J.A., Minkoff, G., Puvanendran, V., 2003. Larviculture of Atlantic cod (*Gadus morhua*):
569 progress, protocols and problems. *Aquaculture* 227, 357-372.

570 Burdge, G.C., 2006. Metabolism of α -linolenic acid in humans. *Prostaglandins Leukot. Essent.*
571 *Fatty Acids* 75, 161-168.

572 Caballero, M.J., Izquierdo, M.S., Kjorsvik, E., Montero, D., Socorro, J., Fernandez, A.J.,
573 Rosenlund, G., 2003. Morphological aspects of intestinal cells from gilthead seabream (*Sparus*
574 *aurata*) fed diets containing different lipid sources. *Aquaculture* 225, 325-340.

575 Chesnokov, I.N., 2007. Multiple functions of the origin recognition complex. *Int. Rev. Cytol.*
576 256, 69-109.

577 Christie, W.W., 1993. Preparation of derivatives of fatty acids for chromatographic analysis, in:
578 Christie, W.W. (Ed.), *Advances in Lipid Methodology-Two*. The Oily Press, Dundee, pp. 69-
579 111.

580 Clark, J.F., 1994. The creatine kinase system in smooth muscle. *Mol. Cell. Biochem.* 133-134,
581 221-232.

582 Cleveland, W.S., Devlin, S.J., 1988. Locally weighted regression - an approach to regression-
583 analysis by local fitting. *J. Am. Statist. Assoc.* 83, 596-610.

584 Cros, N., Muller, J., Bouju, S., Piétu, G., Jacquet, C., Léger, J.J., Marini, J.F., Dechesne, C.A.,
585 1999. Upregulation of M-creatine kinase and glyceraldehyde3-phosphate dehydrogenase: two
586 markers of muscle disuse. *Am. J. Physiol.* 276, R308-316.

587 DiSepio, D., Ghosn, C., Eckert, R.L., Deucher, A., Robinson, N., Duvic, M., Chandraratna, R.A.,
588 Nagpal, S., 1998. Identification and characterization of a retinoid-induced class II tumor
589 suppressor/growth regulatory gene. *Proc. Natl. Acad. Sci. USA* 95, 14811-14815.

590 Dysvik, B., Jonassen, I., 2001. J-Express: exploring gene expression data using Java.
591 *Bioinformatics* 17, 369-370.

592 Edvardsen, R B., Malde, K., Mittelholzer, C., Taranger, G.L., Nilsen, F., 2011. EST resources
593 and establishment and validation of a 16k cDNA microarray from Atlantic cod (*Gadus*
594 *morhua*). *Comp. Biochem. Physiol. Part D* 6, 23-30.

595 Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and
596 purification of total lipids from animal tissues. *J. Biol. Chem.* 226, 497-509.

597 Fried, M.W., Murthy, U.K., Hassig, S.R., Woo, J., Oates, R.P., 1991. Creatine kinase isoenzymes
598 in the diagnosis of intestinal infarction. *Dig. Dis. Sci.* 36, 1589-1593.

599 Green, P.H., Glickman, R.M., 1981. Intestinal lipoprotein metabolism. *J. Lipid Res.* 22, 1153-
600 1173.

601 Gunstone, F.D., 2010. The world's oils and fats. In: Turchini, G.M., Ng, W.-K., Tocher, D.R.
602 (Eds.), *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*. Taylor &
603 Francis, CRC Press, Boca Raton, FL, pp. 61-98.

604 Hansen, Ø.J., Puvanendran, V., Jøstensen, J.P., Ous, C., 2011. Effects of dietary levels and ratio
605 of phosphatidylcholine and phosphatidylinositol on the growth, survival and deformity levels
606 of Atlantic cod larvae and early juveniles. *Aquacult. Res.* 42, 1026-1033.

607 Hardy, R.W., 2010. Utilization of plant proteins in fish diets: effects of global demand and
608 supplies of fishmeal. *Aquacult. Res.* 41, 770-776.

609 Hemre, G.-I., Karlsen, O., Eckhoff, K., Viet, K., Mangor-Jensen, A., Rosenlund, G., 2004. Effect
610 of season, light regime and diet on muscle composition and selected parameters in farmed
611 Atlantic cod, *Gadus morhua* L. *Aquacult. Res.* 35, 683-697.

612 Hsu, Y.C., Chern, J.J., Cai, Y., Liu, M., Choi, K.W., 2007. Drosophila TCTP is essential for
613 growth and proliferation through regulation of dRheb GTPase. *Nature* 445, 785-788.

614 Huang, S.L., Shyu, R.Y., Yeh, M.Y., Jiang, S.Y., 2000. Cloning and characterization of a novel
615 retinoid-inducible gene 1 (RIG1) deriving from human gastric cancer cells. *Mol. Cell*
616 *Endocrinol.* 159, 15-24.

617 Izquierdo, M.S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L., Rosenlund, G., 2003.
618 Dietary lipid sources for seabream and seabass: growth performance, tissue composition and
619 flesh quality. *Aquacult. Nutr.* 9, 397-407.

620 Kalogeris, T.J., Rodriguez, M.D., Tso, P., 1997. Control of synthesis and secretion of intestinal
621 apolipoprotein A-IV by lipid. *J Nutr.* 127, 537S-543S.

622 Kitching, R., Li, H., Wong, M.J., Kanaganayakam, S., Kahn, H., Seth, A., 2003. Characterization
623 of a novel human breast cancer associated gene (BCA3) encoding an alternatively spliced
624 proline-rich protein. *Biochim. Biophys. Acta* 1625, 116-121.

625 Kortner, T.M., Overrein, I., Øie, G., Kjørsvik, E., Arukwe, A., 2011. The influence of dietary
626 constituents on the molecular ontogeny of digestive capability and effects on growth and
627 appetite in the Atlantic cod larvae (*Gadus morhua*). *Aquaculture* 315, 114-120.

628 Kurlansky, M., 1998. *Cod: A Biography of the Fish That Changed the World*. Johnathan Cape,
629 London.

630 Lall, S.P., Nanton, D., 2002. Nutrition of Atlantic cod. *Bull. Aquacult. Assoc. Can.* 102, 23-26.

631 Leaver, M.J., Bautista, J.M., Björnsson, T., Jönsson, E. Krey, G., Tocher, D.R., Torstensen, B.E.,
632 2008a. Towards fish lipid nutrigenomics: current state and prospects for fin-fish aquaculture.
633 Rev. Fisheries Sci. 16(S1), 71-92.

634 Leaver, M.J., Villeneuve, L.A., Obach, A., Jensen, L., Bron, J.E., Tocher, D.R., Taggart, J.B.,
635 2008b. Functional genomics reveals increases in cholesterol biosynthetic genes and highly
636 unsaturated fatty acid biosynthesis after dietary substitution of fish oil with vegetable oils in
637 Atlantic salmon (*Salmo salar*). BMC Genomics 9, 299.

638 Menoyo, D., Izquierdo, M.S., Robaina, L., Gines, R., Lopez-Bote, C.J., Bautista, J.M., 2004.
639 Adaptation of lipid metabolism, tissue composition and flesh quality in gilthead sea bream
640 (*Sparus aurata*) to the replacement of dietary fish oil by linseed and soybean oils. Br. J. Nutr.
641 92, 41-52.

642 Morais, S., Bell, J.G., Robertson, D.A., Roy, W.J., Morris, P.C., 2001. Protein/lipid ratios in
643 extruded diets for Atlantic cod (*Gadus morhua* L.): Effects on growth, feed utilization, muscle
644 composition and liver histology. Aquaculture 203, 101-119.

645 Morais, S., Pratoomyot, J., Taggart, J.B., Bron, J.E., Guy, D.R., Bell, J.G., Tocher, D.R., 2011a.
646 Genotype-specific responses in Atlantic salmon (*Salmo salar*) subject to dietary fish oil
647 replacement by vegetable oil: a liver transcriptomic analysis. BMC Genomics 12, 255.

648 Morais, S., Pratoomyot, J., Torstensen, B.E., Taggart, J.B., Guy, D.R., Bell, J.G., Tocher, D.R.
649 2011b., Diet x genotype interactions in hepatic cholesterol and lipoprotein metabolism in
650 Atlantic salmon (*Salmo salar*) in response to replacement of dietary fish oil with vegetable oil.
651 Br. J. Nutr. In press.

652 Mourente, G., Dick, J.R., Bell, J.G., Tocher, D.R., 2005. Effect of partial substitution of dietary
653 fish oil by vegetable oils on desaturation and oxidation of [1-¹⁴C]18:3n-3 and [1-¹⁴C]20:5n-3
654 in hepatocytes and enterocytes of European sea bass (*Dicentrarchus labrax* L.). Aquaculture
655 248, 173-186.

656 Murphy, E.J., 2011. Versatile Camelina: the future of biofuel and much more. Inform 22, 604-
657 608.

658 Murray, H.M., Lall, S.P., Rajaselvam, R., Boutilier, L.A., Blanchard, B., Flight, R.M., Colombo,
659 S., Mohindra, V., Douglas, S.E., 2010. A nutrigenomic analysis of intestinal response to
660 partial soybean meal replacement in diets for juvenile Atlantic halibut, *Hippoglossus*
661 *hippoglossus*, L. Aquaculture 298, 282-293.

662 National Research Council (NRC), 2011. Nutrient requirements of fish and shrimp. The National
663 Academies Press, Washington D.C..

664 Naylor, R.L., Hardy, R.W., D.P. Bureau, D.P., Chiu, A., Elliot, M., Farrell, A.P., Forster, I.,
665 Gatlin, D.M., Goldburg, R.J., Hua, K., Nichols, P.D., 2009. Feeding aquaculture in an era of
666 finite resources. Proc. Natl. Acad. Sci. USA 106, 15103-15110.

667 Olsen, R.E., Dragsnes, B.T., Myklebust, R., Ringø, R., 2003. Effect of soybean oil and soybean
668 lecithin on intestinal lipid composition and lipid droplet accumulation of rainbow trout,
669 *Oncorhynchus mykiss* Walbaum. Fish Physiol. Biochem. 29, 181-192.

670 Olsvik, P.A., Torstensen, B.E., Berntssen, M.H.G., 2007. Effects of complete replacement of fish
671 oil with plant oil on gastrointestinal cell death, proliferation and transcription of eight genes'
672 encoding proteins responding to cellular stress in Atlantic salmon *Salmo salar* L. J. Fish Biol.
673 71, 550-568.

674 Olsvik, P.A., Søfteland, L., Lie, K.K., 2008. Selection of reference genes for qRT-PCR
675 examination of wild populations of Atlantic cod *Gadus morhua*. BMC Research Notes 1, 47.

676 Panserat, S., Hortopan, G.A., Plagnes-Juan, E., Kolditz, C., Lansard, M., Skiba-Cassy, S.,
677 Esquerre, D., Geurden, I., Médale, F., Kaushik, S., Corraze, G., 2009. Differential gene
678 expression after total replacement of dietary fish meal and fish oil by plant products in
679 rainbow trout (*Oncorhynchus mykiss*) liver. Aquaculture 294, 123-131.

680 Petropoulos, I.K., Thompson, K.D., Morgan, A., Dick, J.R., Tocher, D.R., Bell, J.G., 2009.
681 Effects of substitution of dietary fish oil with a blend of vegetable oils on liver and peripheral
682 blood leukocyte fatty acid composition, plasma prostaglandin E₂ and immune parameters in
683 three strains of Atlantic salmon (*Salmo salar*). Aquaculture Nutr. 15, 596-607.

684 Pfaffl, M.W., Horgan, G.W., Dempfle, L., 2002. Relative expression software tool (REST) for
685 group-wise comparison and statistical analysis of relative expression results in real-time PCR.
686 Nucleic Acids Res. 30, e36.

687 Piedecausa, M.A., Mazon, M.J., Garcia, B., Hernandez, M.D., 2007. Effects of replacement of
688 fish oil by vegetable oils in the diets of sharpnose seabream (*Diplodus puntazzo*). Aquaculture
689 253, 211-219.

690 Ramachandran, A., Madesh, M., Balasubramanian, K.A., 2000. Apoptosis in the intestinal
691 epithelium: its relevance in normal and pathophysiological conditions. J. Gastroenterol.
692 Hepatol. 15, 109-120.

693 Regost, C., Arzel, J., Robin, J., Rosenlund, G., Kaushik, S.J., 2003. Total replacement of fish oil
694 by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*) 1. Growth
695 performance, flesh fatty acid profile, and lipid metabolism. *Aquaculture* 217, 465-482.

696 Rozen, S., Skaletsky, H.J., 2000. Primer3 on the WWW for general users and for biologist
697 programmers, in: Krawetz, S., Misener, S. (Eds.), *Bioinformatics Methods and Protocols:*
698 *Methods in Molecular Biology*. Humana Press, Totowa, NJ, pp. 365-386.

699 Salze, G., Tocher, D.R., Roy, W.J., Robertson, D.A., 2005. Egg quality determinants in cod
700 (*Gadus morhua* L.): egg performance and lipids in eggs from farmed and wild broodstock,
701 *Aquaculture Res.* 36, 1488-1499.

702 Sasaki, T., Gilbert, D.M., 2007. The many faces of the origin recognition complex. *Curr. Opin.*
703 *Cell Biol.* 19, 337-343.

704 Shoshan-Barmatz, V., Israelson, A., Brdiczka, D., Sheu, S.S., 2006. The voltage-dependent anion
705 channel (VDAC): function in intracellular signalling, cell life and cell death. *Curr. Pharm.*
706 *Des.* 12, 2249-2270.

707 Simopoulos, A.P., 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic
708 variation: nutritional implications for chronic diseases. *Biomedicine Pharmacotherapy* 60,
709 502-507.

710 Skugor, S., Grisdale-Helland, B., Refstie, S., Afanasyev, S., Vielma, J., Krasnov, A., 2011. Gene
711 expression responses to restricted feeding and extracted soybean meal in Atlantic salmon
712 (*Salmo salar* L.). *Aquacult. Nutr.* DOI: 10.1111/j.1365-2095.2010.00832.x

713 Stan, S., Delvin, E., Lambert, M., Seidman, E., Levy, E., 2003. Apo A-IV: an update on
714 regulation and physiologic functions. *Biochim. Biophys. Acta* 1631, 177-187.

715 Star, B., Nederbragt, A.J., Jentoft, S., Grimholt, U., Malmstrøm, M., Gregers, T.F., Rounge, T.B.,
716 Paulsen, J., Solbakken, M.H., Sharma, A., Wetten, O.F., Lanzén, A., Winer, R., Knight, J.,
717 Vogel, J.H., Aken, B., Andersen, O., Lagesen, K., Tooming-Klunderud, A., Edvardsen, R.B.,
718 Tina, K.G., Espelund, M., Nepal, C., Previti, C., Karlsen, B.O., Moum, T., Skage, M., Berg,
719 P.R., Gjøen, T., Kuhl, H., Thorsen, J., Malde, K., Reinhardt, R., Du, L., Johansen, S.D.,
720 Searle, S., Lien, S., Nilsen, F., Jonassen, I., Omholt, S.W., Stenseth, N.C., Jakobsen, K.S.,
721 2011. The genome sequence of Atlantic cod reveals a unique immune system. *Nature*, doi:
722 10.1038/nature10342.

723 Taggart, J.B., Bron, J.E., Martin, S.A.M., Seear, P.J., Hoyheim, B., Talbot, R., Villeneuve, L.,
724 Sweeney, G.E., Houlihan, D.F., Secombes, C.J., Tocher, D.R., Teale, A.J., 2008. A

725 description of the origins, design and performance of the TRAITS/SGP Atlantic salmon
726 (*Salmo salar* L.) cDNA microarray. J. Fish Biol. 72, 2071-2094.

727 Teitelbaum, J.E., Allan Walker, W., 2001. Review: the role of omega 3 fatty acids in intestinal
728 inflammation. J. Nutr. Biochem. 12, 21-32.

729 Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev.
730 Fisheries Sci. 11, 107-184.

731 Tocher, D.R., Harvie, D.G., 1988. Fatty acid composition of the major phosphoglycerides from
732 fish neutral tissues: (n-3) and (n-6) polyunsaturated fatty acids in rainbow trout (*Salmo*
733 *gairdneri* L.) and cod (*Gadus morhua* L.) brains and retinas. Fish Physiol. Biochem. 5, 229-
734 239.

735 Tocher, D.R., Fonseca-Madrugal, J., Gordon Bell, J., Dick, J.R., Henderson, R.J., Sargent, J.R.,
736 2002. Effects of diets containing linseed oil on fatty acid desaturation and oxidation in
737 hepatocytes and intestinal enterocytes in Atlantic salmon (*Salmo salar*). Fish Physiol.
738 Biochem. 26, 157-170.

739 Tocher, D.R., Zheng, X., Schlechtriem, C., Hastings, N., Dick, J.R., Teale, A.J., 2006. Highly
740 unsaturated fatty acid synthesis in marine fish: cloning, functional characterization, and
741 nutritional regulation of fatty acyl delta 6 desaturase of Atlantic cod (*Gadus morhua* L.).
742 Lipids 41, 1003-1016.

743 Tocher, D.R., Francis, D.S., Coupland, K., 2010. n-3 Polyunsaturated fatty acid-rich vegetable
744 oils and blends. In: Turchini, G.M., Ng, W.-K., Tocher, D.R. (Eds.), Fish Oil Replacement and
745 Alternative Lipid Sources in Aquaculture Feeds. Taylor & Francis, CRC Press, Boca Raton,
746 pp. 209-244.

747 Torstensen, B.E., Tocher, D.R., 2010. The Effects of fish oil replacement on lipid metabolism of
748 fish. In: Turchini, G.M., Ng, W.-K., Tocher, D.R. (Eds.), Fish Oil Replacement and
749 Alternative Lipid Sources in Aquaculture Feeds. Taylor & Francis, CRC Press, Boca Raton,
750 pp. 405-437.

751 Tso, P., Liu, M., Kalogeris, T.J., Thomson, A.B., 2001. The role of apolipoprotein A-IV in the
752 regulation of food intake. Annu. Rev. Nutr. 21, 231-254.

753 Turchini, G.M., Ng, W.-K., Tocher, D.R., 2010. Fish Oil Replacement and Alternative Lipid
754 Sources in Aquaculture Feeds. Taylor & Francis, CRC Press, Boca Raton.

755 Tuynder, M., Fiucci, G., Prieur, S., Lespagnol, A., Géant, A., Beaucourt, S., Duflaut, D., Besse,
756 S, Susini, L., Cavarelli, J., Moras, D., Amson, R., Telerman, A., 2004. Translationally

757 controlled tumor protein is a target of tumor reversion. Proc. Natl. Acad. Sci. U S A 101,
758 15364-15369.

759 Whelan, J., 2008. The health implications of changing linoleic acid intakes. Prostaglandin
760 Leukotr. Essent. Fatty Acids 79, 165-167.

761 Wu, G., Nomoto, S., Hoque, M.O., Dracheva, T., Osada, M., Lee, C.C., Dong, S.M., Guo, Z.,
762 Benoit, N., Cohen, Y., Rechthand, P., Califano, J., Moon, C.S., Ratovitski, E., Jen, J.,
763 Sidransky, D., Trink, B., 2003. DeltaNp63alpha and TAp63alpha regulate transcription of
764 genes with distinct biological functions in cancer and development. Cancer Res. 63, 2351-
765 2357.

766 Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F.,
767 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging
768 of multiple internal control genes. Genome Biol. 23, 0034.1-0034.11. doi: 10.1186/gb-2002-
769 3-7-research0034.

770 Yan, L., Fei, K., Bridge, D., Sarras, M.P., 2000. A cnidarian homologue of translationally
771 controlled tumor protein (P23/TCTP). Dev. Genes Evol. 210, 507-511.

772 Yang, A., Kaghad, M., Wang, Y., Gillett, E., Fleming, M.D., Dötsch, V., Andrews, N.C., Caput,
773 D., McKeon, F., 1998. p63, a p53 homolog at 3q27-29, encodes multiple products with
774 transactivating, death-inducing, and dominant-negative activities. Mol. Cell 2, 305-316.

775 Zar, J.H., 1999. Biostatistical Analysis 4th Edition Prentice-Hall, New Jersey.

776 Zheng, X., Tocher, D.R., Dickson, C.A., Bell, J.G., Teale, A.J., 2004. Effects of diets containing
777 vegetable oil on expression of genes involved in highly unsaturated fatty acid biosynthesis in
778 liver of Atlantic salmon (*Salmo salar*). Aquaculture 236, 467-483.

779 Zheng, X., Torstensen, B.E., Tocher, D.R., Dick, J.R., Henderson, R.J., Bell, J.G., 2005.
780 Environmental and dietary influences on highly unsaturated fatty acid biosynthesis and
781 expression of fatty acyl desaturase and elongase genes in liver of Atlantic salmon (*Salmo*
782 *salar*). Biochim. Biophys. Acta 1734, 13-24.

783 Zheng, X., Leaver, M.J., Tocher, D.R., 2009. Long-chain polyunsaturated fatty acid synthesis in
784 fish: Comparative analysis of Atlantic salmon (*Salmo salar* L.) and Atlantic cod (*Gadus*
785 *morhua* L.) Delta6 fatty acyl desaturase gene promoters. Comp. Biochem. Physiol. B 154,
786 255-263.

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789 **Figure Captions**

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791 **Fig. 1.** Expression, measured by RT-qPCR, of fatty acyl elongase (*elovl5*) and desaturase (*Δ6fad*)
792 genes in Atlantic cod intestine (A) and liver (B) tissues. Results are normalised expression ratios
793 (average of $n=6 \pm SE$) of the expression of these genes in fish fed each one of the diets containing
794 *Camelina* oil, in relation to those fed diet C0 (100% FO).

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796 **Fig. 2.** Distribution by categories of biological function of genes found to be differentially
797 expressed in the intestine between cod fed diets C0 and C66 (Welch t-test, $p<0.05$; 1.2 fold
798 change cut-off). Non-annotated genes (36% of a total of 289 features) and features corresponding
799 to the same gene are not represented.

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801

801 **Table 1**

802 Formulations (g/Kg) and proximate composition (percentage of total weight) of diets containing
 803 increasing levels of Camelina oil.

804	Component	C0	C33	C66	C100
805	¹ Fish meal	610.6	610.6	610.6	610.6
806	¹ Soya meal	150	150	150	150
807	¹ Wheat	65	65	65	65
808	² Krill meal	50	50	50	50
809	¹ Northern Fish oil	80	53.6	27.2	0
810	³ Camelina oil	0	26.4	52.8	80
811	⁴ Vitamin mix comp	10	10	10	10
812	⁵ Mineral mix (M ₂)	24	24	24	24
813	⁶ Carboxymethyl cellulose	10	10	10	10
814	⁷ Antioxidant mix	0.4	0.4	0.4	0.4
815	<u>Proximate composition</u>				
816	Protein	54.9	55.5	55.0	53.3
817	Lipid	14.2	15.3	14.6	14.3
818	Moisture	9.3	7.2	8.1	6.8
819	Ash	10.6	10.7	10.4	10.6

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 821 C0, control diet containing 100% fish oil. C33, C66 and C100, diets containing 33%, 66% and
 822 100% Camelina oil as a replacement for fish oil,
 823 ¹Ewos Ltd, Bathgate, UK. ²Aker Biomarine, Norway. ³Technology Crops International Inc.,
 824 USA. ⁴Contains g/kg diet, Vitamin A, 2500 IU; Vitamin D, 2400 IU; Vitamin E, 100; Vitamin K,
 825 10; Ascorbic acid 1000; Thiamin, 10; Riboflavin, 20; Pyridoxine, 12; Pantothenic acid, 44;
 826 Nicotinic acid, 150; Biotin, 1; Folic acid, 5; Vitamin B12, 0.02; myo-inositol, 400; Choline
 827 Chloride, 3. ⁵ Contains g/kg diet or as stated, KH₂PO₄, 22; FeSO₄.7H₂O; 1.0; ZnSO₄.7H₂O, 0.13;
 828 CuSO₄5H₂O, 12 mg/kg; MnSO₄.4H₂O, 53 mg/kg; KI, 2 mg/kg; CoSO₄.7H₂O, 2 mg/kg; α-
 829 cellulose, 0.8; ⁶Sigma-Aldrich Ltd, Gillingham, UK. ⁷ Contains g/L; Butylated hydroxyanisole,
 830 60; Propyl gallate, 60; Citric acid, 40; in propylene glycol, added at 0.4g/kg diet.

831

831 **Table 2**

832 Fatty acid compositions (percentage of weight of total fatty acids) of experimental diets.

	C0	C33	C66	C100	
837	14:0	5.5	4.6	3.6	2.5
838	16:0	13.6	12.8	11.9	10.9
839	18:0	1.9	2.1	2.5	2.7
840	Total saturates ¹	22.1	20.8	19.3	17.4
841	16:1n-7	6.3	5.4	4.2	2.1
842	18:1n-9	9.3	10.5	11.5	12.2
843	18:1n-7	2.5	2.3	2.0	1.6
844	20:1 ²	11.6	11.6	11.5	11.2
845	22:1 ³	16.3	13.3	10.0	6.6
846	24:1	0.9	0.9	0.8	0.8
847	Total monoenes ⁴	49.2	45.6	41.3	36.4
848	18:2n-6	3.5	5.7	8.6	11.6
849	20:2n-6	0.3	0.5	0.9	1.2
850	20:4n-6	0.4	0.4	0.3	0.3
851	Total n-6 ⁵	4.4	6.9	9.9	13.1
852	18:3n-3	1.2	6.4	12.6	18.7
853	18:4n-3	3.3	2.8	2.2	1.6
854	20:3n-3	0.1	0.3	0.6	0.8
855	20:4n-3	0.5	0.4	0.3	0.3
856	20:5n-3	8.4	7.2	5.8	4.6
857	22:5n-3	0.7	0.6	0.5	0.4
858	22:6n-3	9.0	8.1	6.9	6.2
859	Total n-3	23.0	26.4	28.7	32.6
860	n-3/n-6 PUFA	5.2	3.8	2.9	2.5
861	Total PUFA ⁶	28.7	33.7	39.4	46.2

864 ¹Includes 15:0, 17:0 and 20:0. ²Includes both 20:1n-9 and 20:1n-7; ³Includes both 22:1n-11 and865 22:1n-9; ⁴Includes 14:1n-5 and 16:1n-9; ⁵Includes 18:3n-6, 20:3n-6, 22:4n-6 and 22:5n-6;866 ⁶Includes C16 PUFA.

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Table 3
Primers used for RT-qPCR analyses.

Transcript	Primer sequence (5'-3')	Fragment	Ta	Accession No./Contig
<i>Δ6fad</i>	AGGCACAACCTACCAGGTGCT CTTAAGCGACCGCACGAC	117 bp	60 °C	DQ054840 ¹
<i>elovl5</i>	ACTGCCAAGACACACACAGC CTGGCGTGATGGTAGATGTG	163 bp	56 °C	GW848298 ¹
<i>elf1a</i>	GTGATCAAGAGCGTCGACAA GATGCGCATTAACCAGTCCT	189 bp	60 °C	CL5Contig2 ²
<i>rpl13</i>	TCAGGTGTCCAACCATCAGA CCTCAGTGCTGTCTCCCTTC	249 bp	60 °C	CL81Contig1 ²
<i>etf3f</i>	GCCACCGTTCTCACCTACAT TTGGGTGAGGTTGGACAAAT	186 bp	60 °C	CL224Contig1 ²
<i>tprg11</i>	TTGTGCGAGCCTCTCCTGTCT GCTCGCCGACTTAACAACCTC	207 bp	60 °C	CL553Contig1 ²
<i>rarres3</i>	ATCTACCATGGCCCAACTT ACAACGGCACAGGAACCTGTC	174 bp	60 °C	CL2455Contig1 ²
<i>rpsa</i>	GCTGACCAGAGAGGTTCTGC CACTCAGCCTGGAACCTCTC	162 bp	60 °C	CL97Contig1 ²
<i>tctp</i>	TTTTTCATCGAGGTGGAAGG CTCTCTGGCTTGGTCTCCTG	248 bp	60 °C	CL18Contig1 ²
<i>casp3</i>	AGCTGATGCAGATCATGACG TTTCTCAGCACCCGTAGTT	216 bp	60 °C	CL1129Contig1 ²
<i>tpm</i>	CCGCTTAGCGTTCAGTTAGG GCTCTGTCCAAGGCATTCTC	158 bp	60 °C	CL54Contig1 ²
<i>tekt4</i>	ATTACGCGATCCTCCAACAG CTGCAACTCCGACTTCAACA	176 bp	60 °C	CL1914Contig1 ²
<i>cdh3</i>	CCTGCCAATCCTGAAGACAT GAGGAGTTGAGGGACGACAG	152 bp	60 °C	CE2051-2005-02-15.ab1 ²
Reference genes				
<i>bact</i>	CTACGAGGGGTATGCTCTGC CTCTCAGCAGTGGTGGTGAA	123 bp	56 °C	AJ555463 ¹
<i>ubq</i> ³	GGCCGCAAAGATGCAGAT CTGGGCTCGACCTCAAGAGT	69 bp	60 °C	EX735613 ¹
<i>gapdh</i> ³	CCATGACAACCTTGGCATCGT AGGGTCCGTCCACTGTCTTCT	83 bp	60 °C	EX725566 ¹

871 ¹ GenBank Accession No (<http://www.ncbi.nlm.nih.gov/>)

872 ² Contig in microarray (based on an assembly of cod ESTs; ArrayExpress: E-TABM-1178)

873 ³ Olsvik et al. (2008)

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875 **Table 4**

876 Growth, feed efficiency and biometry of Atlantic cod fed diets containing increasing proportions
 877 of Camelina oil.

	C0	C33	C66	C100
Initial weight (g)	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.2	1.4 ± 0.0
Final weight (g)	6.4 ± 0.8	7.0 ± 1.0	8.8 ± 0.8	6.6 ± 1.4
SGR	1.8 ± 0.1	1.9 ± 0.1	2.1 ± 0.2	1.8 ± 0.2
TGC	1.0 ± 0.1	1.0 ± 0.2	1.2 ± 0.1	1.0 ± 0.2
FCR	2.2 ± 0.2	1.9 ± 0.1	1.8 ± 0.2	2.0 ± 0.1
HSI	4.8 ± 0.5	5.2 ± 0.5	5.4 ± 0.5	5.5 ± 0.3
VSI	12.5 ± 0.4	14.2 ± 0.6	14.0 ± 0.6	13.8 ± 1.1
Liver lipid (%)	45.2 ± 4.7	44.1 ± 7.5	52.7 ± 9.5	50.9 ± 6.2
Flesh lipid (%)	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.2	1.2 ± 0.0
Intestine lipid (%)	2.8 ± 0.4 ^b	3.2 ± 0.0 ^{ab}	3.5 ± 0.1 ^a	3.2 ± 0.1 ^{ab}

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879 Columns with different letters are significantly different (P < 0.05).

880

880 **Table 5**

881 Fatty acid compositions (percentage weight of total fatty acids) of cod liver after feeding diets
 882 containing increasing levels of Camelina oil.

883		C0	C33	C66	C100
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885					
886	14:0	3.7 ± 0.1 ^a	3.1 ± 0.1 ^b	2.4 ± 0.1 ^c	1.7 ± 0.0 ^d
887	16:0	12.4 ± 0.3 ^a	11.6 ± 0.3 ^{ab}	10.4 ± 0.3 ^{bc}	9.7 ± 0.9 ^c
888	18:0	2.7 ± 0.1	3.0 ± 0.1	2.9 ± 0.3	2.9 ± 0.2
889	Total saturates ¹	19.6 ± 0.3 ^a	18.3 ± 0.4 ^a	16.4 ± 0.5 ^b	15.0 ± 0.8 ^c
890	16:1n-7	6.4 ± 0.2 ^a	5.6 ± 0.1 ^b	4.4 ± 0.1 ^c	3.5 ± 0.4 ^d
891	18:1n-9	15.9 ± 0.4 ^b	16.5 ± 0.5 ^{ab}	17.1 ± 0.6 ^{ab}	17.4 ± 0.5 ^a
892	18:1n-7	4.1 ± 0.1 ^a	3.8 ± 0.1 ^a	3.2 ± 0.1 ^b	2.7 ± 0.3 ^c
893	20:1 ²	12.0 ± 0.4 ^a	11.3 ± 0.5 ^{ab}	10.7 ± 0.2 ^{bc}	10.1 ± 0.3 ^c
894	22:1 ³	8.9 ± 0.1 ^a	6.8 ± 0.4 ^b	4.8 ± 0.2 ^c	3.0 ± 0.2 ^d
895	24:1	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.2
896	Total monoenes ⁴	50.6 ± 0.4 ^a	46.7 ± 0.4 ^b	42.6 ± 0.3 ^c	39.0 ± 1.0 ^d
897	18:2n-6	3.9 ± 0.1 ^d	6.3 ± 0.1 ^c	9.3 ± 0.4 ^b	12.0 ± 0.2 ^a
898	20:2n-6	0.5 ± 0.0 ^d	0.8 ± 0.1 ^c	1.2 ± 0.1 ^b	1.6 ± 0.1 ^a
899	20:4n-6	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.1
900	Total n-6 PUFA ⁵	5.2 ± 0.1 ^d	7.8 ± 0.2 ^c	11.1 ± 0.4 ^b	14.2 ± 0.3 ^a
901	18:3n-3	1.3 ± 0.1 ^d	6.5 ± 0.2 ^c	12.7 ± 0.6 ^b	18.0 ± 0.7 ^a
902	18:4n-3	3.0 ± 0.1 ^a	2.7 ± 0.1 ^b	2.2 ± 0.1 ^c	1.6 ± 0.1 ^d
903	20:3n-3	0.2 ± 0.0 ^d	0.5 ± 0.0 ^c	0.8 ± 0.0 ^b	1.1 ± 0.0 ^a
904	20:4n-3	0.8 ± 0.1 ^a	0.6 ± 0.0 ^b	0.5 ± 0.0 ^{bc}	0.4 ± 0.0 ^c
905	20:5n-3	8.7 ± 0.2 ^a	7.5 ± 0.3 ^b	6.1 ± 0.2 ^c	4.6 ± 0.3 ^d
906	22:5n-3	0.9 ± 0.0 ^a	0.8 ± 0.0 ^a	0.6 ± 0.0 ^b	0.5 ± 0.0 ^b
907	22:6n-3	8.9 ± 0.3 ^a	7.7 ± 0.2 ^b	6.3 ± 0.5 ^c	5.2 ± 0.2 ^d
908	Total n-3 PUFA	23.7 ± 0.6 ^d	26.3 ± 0.6 ^c	29.1 ± 0.6 ^b	31.3 ± 1.1 ^a
909	n-3/n-6 PUFA	4.4 ± 0.3 ^a	3.3 ± 0.3 ^b	2.6 ± 0.4 ^{bc}	2.2 ± 0.6 ^c
910	Total PUFA ⁶	29.8 ± 0.7 ^d	35.0 ± 0.7 ^c	41.0 ± 0.7 ^b	46.0 ± 1.2 ^a
911					

912 Values are means ± SD (n = 3). Columns with different letters are significantly different (P <
 913 0.05). ¹Includes 15:0, 17:0 and 20:0. ²Includes both 20:1n-9 and 20:1n-7; ³Includes both 22:1n-11
 914 and 22:1n-9; ⁴Includes 14:1n-5 and 16:1n-9; ⁵Includes 18:3n-6, 20:3n-6, 22:4n-6 and 22:5n-6;
 915 ⁶Includes C16 PUFA.

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Table 6

Fatty acid composition (percentage weight of total fatty acids) of cod intestine after feeding diets containing increasing levels of Camelina oil.

	C0	C33	C66	C100
14:0	2.1 ± 0.6	2.5 ± 0.2	2.1 ± 0.1	1.7 ± 0.1
16:0	14.6 ± 0.4 ^a	13.4 ± 0.1 ^b	12.5 ± 0.2 ^{bc}	12.0 ± 0.7 ^c
18:0	4.0 ± 0.5	3.4 ± 0.1	3.6 ± 0.2	3.7 ± 0.3
Total saturates ¹	21.6 ± 0.2 ^a	20.2 ± 0.2 ^{ab}	18.9 ± 0.3 ^{bc}	18.4 ± 1.3 ^c
16:1n-7	3.2 ± 0.5 ^{ab}	3.6 ± 0.2 ^a	3.1 ± 0.2 ^{ab}	2.5 ± 0.2 ^b
18:1n-9	11.2 ± 0.1 ^b	11.2 ± 0.6 ^b	12.2 ± 0.5 ^{ab}	12.7 ± 0.5 ^a
18:1n-7	2.8 ± 0.2 ^a	2.7 ± 0.1 ^a	2.3 ± 0.1 ^b	1.8 ± 0.1 ^c
20:1 ²	6.8 ± 0.7 ^b	7.8 ± 0.3 ^{ab}	8.1 ± 0.1 ^a	8.2 ± 0.5 ^a
22:1 ³	4.7 ± 1.9	4.9 ± 0.8	4.0 ± 0.2	3.3 ± 0.4
24:1	2.2 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	2.0 ± 0.5
Total monoenes ⁴	31.2 ± 2.7	32.3 ± 0.8	31.7 ± 0.4	30.9 ± 1.4
18:2n-6	2.7 ± 1.0 ^d	4.5 ± 0.2 ^c	6.9 ± 0.2 ^b	9.4 ± 0.7 ^a
20:2n-6	0.5 ± 0.0 ^d	0.9 ± 0.1 ^c	1.3 ± 0.1 ^b	1.6 ± 0.0 ^a
20:4n-6	1.7 ± 0.2 ^a	1.2 ± 0.1 ^b	1.0 ± 0.1 ^b	0.9 ± 0.1 ^b
Total n-6 PUFA ⁵	5.4 ± 0.8 ^d	7.0 ± 0.1 ^c	9.6 ± 0.1 ^b	12.3 ± 0.7 ^a
18:3n-3	0.8 ± 0.3 ^d	4.1 ± 0.3 ^c	8.5 ± 0.4 ^b	12.5 ± 1.3 ^a
18:4n-3	1.2 ± 0.4	1.5 ± 0.2	1.3 ± 0.1	1.0 ± 0.1
20:3n-3	0.1 ± 0.0 ^d	0.4 ± 0.0 ^c	0.7 ± 0.0 ^b	1.0 ± 0.2 ^d
20:4n-3	0.5 ± 0.0 ^a	0.6 ± 0.0 ^a	0.5 ± 0.1 ^a	0.3 ± 0.1 ^b
20:5n-3	10.1 ± 0.3 ^a	9.9 ± 0.3 ^a	8.4 ± 0.2 ^b	6.8 ± 0.3 ^c
22:5n-3	1.2 ± 0.1 ^a	1.0 ± 0.1 ^{ab}	0.8 ± 0.1 ^{bc}	0.7 ± 0.0 ^c
22:6n-3	24.1 ± 3.9 ^a	18.9 ± 1.4 ^{ab}	16.3 ± 0.5 ^b	13.9 ± 0.9 ^b
Total n-3 PUFA	38.0 ± 3.7	36.5 ± 1.3	36.5 ± 0.3	36.3 ± 1.5
n-3/n-6 PUFA	7.2 ± 1.8 ^a	5.2 ± 0.2 ^a	3.8 ± 0.1 ^b	2.9 ± 0.1 ^c
Total PUFA ⁶	44.1 ± 2.9 ^b	44.2 ± 1.3 ^{ab}	46.8 ± 0.3 ^{ab}	49.0 ± 2.1 ^a

Values are means ± SD (n = 3). Columns with different letters are significantly different (P < 0.05). ¹Includes 15:0, 17:0 and 20:0. ²Includes both 20:1n-9 and 20:1n-7; ³Includes both 22:1n-11 and 22:1n-9; ⁴Includes 14:1n-5 and 16:1n-9; ⁵Includes 18:3n-6, 20:3n-6, 22:4n-6 and 22:5n-6; ⁶Includes C16 PUFA.

957 **Table 7**

958 Fatty acid compositions (percentage weight of total fatty acids) of cod muscle after feeding diets
 959 containing increasing levels of Camelina oil.

	C0	C33	C66	C100
963 14:0	1.6 ± 0.0 ^a	1.7 ± 0.4 ^a	1.5 ± 0.1 ^{ab}	1.0 ± 0.2 ^b
964 16:0	15.8 ± 0.5 ^a	14.5 ± 1.2 ^{ab}	14.9 ± 1.0 ^{ab}	13.2 ± 0.4 ^b
965 18:0	3.3 ± 0.1	3.3 ± 0.3	4.1 ± 0.8	3.6 ± 0.2
966 Total saturates ¹	21.7 ± 0.4 ^a	19.8 ± 1.0 ^{ab}	20.4 ± 1.5 ^{ab}	18.2 ± 0.6 ^b
967 16:1n-7	2.8 ± 0.0 ^a	2.6 ± 0.3 ^a	2.1 ± 0.1 ^b	1.6 ± 0.2 ^c
968 18:1n-9	11.4 ± 1.4	11.3 ± 0.9	11.3 ± 0.7	11.2 ± 0.7
969 18:1n-7	3.2 ± 0.2 ^a	2.8 ± 0.1 ^b	2.5 ± 0.1 ^{bc}	2.2 ± 0.1 ^c
970 20:1 ²	4.5 ± 0.1	5.1 ± 1.0	4.5 ± 0.2	4.5 ± 0.2
971 22:1 ³	2.3 ± 0.2 ^{ab}	2.6 ± 0.7 ^a	1.7 ± 0.5 ^{ab}	1.4 ± 0.1 ^b
972 24:1	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
973 Total monoenes ⁴	26.7 ± 2.2	27.0 ± 3.5	24.5 ± 1.3	22.7 ± 1.3
974 18:2n-6	3.0 ± 0.1 ^d	4.8 ± 0.3 ^c	6.6 ± 0.4 ^b	8.8 ± 0.3 ^a
975 20:2n-6	0.4 ± 0.0 ^d	0.7 ± 0.0 ^c	1.0 ± 0.1 ^b	1.4 ± 0.0 ^a
976 20:4n-6	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.0	1.1 ± 0.1
977 Total n-6 PUFA ⁵	5.0 ± 0.2 ^d	7.3 ± 0.2 ^c	9.3 ± 0.4 ^b	11.9 ± 0.3 ^a
978 18:3n-3	0.9 ± 0.0 ^d	4.3 ± 0.7 ^c	7.5 ± 0.4 ^b	11.0 ± 0.7 ^a
979 18:4n-3	1.5 ± 0.2 ^a	1.4 ± 0.4 ^{ab}	1.1 ± 0.1 ^{ab}	0.8 ± 0.1 ^b
980 20:3n-3	0.1 ± 0.0 ^d	0.4 ± 0.0 ^c	0.7 ± 0.0 ^b	1.0 ± 0.0 ^a
981 20:4n-3	0.7 ± 0.0 ^a	0.6 ± 0.0 ^{ab}	0.5 ± 0.0 ^{ab}	0.4 ± 0.0 ^b
982 20:5n-3	14.5 ± 1.6 ^a	13.5 ± 0.9 ^a	11.9 ± 0.4 ^{ab}	10.8 ± 0.7 ^b
983 22:5n-3	1.6 ± 0.2	1.5 ± 0.2	1.3 ± 0.0	1.3 ± 0.1
984 22:6n-3	25.5 ± 3.8	23.4 ± 2.7	21.7 ± 1.0	21.3 ± 1.4
985 Total n-3 PUFA	43.1 ± 4.4	44.9 ± 3.3	44.7 ± 1.0	46.6 ± 1.8
986 n-3/n-6 PUFA	8.6 ± 2.1 ^a	6.2 ± 1.1 ^{ab}	4.8 ± 0.7 ^b	3.9 ± 0.8 ^b
987 Total PUFA ⁶	50.3 ± 5.1	52.6 ± 3.2	54.3 ± 1.1	58.6 ± 1.8

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 990 Values are means ± SD (n = 3). Columns with different letters are significantly different (P <
 991 0.05). ¹Includes 15:0, 17:0 and 20:0. ²Includes both 20:1n-9 and 20:1n-7; ³Includes both 22:1n-11
 992 and 22:1n-9; ⁴Includes 14:1n-5 and 16:1n-9; ⁵Includes 18:3n-6, 20:3n-6, 22:4n-6 and 22:5n-6;
 993 ⁶Includes C16 PUFA.

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994 **Table 8**

995 Transcripts corresponding to the top 100 most significant features exhibiting differential
 996 expression in cod intestine between diets C0 and C66 (fold change given as C66 / C0). Annotated
 997 features (66%) are arranged by categories of biological function and, within these, by decreasing
 998 significance (p-value, assessed by Welch t-test). Indicated is also the percentage of gene
 999 distribution by functional categories, after removing features representing the same gene.

Probe No.	Accession No.	SwissProt Annotation	Fold change (C66/C0)	p-value
<i>Lipid metabolism (5%)</i>				
CE2148	GO377376	Apolipoprotein A-IV	1.48	0.0018
CPY1052	GO387010	Apolipoprotein A-IV	1.50	0.0034
CPY442	GO387753	Apolipoprotein A-IV	1.53	0.0048
CPY1606	GO387357	Apolipoprotein A-IV	1.51	0.0049
CPY1409	GO387232	Apolipoprotein A-IV	1.54	0.0057
CPY465	GW857638	Apolipoprotein A-IV	1.50	0.0057
CHY224	GO383431	Apolipoprotein A-IV	1.59	0.0057
CPY1627	GO387370	Apolipoprotein A-IV	1.45	0.0064
CLE718	GW849678	Lipovitellin-2	- 1.21	0.0098
CE1608	GW843824	Apolipoprotein A-IV	1.53	0.0075
CPY225	GO387561	Apolipoprotein A-IV	1.48	0.0104
CPY205	GO387527	Apolipoprotein A-IV	1.40	0.0141
CTA293	N/A	Apolipoprotein A-IV	1.51	0.0161
<i>Energy metabolism (5%)</i>				
CHO292	GO382345	Creatine kinase B-type	1.62	0.0104
CE1110	GO376622	Creatine kinase B-type	1.59	0.0138
CTA327	GO390332	Creatine kinase B-type	1.37	0.0146
CE2531	GW843201	Creatine kinase M-type	1.28	0.0152
CTE1039	GO390859	Creatine kinase B-type	1.51	0.0174
CTE1378	GW861048	Creatine kinase B-type	1.60	0.0178
<i>Proteolysis (5%)</i>				
CLE650	GW849656	Carboxypeptidase N polypeptide 1	- 1.84	0.0052
CE1303	GO376762	F-box and leucine-rich repeat protein 18	- 1.21	0.0082
<i>Protein folding (5%)</i>				
CSMI546	GO388637	Peptidyl-prolyl cis-trans isomerase FKBP5	- 1.24	0.0022
CPY173	GO387434	Heat shock cognate 70 kDa protein	- 1.24	0.0081
<i>Regulation of transcription (5%)</i>				
NM814	N/A	Transcription factor IIIA	- 1.49	0.0131
NM115	N/A	Protein strawberry notch homolog 1	1.23	0.0172
<i>Translation (25%)</i>				
CE2965	GO378001	40S ribosomal protein S15a	- 1.23	0.0006
CE2958	GW843393	60S ribosomal protein L13	- 1.33	0.0011
CE2753	GW843313	Elongation factor 1-alpha	- 1.22	0.0038
CE102	GO376549	60S ribosomal protein L27a	- 1.26	0.0055
CE916	GO378560	60S ribosomal protein L27	- 1.22	0.0068

CE1965	GW843877	60S ribosomal protein L38	- 1.23	0.0077
CHO1803	GO381721	Elongation factor 1-beta	- 1.20	0.0098
CE2064	GW842964	60S ribosomal protein L6	- 1.22	0.0111
CHY916	GO383943	Eukaryotic translation initiation factor 3 subunit F	- 1.27	0.0115
CLE430	GO384373	Polyadenylate-binding protein 1	- 1.24	0.0137
CSMI161	GO388318	60S ribosomal protein L35	- 1.22	0.0154
CE2959	GO377996	40S ribosomal protein S17	- 1.21	0.0166

Cell proliferation, differentiation and apoptosis (20%)

CTE684	GW861483	Tumor protein p63 regulated 1	1.22	0.0007
CTE495	GO391915	40S ribosomal protein SA	2.03	0.0011
NM303	N/A	Tumor suppressor candidate 2	- 1.22	0.0017
NM253	N/A	Proline-rich protein BCA3-like	- 1.37	0.0032
CHJ1269	GO380651	Translationally-controlled tumor protein homolog	- 1.26	0.0040
NM449	N/A	origin recognition complex, subunit 2-like	2.32	0.0096
CHJ1272	GO380654	Translationally-controlled tumor protein homolog	- 1.23	0.0104
CHO1630	GW846809	Caspase 3	1.21	0.0106
CTA632	GO390541	Translationally-controlled tumor protein homolog	- 1.22	0.0107
CTA484	GO390436	Translationally-controlled tumor protein homolog	- 1.23	0.0108
CPY269	GO387613	Retinoic acid receptor responder protein 3	2.38	0.0110
CSMI714	GW860190	Translationally-controlled tumor protein homolog	- 1.24	0.0111
COV1041	GW855020	Voltage-dependent anion-selective channel protein 1	1.22	0.0135
CE1707	GO377048	Translationally-controlled tumor protein homolog	- 1.27	0.0161
CTE1915	GO391572	Caspase-3 subunit p12	1.38	0.0176

Structural molecule (9%)

CTE1047	GO390866	Tektin-4	1.26	0.0067
CE2716	GO377798	Tropomyosin alpha-1 chain	1.53	0.0110
CE2051	GW842958	Cadherin 3, type 1	- 1.20	0.0120
COV757	GW855647	Claudin 4	- 1.33	0.0124

Immune response (9%)

CPY1238	GO387126	Viperin	1.37	0.0159
CTE1043	GO390863	Barrier-to-autointegration factor	1.21	0.0159
CSMI679	GO388738	Interferon-induced protein 44	1.60	0.0166
CHO1910	GW846481	High affinity immunoglobulin epsilon receptor subunit gamma	- 1.38	0.0177

Miscellaneous or unknown function (14%)

CE2960	GW843396	Autophagy-related protein 16-1	- 1.22	0.0052
CTE324	GO391798	Endoribonuclease	1.65	0.0124
CLE567	GO384459	Processed zona pellucida sperm-binding protein 3	- 1.37	0.0129
NM762	N/A	Omega-amidase NIT2-B	1.61	0.0140
CHO673	GO382599	Hemoglobin beta 1 chain	- 1.68	0.0153
CTE829	GW861558	Tetraspanin-14	1.21	0.0175

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 1006 **Table 9**
 1007 GO analysis results showing significant enrichment, at an adjusted p-value of 1.825E-4, of
 1008 features for the molecular function category GO:0003779; actin binding (14.9% count in
 1009 selection; 1.8% count in total).

Probe No.	Accession No.	SwissProt Annotation	Fold change (C66/C0)
CE1462	GW842770	Myosin heavy chain	1.38
CE2112	GO377351	Tropomyosin 1 (alpha)	1.42
CE2636	GW843270	Actin, alpha cardiac muscle 1	- 1.21
CE2716	GO377798	Tropomyosin 1 (alpha)	1.53
CE457	GO378247	Tropomyosin 1 (alpha)	1.43
CE551	GO378302	Tropomyosin 1 (alpha)	1.46
CHJ685	GO381113	Actin, alpha cardiac muscle 1	- 1.26
CHO2151	GO382012	Actin, alpha cardiac muscle 1	- 1.21
CHY703	GO383785	Actin, alpha cardiac muscle 1	- 1.23
COV1757	GW855413	Cofilin 1 (non-muscle)	1.82
COV702	GW855592	Ectodermal-neural cortex (with BTB-like domain)	1.80
CSMI989	GW859865	Actin, alpha cardiac muscle 1	- 1.21
CTA516	GO390453	Tropomyosin 1 (alpha)	1.41
CTE369	GO391832	Transgelin	1.57
CTE807	GO392129	Transgelin	1.60

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 1012 **Table 10**
 1013 Validation, by RT-qPCR, of selected genes found to be significantly different when expressed in
 1014 the microarray analysis. Indicated is the fold change (FC) between expression levels in diets C66
 1015 and C0 and p-value (assessed by REST2008) of the RT-qPCR analysis. Asterisks indicate fold
 1016 changes, assessed by RT-qPCR, that are statistically significant.

Genes	Microarray	RT-qPCR	
	FC (C66/C0)	FC (C66/C0)	p-value
Elongation factor 1-alpha (<i>elf1a</i>)	-1.22	1.18	0.095
60S ribosomal protein L13 (<i>rpl13</i>)	-1.33	-1.00	0.983
Eukaryotic translation initiation factor 3 subunit F (<i>etf3f</i>)	-1.27	-1.00	0.985
Tumor protein p63 regulated 1-Like (<i>tprg1l</i>)	1.22	1.14	0.256
Retinoic acid receptor responder protein 3 (<i>rarres3</i>)	2.38	*7.81	0.014
40S ribosomal protein SA (<i>rpsa</i>)	2.03	1.03	0.658
Translationally-controlled tumor protein homolog (<i>tctp</i>)	1.26	-1.23	0.136
Caspase 3 (<i>casp3</i>)	1.21	*1.89	0.000
Tropomyosin alpha-1 chain (<i>tpm</i>)	1.53	*1.55	0.036
Tektin-4 (<i>tekt4</i>)	1.26	-1.33	0.341
Cadherin 3 type 1 (<i>cdh3</i>)	-1.20	1.01	0.921

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