

1 **Oil from transgenic *Camelina sativa* containing over 25 % n-3**
2 **long-chain polyunsaturated fatty acids as the major lipid source**
3 **in feed for Atlantic salmon (*Salmo salar*)**

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21

22 **Abstract**

23 Facing a bottleneck in the growth of aquaculture, and a gap in the supply and demand of the
24 highly beneficial omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA),
25 sustainable alternatives to traditional marine-based feeds are required. Therefore, in the
26 present trial, a novel oil obtained from a genetically engineered oilseed crop, *Camelina*
27 *sativa*, that supplied over 25 % n-3 LC-PUFA was tested as a sole dietary added lipid source
28 in Atlantic salmon (*Salmo salar*) feed. Three groups of fish were fed for 12 weeks three
29 experimental diets with the same basal composition and containing 20 % added oil supplied
30 by either a blend of fish oil and rapeseed oil (1:3) (COM) reflecting current commercial
31 formulations, wild-type *Camelina* oil (WCO) or the novel transgenic *Camelina* oil (TCO).
32 There were no negative effects on the growth, survival rate or health of the fish. The whole
33 fish and flesh n-3 LC-PUFA levels were highest in fish fed TCO with levels almost and more
34 than 2-fold higher compared to those of fish fed the COM and WCO diets, respectively. Diet
35 TCO had no negative impacts on the evaluated immune and physiological parameters of head
36 kidney monocytes. The transcriptomic responses of liver and mid-intestine showed only mild
37 effects on metabolism genes. Overall, the results clearly indicated that the oil from transgenic
38 *Camelina* was highly efficient in supplying n-3 LC-PUFA providing levels double that
39 obtained with a current commercial standard, and similar to those a decade ago prior to
40 substantial dietary fishmeal and oil replacement.

41

42 **1. Introduction**

43 Fish is recognized as a nutritious and healthy food in part due to the fact that they are
44 naturally rich in the health promoting omega-3 (n-3) long-chain polyunsaturated fatty acids
45 (LC-PUFA), eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3;
46 DHA)^(1,2). Much of the fish consumed today is farmed⁽³⁾, many reared on feeds containing
47 high levels of more sustainable raw materials such as plant meals and oils of agricultural
48 origin. Such terrestrial ingredients are devoid of n-3 LC-PUFA and, therefore, the use of
49 these plant ingredients translates into reduced levels of beneficial n-3 LC-PUFA in the fillet
50 of the fish, as has been documented in farmed Atlantic salmon (*Salmo salar*) from 2006
51 onwards⁽⁴⁾. This means that larger or more portions of farmed fish must be consumed in order
52 to obtain the recommended EPA and DHA intake suggested by different agencies (e.g. 5 and
53 6).

54 Oilseed crops are major agricultural products with a highly organised and well-
55 established infrastructure for their cultivation, harvest, and processing, as well as distribution,
56 marketing and utilisation of vegetable oils (VO)⁽⁷⁾. Thus, VO have been the main alternatives
57 to dietary fish oil (FO) in aquafeeds and, while these oils are devoid of n-3 LC-PUFA, they
58 can be rich in shorter chain PUFA such as 18:3n-3. This opens up the possibility for genetic
59 modification to enable the desaturation and elongation of 18:3n-3 to EPA and DHA.
60 Recently, transgenic *Camelina sativa* crops, capable of producing either EPA or EPA and
61 DHA in their seeds, have been developed^(8,9). Two such oils have been evaluated as
62 replacements for dietary FO in feeds for Atlantic salmon (*Salmo salar*)⁽¹⁰⁻¹³⁾ and gilthead sea
63 bream (*Sparus aurata*)⁽¹⁴⁾ showing promising results, enabling good fish growth and
64 deposition of n-3 LC-PUFA in tissues including flesh. In contrast, feeds formulated with high
65 levels of conventional plant ingredients, including VO, can adversely affect fish health
66 through impacts on immune and stress responses⁽¹⁵⁾. A well-balanced dietary fatty acid
67 profile, particularly in terms of LC-PUFA, is a determinant of animal health and welfare and,
68 thus, the replacement of FO by VO can alter this balance. In previous trials, no major impacts
69 on fish health were observed after feeding fish with oil from transgenic *Camelina* supplying
70 12% EPA+DHA with, specifically, no detrimental effects on immune function⁽¹²⁾ or response
71 after an environmental stress⁽¹³⁾. However, the outcome of stress responses in fish fed VO
72 will depend on the intensity and type of the stressor as well as its duration⁽¹⁶⁾ and, therefore,
73 different stressors may have different effects on particular immune or inflammatory
74 responses.

75 In previous studies, oils from transgenic Camelina supplying either 20% EPA or 6% each
76 of EPA and DHA were tested in Atlantic salmon against “gold standard” feeds formulated
77 with high fishmeal and FO⁽¹⁰⁻¹²⁾. The oil supplying 6% each of EPA and DHA was
78 subsequently tested in Atlantic salmon against feeds formulated with lower fishmeal and
79 FO⁽¹³⁾. In the present study, a third generation oil supplying levels of EPA, DHA and total n-3
80 LC-PUFA greater than most FO was tested in feeds for Atlantic salmon with even lower
81 levels of fishmeal and FO reflecting current commercial salmon feeds. Thus, triplicate groups
82 of Atlantic salmon were fed one of three experimental diets formulated with 30 % fishmeal
83 and 43 % plant meals that contained either a mix of FO and rapeseed oil (1:3) (diet COM),
84 wild-type Camelina oil (diet WCO) or transgenic Camelina oil (diet TCO) in order to
85 evaluate the capability of the new transgenic oil to restore EPA and DHA levels in farmed
86 salmon to those of a decade ago before the development of sustainable feeds⁽⁴⁾. Therefore,
87 feeds were formulated with lower levels of fishmeal (30 % vs. 49 or 35 % previously) and, in
88 the reference (COM) diet, FO (5 % vs. 17.5 or 10 % previously) to reflect current feed
89 formulations and n-3 LC-PUFA levels. The impacts of diet TCO on fish growth performance
90 and tissue fatty acid composition were assessed, and influences on fish metabolism were
91 determined by investigating gene expression via liver and pyloric caeca (PC) transcriptomic
92 responses. Additionally, at the end of the experimental trial an *ex vivo* challenge was
93 performed on head kidney monocytes in order to assess potential impacts on the immune
94 response.

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98 **2. Materials and Methods**

99 *2.1 Diets and feeding trial*

100 The feeding trial was carried out at the facilities of the Institute of Marine Research (Matre,
101 Norway) from December 2016 to February 2017. A total of 297 post-smolt Atlantic salmon
102 (initial weight 133.0 ± 1.6) were distributed into nine 400 L squared flow-through seawater
103 tanks (33 fish per tank) and fed twice daily with one of the three feeds in triplicate for 12
104 weeks. Fish were fed to satiety+ to ensure that feed availability did not restrict growth. Water
105 temperature was maintained at 13 °C throughout the experimental period. The three feeds
106 were formulated to be essentially isonitrogenous and isolipidic and produced by vacuum
107 coating identical basal extruded pellets with either FO/rapeseed oil (COM), wild-type
108 Camelina oil (WCO) or high EPA+DHA-Camelina oil (TCO) (Table 1). Fatty acid profiles of
109 the constituent oils used in the present trial (Supplementary Table 1) resulted in percentages
110 of both EPA and DHA in diet TCO that were almost 3-fold higher than in the other two
111 dietary treatments (Table 1). The proportions of n-6 and total PUFA were also higher in the
112 TCO diet whereas this feed showed the lowest levels of monounsaturated fatty acids. Yttrium
113 oxide (Y_2O_3 , >99.9 % purity, Strem chemicals, Bischheim, France) was added to the feeds as
114 an inert marker in order to determine lipid and fatty acid digestibility. All procedures were
115 approved by the Norwegian Animal Experiment Committee (Forsøksdyrutvalget),
116 experiment ID.8089.

117

118 *2.2 Sample collection*

119 At the end of the feeding trial 13 fish per tank were killed by an overdose of metacaine
120 sulphonate ($> 150 \text{ mg l}^{-1}$, FINQUEL vet., ScanAqua AS, Årnes, Norway). Fish were
121 measured (weight and length) and blood from 4 fish per tank collected via the caudal vein
122 using 1 ml heparinised syringes fitted with 20G needles, and whole blood used for
123 haematocrit determination. Two whole fish per tank were frozen (-20 °C) for biochemical
124 proximate composition analysis, and liver and pyloric caeca were collected from 4 fish per
125 tank were stabilized in RNA Later (Sigma, Poole, UK) and frozen at -20 °C until further
126 RNA extraction. After sampling the fish remaining in the tanks continued to be fed the same
127 feeds as prior to sampling for a further three days at which point faeces were collected and
128 pooled by tank ($n = 3$). Briefly, fish were killed by an overdose of metacaine sulphonate as

129 above and faecal samples collected from the hind gut region by gently squeezing the ventral
130 abdominal area⁽¹⁷⁾. Faeces were initially stored at -20 °C and freeze dried prior to analyses.

131 *2.3 Proximate composition*

132 Feeds were ground and whole fish were pooled per tank (n = 3 per dietary treatment) and
133 homogenised before determination of proximate composition according to standard
134 procedures⁽¹⁸⁾. Moisture contents were obtained after drying in an oven for 24 h at 110 °C.
135 Ash content was determined after incinerating the samples at a temperature of 600 °C for 20
136 h. Crude protein content was measured by determining nitrogen content (N x 6.25) using
137 automated Kjeldahl analysis (Tecator Kjeltac Auto 1030 analyzer, Foss, Warrington, UK)
138 and crude lipid determined gravimetrically after Soxhlet lipid extraction with petroleum ether
139 (Fisher Scientific UK, Loughborough) using a Tecator Soxtec system 2050 Auto Extraction
140 apparatus (Foss, Warrington, UK).

141 *2.4 Calculations*

142 Biometric parameters were estimated as follows: Fulton's condition factor (k) = 100 *
143 (W/L³), where W is the final weight (g) and L is the total length (cm). Specific growth rate
144 (SGR) = 100 * (lnWo - ln Wf) * D⁻¹, where Wo and Wf are the initial and final weights
145 (tanks means, n = 3), respectively, and D represents the number of feeding days.

146 *2.5 Lipid content and fatty acid composition*

147 Total lipid was extracted from feeds, faeces and whole fish, and flesh, liver, head kidney,
148 midgut, and hindgut homogenates prepared from three pooled fish per tank (n = 3 per
149 treatment) by homogenising approximately 1 g samples in chloroform/methanol (2:1, v/v)
150 using an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK), with content
151 determined gravimetrically⁽¹⁹⁾. Fatty acid methyl esters (FAME) were prepared from total
152 lipid by acid-catalysed transesterification at 50 °C for 16 h⁽²⁰⁾, and FAME extracted and
153 quantified by a gas chromatography (AutoSystem XL, Perkin Elmer, Waltham, MA) with
154 Total Chrom Version 6.3.1 software. The GC was equipped with a fused silica capillary
155 column coated with chemically-bonded polyethylene glycol (CP-Wax 52CB, 25 m x 0.25
156 mm i.d; Varian, Palo Alto, CA), auto-injector (1 µl, inlet temperature 250 °C) and a flame
157 ionisation detector (FID, 280 °C). Helium was the carrier gas and the oven temperature
158 program was 90 °C for 1 min, increased to 150 °C at 30 min⁻¹ and finally raised to 225 °C at 3

159 °C min⁻¹ and held for 7 min.. Individual methyl esters were identified by comparison with
160 known standards and a well-characterised fish oil, and also by reference to published data⁽²¹⁾.

161 *2.6 Digestibility calculation:*

162 The apparent digestibility coefficients (ADC) of lipid and selected fatty acids were calculated
163 as: $100 - [100 \times (Y_2O_3 \text{ concentration in feed} / Y_2O_3 \text{ concentration in faeces}) \times (\text{lipid or fatty}$
164 $\text{acid concentration in faeces} / \text{lipid or fatty acid concentration in feed})]$. The concentration of
165 individual fatty acids in diets and faeces were calculated based on the relative proportion of
166 each fatty acid compared with a known amount of internal standard (17:0) added and the total
167 lipid content determined in the samples.

168 *2.7 Isolation and primary culture of head kidney monocytes/macrophages*

169 Four fish per tank were killed by an overdose of metacaine sulphonate (as above) and head
170 kidneys removed, pooled (n = 2 per tank) and transferred to Leibovitz L-15 medium (Sigma,
171 Oslo, Norway) supplemented with 10 U ml⁻¹ heparin, 1.8 mM glucose, 10 mM HEPES, 20 U
172 Penicillin ml⁻¹, 20 µg Streptomycin ml⁻¹ and 0.05 µg Amphotericin B ml⁻¹. Head kidneys
173 were carefully sieved through a 100 µm nylon cell strainer and the cell suspension loaded
174 onto discontinuous 36 % / 54 % Percoll (GE Healthcare, Oslo, Norway) gradients followed
175 by centrifugation at 400 g for 30 min at 4 °C. The interface was collected and washed twice
176 with L-15 medium (without heparin) followed by centrifugation at 400 g for 5 min at 4 °C.
177 Finally, cells were diluted in L-15 medium and seeded at a density of 1×10^7 cells per well in
178 96-well or 6-well culture plates (Nunc, Roskilde, Denmark) in aliquots of 50 µl well⁻¹ or 1 ml
179 well⁻¹, respectively. The culture plates were incubated at 12 °C overnight and the cells
180 washed twice in 50 ml L-15 and incubated in L-15 for further studies.

181 *2.8 LPS challenge*

182 Isolated macrophage cultures (6-well plates; n = 6) were stimulated with 20 µg ml⁻¹ crude *E.*
183 *coli* LPS (0111:B4; Sigma Aldrich, Oslo, Norway). Samples were collected prior to the
184 addition of LPS (0 h) and after incubation at 12 °C for sequential sampling points (6 and 24
185 h) cells were harvested by adding 1 ml of TRI Reagent[®] (Sigma, Oslo, Norway) lysis buffer
186 and stored at -70 °C until RNA extraction.

187 *2.9 Respiratory burst activity*

188 Respiratory burst activity was determined by the reduction of nitroblue tetrazolium (NBT) to
189 formazan^(22,23) Cells were incubated in L-15 medium supplemented with 1 mg ml⁻¹ NBT and
190 1 µg ml⁻¹ phorbol myristate acetate (PMA) for 30 min at 12 °C. Cells were then washed twice
191 with pre-warmed phosphate-buffered saline (PBS) and fixed in absolute methanol for 10 min,
192 washed once with 70 % methanol to remove extracellular formazan, air-dried and then
193 intracellular formazan was dissolved in 120 µl 2 M KOH and 140 µl DMSO. Absorbance
194 was measured in a microplate reader at 630 nm using a KOH/DMSO blank. The mean
195 number of cells, obtained from at least three wells was determined by counting nuclei after
196 removal of medium and addition of 100 µl lysis buffer containing 0.1 M citric acid, 1 %
197 Tween 20 and 0.05 % crystal violet. Results were presented as absorbance per 10⁵ cells.

198 *2.10 Phagocytosis*

199 Phagocytic activity was determined by a modification of the method of Pulsford et al.⁽²⁴⁾
200 using opsonised yeast as engulfed material. For opsonisation, yeast was sterilized in boiling
201 water and, after several washes with PBS, yeast were incubated overnight with foetal bovine
202 serum (FBS) at room temperature⁽²⁵⁾. Head kidney cells were mixed with opsonised yeast to
203 obtain an approximate ratio of 10:1 yeast to kidney cells. Phagocytosis proceeded for 1 h at
204 26 °C, and was then terminated by washing with ice-cold PBS. Phagocytosis was evaluated
205 by observation by inverted light microscopy⁽²⁶⁾. At least one hundred cells were counted and
206 phagocytosis was expressed as Phagocytosis percentage (PP), which was calculated:

$$PP = \left(\frac{\text{no of cells ingesting yeasts}}{\text{no of adherent cells observed}} \right) \times \left(\frac{\text{no of yeast ingested}}{\text{no of adherent cells observed}} \right)$$

207

208 *2.11 RNA extraction and cDNA synthesis*

209 Individual liver and pyloric caeca samples from eighteen fish per dietary treatment were
210 homogenised in 1 ml of TriReagent[®] (Sigma-Aldrich, Dorset, UK) to extract total RNA
211 following the producer's protocol. Quantity and quality of RNA was determined by
212 spectrophotometry using a Nanodrop ND-1000 (Labtech Int., East Sussex, UK). The same
213 protocol was used to extract RNA from macrophage samples. cDNA was synthesised using a
214 high capacity reverse transcription kit utilising 2 µg of total RNA and random primers in a
215 total reaction volume of 20 µl following the manufacturer's protocol (Applied Biosystems,

216 Warrington, UK). The samples were pooled to obtain n = 6 per dietary treatment. A dilution
217 of 1:20 was applied to the resulting cDNA using milliQ water.

218 *2.12 Liver and pyloric caeca transcriptome - microarray hybridisations and image analysis*

219 Transcriptomic analysis of liver and pyloric caeca was performed using a custom-designed 4
220 x 44 k Atlantic salmon oligo microarray (Agilent Technologies, Wokingham, UK;
221 ArrayExpress accession no. A-MEXP-2065). The salmon microarray and laboratory
222 procedures utilised have been widely used and validated in many previous studies^(10-11; 27-30).
223 Replicate RNA samples were amplified using TargetAmp™ 1-Round Aminoallyl-aRNA
224 Amplification Kit, (Epicentre Technologies Corporation, Madison, Wisconsin, USA)
225 following recommended procedures. Aminoallyl-amplified RNA (aRNA) samples were
226 labelled with Cy3 dye (GE HealthCare Life Sciences, Buckinghamshire, UK) while a pool of
227 all aRNA samples was labelled with Cy5 dye (GE HealthCare Life Sciences) and used as a
228 common reference in a dual-label common reference design, and finally hybridised. Scanning
229 was performed using a GenePix 4200 AL Scanner (Molecular Devices (UK) Ltd.,
230 Wokingham, UK), and the resulting images analysed with Agilent Feature Extraction
231 Software v.9.5 (Agilent Technologies) to extract intensity values and identify features.
232 Features considered outliers (i.e., defined as those probes whose background intensity was
233 between the 0.05th and 99.95th percentile of the distribution) in two or more replicates within
234 at least one treatment were excluded from further analyses. Additionally, features
235 consistently expressed just above background noise (defined as those features whose intensity
236 was lower than the 5th percentile of the distribution in 75 % or more of the analysed samples)
237 were also removed. The full protocol for microarray and data analysis has been reported
238 previously⁽¹⁰⁾. The full data set supporting the results is available in MIAME-compliant
239 format in the ArrayExpress repository under accession number E-MTAB-6228.

240 *2.13 Quantitative PCR gene expression*

241 Gene expression levels were determined by real-time quantitative RT-PCR in liver, pyloric
242 caeca and macrophages as described previously⁽³¹⁾. Genes involved in LC-PUFA biosynthesis
243 (*fads2d6*, *delta-6 fatty acyl desaturase*; *fads2d5*, *delta-5 fatty acyl desaturase*; *elovl2*, *fatty*
244 *acyl elongase 2*; *elovl5a*, *fatty acyl elongase 5 isoform a*; *elovl5b*, *fatty acyl elongase isoform*
245 *b*) were measured in liver and pyloric caeca whereas genes involved in inflammation (*il1b*,
246 *interleukin 1 beta*; *cox2*, *cyclooxygenase 2*, *inos*, *inducible nitric oxide synthase* and *tnfa*,
247 *tumor necrosis factor alpha*;) and antibacterial activity (*cath*, *cathelicidin* and *hepc*, *hepcidin*)

248 were measured in macrophages (Supplementary Table 2). Results were normalised using
249 reference genes, *cofilin 2 (cfl2)*, *elongation factor 1 alpha (ef1a)* and *ribosomal protein L2*
250 (*rpl2*). Primers were designed using Primer 3 in regions that included the microarray
251 probes⁽³²⁾. Quantitative PCR was performed using a Biometra TOptical Thermocycler
252 (Analytik Jena, Goettingen, Germany) in 96-well plates in duplicate 20 µl reaction volumes
253 containing 10 µl of Luminaris Color HiGreen qPCR Master Mix (Thermo Scientific), 1 µl of
254 primer corresponding to the analysed gene (10 pmol), 3 µl of molecular biology grade water
255 and 5 µl of cDNA, with the exception of the reference genes, which were determined using 2
256 µl of cDNA. In addition, amplifications were carried out with a systematic negative control
257 (NTC-no template control) containing no cDNA. Standard amplification parameters
258 contained an UDG pre-treatment at 50 °C for 2 min, an initial activation step at 95 °C for 10
259 min, followed by 35 cycles: 15 s at 95 °C, 30 s at the annealing T_m and 30 s at 72 °C.

260 2.12 Statistical Analysis:

261 All data are means ± S.D. (n = 3) unless otherwise specified. Percentage data were subjected
262 to arcsin square-root transformation prior to statistical analyses. Data were tested for
263 normality and homogeneity of variances with Levene's test prior to one-way analysis of
264 variance (ANOVA) followed by a Tukey and post-hoc test. All statistical analyses including
265 the gene expression results were performed using SPSS software (IBM SPSS Statistics 23;
266 SPSS Inc., Chicago, IL, USA).

267

268 3. Results

269 3.1 Fish growth performance and biochemical composition

270 No significant differences were observed between the three dietary groups at the end of the
271 feeding period in any of the evaluated growth or biometric parameters (Table 2). There was a
272 100 % survival rate and no presence of disease was observed. Fish fed diet TCO had a lower
273 lipid content than fish fed the COM and WCO diets, but there were no differences in protein,
274 ash or dry matter contents of whole fish (Table 2).

275

276 3.2 Lipid and fatty acid digestibility

277 Differences in apparent digestibility coefficient (ADC) of dietary lipid were observed among
278 the dietary treatments with TCO showing the lowest value (Table 3). The ADCs of saturated

279 and monounsaturated fatty acids varied between about 87 - 94 % and 91 – 98 %, respectively,
280 with the rank order generally being COM > WCO > TCO (Table 3). The ADC for n-6, n-3
281 and total PUFA were all 97 - 98 % with few significant differences between feeds although
282 the diet WCO showed the lowest ADC for EPA, DHA, 22:5n-3 (docosapentaenoic acid,
283 DPA) and 20:4n-6 (arachidonic acid, ARA). The COM diet showed the lowest ADC for
284 20:3n-3, probably reflecting the very low dietary content of this fatty acid (Table 1).

285

286 *3.3 Lipid contents and fatty acid compositions of whole fish and tissues*

287 The lower lipid content of whole fish fed diet TCO compared to fish fed COM (Table 2) was
288 reflected in the lipid content of flesh (whole muscle) although there was no significant
289 difference to fish fed WCO (Table 4). In contrast, diet had no effect on the lipid contents of
290 liver and head kidney (Table 5).

291

292 *3.4 Fatty acid compositions of whole fish and tissues*

293 The proportion of total n-3 LC-PUFA in whole body of Atlantic salmon fed diet TCO was
294 around double that of fish fed diets WCO or COM (Table 4). Specifically, the relative
295 contents of EPA, DPA and DHA were highest in fish fed TCO with fish fed COM and WCO
296 showing similar lower values. The proportions of 18:2n-6, ARA and n-6 PUFA were also
297 highest in TCO-fed fish with COM-fed fish showing the lowest proportions of 18:2n-6 and
298 total n-6 PUFA. Differences were also observed in total saturated and monoenes with COM-
299 fed fish showing the lowest and highest contents respectively.

300 Flesh of fish fed TCO displayed the highest contents of the n-3 LC-PUFA, EPA, DPA and
301 DHA, and ARA (Table 4). In COM-fed fish, flesh showed the highest proportions of total
302 saturates and 18:1n-9, and the lowest contents of 18:3n-3, whereas WCO-fed fish contained
303 the highest percentage of this fatty acid. Relative levels of total n-3 PUFA, EPA and DPA in
304 liver and head kidney were highest in fish fed TCO, although total n-3 LC-PUFA levels in
305 TCO-fed fish was not different to WCO-fed fish. Although DHA level was highest in fish fed
306 TCO in both liver and head kidney, variation in the data rendered the dietary differences not
307 significant (Table 5).

308 A PCA analysis was applied to the whole fish and tissue fatty acid profiles (percentage) of
309 fish fed the three experimental feeds. The first two principal components explained 82.8 % of
310 variance (Fig. 1). The score plot showed some separation between tissues of fish fed COM

311 and those fed both WCO and TCO. There was an overlap between tissues of fish fed WCO
312 and TCO, although liver from fish fed TCO showed a distinct profile, clustering at the left
313 side of the plot (red diamonds). The fatty acid vectors representing the MUFA 16:1n-7,
314 18:1n-7, 18:1n-9 and 22:1n-11 appeared to be correlated with fish fed the COM diet. The n-6
315 and n-3 PUFA (18:2n-6, 18:3n-3 and 18:4n-3) vectors lie towards fish fed WCO whereas the
316 n-6 and n-3 LC-PUFA (20:4n-6, 20:5n-3, and 22:6n-3) point towards TCO-fed fish, as well
317 as livers from WCO-fed fish.

318

319 *3.5 Liver transcriptome*

320 In liver, a total of 312 unique genes were differentially expressed ($p < 0.05$; fold-change, FC
321 > 1.3) in salmon fed the TCO diet versus the COM diet whereas 249 genes were affected
322 when comparing WCO-fed fish with COM-fed fish (Table 6). The highest number (506) of
323 differentially expressed genes (DEG) was observed when comparing the hepatic
324 transcriptomes of TCO-fed and WCO-fed fish. Among these unique probes, 86 DEG were
325 commonly regulated when comparing fish fed TCO with fish fed either COM or WCO,
326 whereas 45 were found when TCO- and WCO-fed fish were compared to COM-fed fish and
327 only 37 when fish fed TCO and COM were compared to those fed WCO (Fig. 2A).

328 In order to elucidate the genes exclusively affected by TCO, KEGG Orthology (KO)
329 numbers were assigned to the 86 genes commonly affected by TCO (Supplementary Table 3),
330 and genes mapped to a known compendium of metabolic pathways (KEGG). Enrichr, an
331 integrative web-based software application⁽³³⁾, was used to build a network with the enriched
332 gene sets (Fig. 3A). Many of the categories represented belonged to metabolism with high
333 representation of lipid metabolism including “fatty acid degradation”, “sphingolipid
334 metabolism” and “terpenoid backbone biosynthesis”, as well as amino acid metabolism
335 including “alanine, aspartate and glutamate metabolism” and “other glycan degradation”.
336 Genes belonging to other categories such as “protein processing in the endoplasmic
337 reticulum”, and “DNA replication” were also highly represented. At a gene level, all the
338 genes presented the same direction of regulation (up or down) between both contrasts and a
339 similar FC. Expression of a set of candidate LC-PUFA biosynthesis genes was determined by
340 qPCR with *fads2d5* and *fads2d6* showing up-regulation in fish fed WCO, with no difference
341 between fish fed TCO and COM (Fig. 4). No differences were observed among the
342 expression of the other LC-PUFA biosynthesis genes evaluated by qPCR.

343

344 *3.6 Pyloric caeca transcriptome*

345 A greater number of DEG was found in pyloric caeca compared to liver when comparing fish
346 fed either diets TCO or WCO to fish fed COM (804 and 971 DEG, respectively) (Table 6).
347 However when comparing fish fed TCO with fish fed WCO, the number of DEG was much
348 lower in pyloric caeca compared to liver (279 versus 506). There were differences in the
349 distribution of genes depending on FC, with a high number of DEG showing FC >2.5,
350 particularly among down-regulated genes.

351 Only 61 genes were commonly regulated in the pyloric caeca of fish fed TCO compared
352 with fish fed COM and WCO (Fig. 2B). Network analysis of the enriched categories showed
353 that most of the genes were related to metabolism, although many also belonged to “mTOR
354 signalling pathway” and “regulation of autophagy” (Fig. 3B). After removing non-annotated
355 genes, 24 unique genes could be identified as being commonly affected by TCO in pyloric
356 caeca (Supplementary Table 4). All the genes presented the same direction of regulation and
357 similar FC in both contrasts (TCO v. COM and TCO v. WCO). High FCs were observed
358 particularly in genes related to carbohydrate metabolism (*N-acetylgalactosaminide alpha-2,6-*
359 *sialyltransferase* and *beta-mannosidase*), which were up-regulated in TCO-fed fish,
360 particularly when compared with WCO-fed fish (FC of 12.7 and 6.8, respectively). In pyloric
361 caeca, while there appeared to be a trend for lower expression of most of the LC-PUFA
362 biosynthesis genes in fish fed TCO compared to fish fed COM, but this was only significant
363 with *elovl5b* (Fig. 5).

364

365 3.7 Head kidney cell LPS challenge and gene expression

366 Diet did not significantly affect leucocyte respiratory burst activity, although there was a
367 trend toward lower values in TCO-fed fish ($p = 0.057$; Table 7). Similarly, no differences
368 were observed in phagocytic activity of monocytes/macrophages from fish fed the three
369 dietary treatments ($p = 0.854$; Table 7).

370 Atlantic salmon monocytes were exposed to LPS to study the effect on gene expression at
371 0, 6 and 24 h after addition. The exposure to LPS triggered a response in all of the genes
372 evaluated with highest expression levels observed 24 h after application of the challenge (Fig.
373 6). The FC varied among the studied genes, with the strongest regulation in *cox2* (approx.
374 2500 FC) and the lowest in *inos* (approx. 4 FC). Two-way ANOVA showed gene expression
375 was highly affected by sampling time ($p < 0.001$), but diet did not affect expression of any of
376 the studied genes and there was no interaction between diet and sample time.

377

378 Discussion

379 The benefits of n-3 LC-PUFA on several aspects of human health are widely known and
380 appreciated (Calder, 2017). However, recent studies have highlighted the gradual decrease of
381 these essential fatty acids in the flesh of farmed salmon, reflecting the necessary development
382 of sustainable feeds increasingly formulated with lower levels of marine ingredients that
383 supply the n-3 LC-PUFA⁽⁴⁾. Previous studies demonstrated the feasibility of oils obtained *de*
384 *novo* from transgenic *Camelina sativa* to supply n-3 LC-PUFA in feeds for Atlantic salmon
385 and sea bream, maintaining growth and enhancing n-3 LC-PUFA contents in fillet compared
386 to fish reared on feeds formulated with conventional VO⁽¹⁰⁻¹⁴⁾. The earlier oils from
387 transgenic *Camelina* supplied either 20 % EPA or 6 % each of EPA and DHA and were
388 compared to feeds formulated with high fishmeal and FO as “gold standards”^(10-12,14) and also
389 a feed with lower fishmeal and FO⁽¹³⁾. In the present study, the latest generation of transgenic
390 oil supplied levels of EPA, DHA and total n-3 LC-PUFA as high as many FO, or higher than
391 those from the northern hemisphere, and was specifically tested in order to evaluate its
392 capability to maintain the sustainability of feeds while simultaneously restoring levels of n-3
393 LC-PUFA in farmed salmon to those of a decade ago, before the development of low marine
394 feeds⁽⁴⁾.

395 In the present study, the COM diet was formulated to reflect current commercial feed
396 formulations for salmon and thus included levels of fishmeal and FO that resulted in a dietary
397 n-3 LC-PUFA level of around 7 % of total dietary fatty acids. Replacing the added oil with
398 the oil from transgenic *Camelina* resulted in the n-3 LC-PUFA content of the TCO diet being
399 almost 24 % of total fatty acids that, in turn, almost doubled the EPA+DHA and n-3 LC-
400 PUFA contents of flesh of the TCO-fed fish compared to fish fed the COM diet. While the
401 fish in the present trial were not market size and so the data obtained are not directly
402 comparable to the data presented in (4), they nonetheless indicate the potential of this 3rd
403 generation transgenically-derived oil to supply sufficient n-3 LC-PUFA to restore EPA+DHA
404 levels in farmed salmon to levels last seen a decade ago, thus retaining all the positive health
405 benefits associated with the consumption of fish.

406 As in previous trials, no differences in term of growth were observed between fish fed the
407 different feeds, which was not unexpected given that the levels of n-3 LC-PUFA contained in
408 all the feeds were sufficient to cover the requirements for this species. For instance, total n-3
409 LC-PUFA in fish fed diet WCO was still ~5.5 % of total fatty acids representing ~ 1.1 % of
410 diet, already above the reported EFA requirement level without even considering the high
411 dietary level of 18:3n-3⁽³⁴⁾. In agreement with previous trials, Atlantic salmon fed TCO

412 showed a reduced deposition of lipid in whole body and flesh, and, although not always
413 significant, other tissues⁽¹²⁾, which could be attributed to the higher contents of EPA and
414 DHA that are known to have anti-adipogenic effects in mammals⁽³⁵⁾. Additionally, the
415 microarray revealed that *acsl*, a gene involved in lipid biosynthesis, was commonly down-
416 regulated in TCO-fed fish when compared to fish fed COM or WCO, which could indicate
417 inhibition of lipogenesis. Interestingly, the expression of *lpl* was also down-regulated in
418 TCO-fed fish, which may or may not be consistent with lower lipid levels in tissues.
419 However, at least three different transcripts exist for *lpl* in Atlantic salmon and different
420 patterns of nutritional regulation for each isoform have been demonstrated⁽³⁶⁾.

421 Although there were no effects on growth performance, some differences were observed in
422 lipid and fatty acid digestibility. The ADC for lipid was generally high and affected by
423 dietary lipid source, being slightly higher in the COM and WCO feeds than in TCO feed.
424 Previous studies in several teleost species generally reported highest lipid ADC in feeds
425 containing FO^(37,38). Indeed, in our previous studies, feeds for Atlantic salmon formulated
426 with either wild-type or transgenic Camelina oil displayed higher lipid ADC than feeds
427 containing FO^(11,12). However the FO-based feeds were formulated with high levels of
428 fishmeal (49 %) and FO (18 %) and thus the content of n-3 LC-PUFA was much higher than
429 in the VO-based feeds, whereas in the present trial diet TCO contained the highest contents of
430 n-3 LC-PUFA. These data suggest that lipid ADC is dependent on the fatty acid profile of the
431 feeds and high contents of n-3 LC-PUFA enhance lipid digestibility as these fatty acids
432 generally have high ADC^(39,40). Consistent with this, the ADC for the n-3 LC-PUFA were
433 high in the present trial and highest in fish fed the TCO diet. Individual fatty acid ADC were
434 also consistent with previous studies where digestibility decreased with increasing chain
435 length, but increased with increasing degree of unsaturation^(39,41-42). While water temperature
436 can also impact the ADC of fatty acids, particularly when dietary FO is substituted by VO⁽⁴²⁾,
437 the trial was performed at a constant controlled temperature throughout and so differences in
438 digestibility of individual fatty acids between diets cannot be attributed to water temperature.
439 Overall, diet TCO had no major negative effects on fatty acid digestibilities and differences
440 between diets could be attributed to the different fatty acid profile of the feeds.

441 Although diet WCO contained the lowest levels of DHA and n-3 LC-PUFA, fish fed this
442 feed did not show major differences in the contents of DHA in whole fish or tissues
443 compared to fish fed the COM diet. Anadromous species, such as the salmonids, have the
444 capability to biosynthesize LC-PUFA, with liver and intestine being the most active
445 metabolic sites^(43,44). In this respect, fish fed WCO showed up-regulation of hepatic *fads2d6*

446 and *fad62d5* as well as intestinal *elovl5b*, which could have contributed in the levels of n-3
447 LC-PUFA being similar in COM- and WCO-fed fish. Although the COM diet had only a
448 slightly higher level of DHA compared to WCO (3.7 and 2.7 %, respectively) and much
449 lower than in TCO (9.0 %) expression levels of desaturases and elongases were similar to that
450 in fish fed TCO. However, the level of 18:3n-3 was almost 4 times higher in diet WCO than
451 in the COM diet and it has been suggested that up-regulation of the LC-PUFA biosynthetic
452 pathway in fish fed VO was induced by the level of dietary precursor (18:3n-3) as well as low
453 levels of pathway products (e.g. DHA)⁽⁴⁵⁾. Therefore, the lower expression of these enzymes
454 in liver and pyloric caeca of fish fed COM compared to fish fed WCO could be related to the
455 low dietary level of the substrate 18:3n-3.

456 The PCA analysis partly reflected the physiological functions of each tissue. In general
457 terms, the fatty acid profiles of whole fish and tissues largely reflected that of the diet,
458 consistent with previous studies in Atlantic salmon^(31,46,47). The four distinct quarters of the
459 plot represent significantly different fatty acid profiles, distinguishing three groups, one per
460 dietary treatment. The fatty acid vectors representing n-6 and n-3 LC-PUFA have significant
461 loadings on the “TCO” side of the plot, which is directly related to the diet fatty acid profiles.
462 This also explains why 18:1n-9 vector is placed towards “COM” as this fatty acid is in high
463 concentration in rapeseed oil, which constitutes 75 % of the added oil in that diet. Liver fatty
464 acid profiles from fish fed WCO are located towards the left side of the plot, where fish fed
465 TCO are placed, which reflects the capacity of liver for the synthesis of LC-PUFA. Head
466 kidney of fish fed diet TCO tended to cluster on the left panel reflecting differences in head
467 kidney DHA contents among fish fed the different diets. Although these differences were not
468 significant in the present trial they were consistent with previous studies in Atlantic salmon,
469 which showed DHA levels in head kidney reflected the reduced DHA content of VO-based
470 diets⁽⁴⁸⁾.

471 The head kidney in fish is an integral tissue of the immune system^(49,50), and dietary lipid
472 and PUFA content are known to influence immune function and thus health status of fish<sup>(51-
473 53)</sup>. In the present study, no differences were observed in the cellular immune functions
474 evaluated, such as monocyte phagocytic activity or respiratory burst. Previous studies with
475 sustainable feeds have found contradictory results regarding these immune parameters. While
476 Atlantic salmon fed diets with different VO (100 % and 50 % substitution of FO,
477 respectively) did not show altered phagocytic activity nor respiratory burst^(54,55) and rainbow
478 trout (*Oncorhynchus mykiss*) fed increasing concentrations of ARA showed no differences in
479 phagocytosis⁽⁵⁶⁾, sea bream fed diets containing VO (100 % substitution of FO) showed

480 reduced phagocytic activity⁽⁵⁷⁾. Differences in the dietary ARA:EPA ratio can alter the
481 production of immunologically active eicosanoids derived from these fatty acids⁽⁵⁸⁾, affecting
482 signal transduction and regulation of gene expression⁽⁵⁹⁾. Despite this, the functional assays
483 show no negative effects of the oil from transgenic Camelina on the fish immune system.
484 Furthermore, the expression of several genes related to inflammation and antibacterial
485 activity evaluated in monocytes isolated from fish after LPS challenge showed no differences
486 in any of the genes among the dietary treatments at either sampling point. Previously, the
487 expression of the pro-inflammatory cytokines *illb* and *tnfa* was not changed 12 h after LPS
488 stimulation in Atlantic salmon after modulation of membrane lipids⁽⁵⁵⁾. Therefore, our results
489 were consistent with replacement of dietary FO by VO in Atlantic salmon feeds having
490 relatively little influence on inflammatory functions of leukocytes. However, in other teleost
491 species, dietary VO can have adverse effects on health/immune system through increasing
492 time to moderate the response up to 7 days after challenge when 70 % of FO was substituted
493 by VO⁽⁶⁰⁾. In this respect, the expression of several inflammatory cytokines as well as the
494 anti-bactericidal gene *cath* started to return basal levels only 72 h after challenging Atlantic
495 salmon monocytes with LPS⁽⁶¹⁾.

496 Transcriptomic analyses showed that diet regulated expression of a lower number of
497 genes in liver than in pyloric caeca, with most of the pathways enriched in liver belonging to
498 metabolism, which was consistent given the functions of this tissue. One of the genes
499 commonly regulated by TCO was *acsl*, which was down-regulated in fish fed TCO compared
500 to fish fed the other two diets. This gene was also down-regulated in salmon fed a similar
501 EPA+DHA-containing Camelina oil in a previous trial⁽¹²⁾. After LC-PUFA enters a cell, *acsl*
502 converts them to fatty acyl-CoAs that can have numerous metabolic fates, including
503 incorporation into triacylglycerol or phospholipids, or substrates for β -oxidation and protein
504 acylation⁽⁶²⁾. Interestingly, a recent study reported up-regulation of *acsl3* in hepatopancreas of
505 grass carp (*Ctenopharyngodon idellus*) with increasing levels of dietary ARA⁽⁶³⁾, which is the
506 opposite trend to that described here, as the TCO feed contained a higher level of ARA than
507 the other diets. However, previous studies in Atlantic salmon showed no differences in the
508 expression of *acsl* in liver between fish fed FO and VO, which had different dietary ARA
509 contents⁽⁶⁴⁾. These authors suggested that this gene may function as a general fatty acid
510 activator for several lipid metabolism pathways not directly related to β -oxidation. This was
511 consistent with the present results, given that TCO-fed fish had the lowest lipid content in
512 body and flesh that, in turn, could be related to enhanced β -oxidation in fish fed this diet.
513 Among the DEG commonly regulated in fish fed TCO, a transcription factor, *ppara*, was

514 down-regulated in TCO compared to fish fed the other diets. Up-regulation in the expression
515 of this enzyme, concomitant with the expression of *fads2*, was observed previously in
516 rainbow trout after substitution of dietary FO with VO⁽⁶⁵⁾.

517 The intestine is not only the site of nutrient uptake, but also plays an active role in
518 metabolism. Indeed, two genes of carbohydrate metabolism were up-regulated in fish fed
519 TCO compared to fish fed the other diets despite the feeds not differing in carbohydrate
520 content. In an earlier trial with salmon fed a previous version of transgenic Camelina oil,
521 intestinal expression of genes of carbohydrate metabolism such as *glucose-6-phosphate*
522 *isomerase (g6pd)* and *glycerol kinase 5 (gk5)* was up-regulated⁽¹³⁾. Previous studies also
523 reported similar effects, probably reflecting that pathways of lipogenesis, lipolysis,
524 glycolysis, gluconeogenesis and pentose phosphate shunt are all interrelated in the regulation
525 of energy homeostasis⁽⁶⁶⁾. No genes were found to be commonly regulated in liver and
526 pyloric caeca in fish fed TCO, probably reflecting the differing functional roles of each
527 tissue.

528 The present study showed that salmon fed the new oil from transgenic *Camelina sativa*
529 supplying ~ 24 % n-3 LC-PUFA could accumulate almost double the level of EPA and DHA
530 in their body and fillet than fish fed a feed reflecting current commercial formulations
531 containing low levels of marine ingredients. This oil also supported good growth without
532 apparently compromising fish health and immune response as shown by the *ex vivo*
533 macrophage challenge and tissue transcriptomic responses. Although fish were not of harvest
534 size (final weight, ~400 g), these data suggest that the new oil from a transgenic oilseed crop
535 has the potential to provide a new generation of salmon feeds that, while maintaining current
536 levels of sustainability, are also able to restore the EPA+DHA levels in harvest-size (~ 4-5
537 Kg) farmed salmon to those last seen a decade ago before the development of low marine
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539

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548

549 **Conflict of Interest**

550 None

551

552 **Authorship**

553 The authors contributed as follows to the manuscript: study concept and design: J.A.N., O.T.,
554 D.R.T., R.E.O.; formal analysis: M.B.B., K.L., M.S., L.H.; funding acquisition: J.A.N.,
555 D.R.T., R.E.O.; writing – original draft: M.B.B., D.R.T.; writing – review and editing: K.L.,
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557

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745

746 **Figure Legends**

747 **Fig. 1.** Principal component analysis (PCA) of fatty acid profiles (percentage of total fatty
748 acids) of tissues from Atlantic salmon fed the experimental feeds for 12 weeks. Fish fed fish
749 /rapeseed oil (COM), black; Fish fed wild-type Camelina oil, light grey (WCO); Fish fed
750 transgenic Camelina oil, dark grey (TCO); filled circle, head kidney; filled square, flesh;
751 diamond, liver.

752 **Fig. 2.** Venn diagram representing genes differentially expressed in liver (A) and pyloric
753 caeca (B) of Atlantic salmon fed the experimental diets (Welch t-test; $p < 0.05$, $FC > 1.3$).
754 Non-annotated genes and features corresponding to the same gene are not represented. COM,
755 fish/rapeseed oil feed; TCO, transgenic Camelina oil feed; WCO, wild-type camelina oil
756 feed.

757 **Fig. 3.** Metabolic categories enriched with genes commonly regulated in fish fed transgenic
758 Camelina oil. Gene networks in liver (A) and pyloric caeca (B) were produced using Enrichr
759 web-application.

760 **Fig. 4.** Expression of genes of the LC-PUFA biosynthesis pathway in liver of Atlantic salmon
761 as determined by qPCR. Results are normalised expression ratios (means \pm SEM; $n = 6$).
762 Different superscript letters denote differences among treatments as identified by one-way
763 ANOVA. COM, fish/rapeseed oil feed; TCO, transgenic camelina oil feed; WCO, wild type
764 camelina oil feed. *elovl2*, fatty acyl elongase 2; *elovl5a*, fatty acyl elongase 5 isoform a;
765 *elovl5b*, fatty acyl elongase 5 isoform b; *fads2d6*, delta-6 fatty acyl desaturase; *fads2d5*,
766 delta-5 fatty acyl desaturase.

767 **Fig. 5.** Expression of genes of the LC-PUFA biosynthesis pathway in pyloric caeca of
768 Atlantic salmon as determined by qPCR. Results are normalised expression ratios (means \pm
769 SEM; $n = 6$). Different superscript letters denote differences among treatments identified by
770 one-way ANOVA. COM, fish/rapeseed oil feed; TCO, transgenic camelina oil feed; WCO,
771 wild-type camelina oil feed. Gene abbreviations as described in legend to Fig. 4.

772 **Fig. 6.** Expression of genes of inflammation and antibacterial activity in head kidney
773 monocytes/macrophages of Atlantic salmon at the end of the experimental trial before (0 h),
774 or 6 h and 24 h after challenging the cells with LPS. Results are expressed as normalised
775 expression ratios (means \pm SEM; $n = 6$). COM, fish/rapeseed oil feed; TCO, transgenic
776 camelina oil feed; WCO, wild-type camelina oil feed; *il1b*, interleukin 1 beta; *tnfa*, tumor

777 necrosis factor alpha; *cox2*, cyclooxygenase 2; *inos*, inducible nitric oxide synthase; *cath*,
778 cathelicidin; *hepc*, hepcidin.

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780 **Table 1.** Formulations, analysed proximate compositions and selected fatty acid profiles of
 781 the experimental diets.

	COM	WCO	TCO
<i>Feed Ingredients (%)</i>			
Fish Meal	30.00	30.00	30.00
Soy Protein Concentrate (> 62 %)	10.00	10.00	10.00
Pea Protein (> 72 %)	10.00	10.00	10.00
Wheat Gluten	5.00	5.00	5.00
Maize Gluten	5.00	5.00	5.00
Wheat	12.95	12.95	12.95
Fish oil	5.00	0.00	0.00
Rapeseed oil	15.00	0.00	0.00
Camelina oil (wild-type)	0.00	20.00	0.00
Camelina oil (transgenic)	0.00	0.00	20.00
Mineral and vitamin premixes	2.05	2.05	2.05
Yttrium oxide	0.05	0.05	0.05
<i>Analysed composition</i>			
Dry matter (%)	92.6	93.9	92.2
Protein (%)	45.1	46.4	48.6
Lipid (%)	23.4	19.7	18.6
Ash (%)	7.1	7.4	7.6
Energy (kJ g ⁻¹)	23.0	22.1	22.4
<i>Analysed fatty acid profile (%)</i>			
16:0	9.3	8.6	9.5
Total saturated*	14.1	14.2	19.9
18:1n-9	42.4	15.8	7.8
Total monounsaturated[†]	54.2	35.7	18.6
18:2n-6	15.7	18.2	19.8
20:4n-6	0.2	0.2	2.2
Total n-6 PUFA[‡]	16.2	19.9	25.9
18:3n-3	6.8	22.8	8.7
20:3n-3	0.1	0.8	0.9
20:5n-3	3.2	2.4	9.4
22:5n-3	0.4	0.3	3.9
22:6n-3	3.7	2.7	9.0
Total n-3 PUFA[‡]	15.0	29.7	35.2
Total PUFA	31.7	50.1	61.5
Total n-3 LC-PUFA	7.5	5.5	23.9

782 COM, fish oil/rapeseed oil feed; WCO, wild-type camelina oil feed; TCO, transgenic
 783 camelina oil feed; LC-PUFA, long-chain polyunsaturated fatty acids (sum of 20:4n-3, 20:5n-
 784 3, 22:5n-3, 22:6n-3). *Contains 14:0, 18:0 and 20:0; [†]Contains 16:1n-7, 18:1n-7, 20:1n-11,
 785 20:1n-9, 20:1n-6, 22:1n-11 and 22:1n-9; [‡]Contains 18:3n-6, 20:2n-6, 20:3n-6 and 22:4n-
 786 6. [‡]Contains 18:4n-3, 20:3n-3 and 20:4n-3.

787 **Table 2.** Growth performance, biometric parameters, and biochemical composition of whole
 788 fish after feeding the experimental diets for 12 weeks. Data are presented as means \pm SD (n =
 789 3).

	COM		WCO		TCO		p value
	Mean	SD	Mean	SD	Mean	SD	
Final weight (g)	412.2	5.6	397.7	9.2	406.2	18.2	0.447
Length (cm)	31.4	0.3	31.2	0.2	31.6	0.5	0.400
Gutted (g)	367.1	14.4	349.5	15.2	365.2	17.6	0.388
HSI	1.0	0.0	1.0	0.0	1.0	0.1	0.856
VSI	6.2	0.2	6.1	0.2	5.9	0.2	0.177
Haematocrit	33.7	0.6	32.4	0.8	33.9	0.8	0.231
<i>Whole body composition (% wet weight)</i>							
Crude protein	17.9	0.3	18.2	0.2	18.0	0.6	0.640
Crude lipid	11.7 ^a	0.5	10.9 ^a	0.3	9.8 ^b	0.2	0.013
Ash	1.6	0.0	1.6	0.0	1.7	0.0	0.178
Dry matter	31.5	0.2	32.3	0.6	31.0	0.5	0.270

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 791 COM, fish oil/rapeseed oil feed; WCO, diet containing wild-type camelina; TCO, transgenic
 792 camelina oil feed; HSI, hepato-somatic index; VSI, viscero-somatic index.

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796 **Table 3.** Apparent digestibility coefficients (ADC) of total lipid and individual fatty acids.

797 Data are presented as means \pm SD (n = 3).

	COM		WCO		TCO	
	Mean	SD	Mean	SD	Mean	SD
Total lipid	94.6 ^a	0.3	93.7 ^a	0.1	90.7 ^b	0.7
14:0	95.9 ^a	0.4	95.0 ^a	1.6	92.5 ^b	0.1
15:0	94.6 ^a	0.2	92.7 ^{ab}	2.1	89.6 ^b	0.8
16:0	93.9 ^a	0.4	92.4 ^{ab}	2.3	89.7 ^b	0.8
18:0	91.8 ^a	0.8	89.6 ^{ab}	3.6	85.1 ^b	1.5
Total saturated	93.7 ^a	0.5	91.4 ^{ab}	2.8	86.7 ^b	1.3
16:1n-7	97.9 ^a	0.5	97.1 ^{ab}	0.9	96.0 ^b	0.7
18:1n-9	98.1 ^a	0.4	96.7 ^a	0.2	94.4 ^b	1.1
18:1n-7	97.7 ^a	0.4	95.8 ^b	0.2	95.6 ^b	0.6
20:1n-9	96.5	0.7	97.0	0.2	96.0	0.5
20:1n-7	95.4	0.7	96.5	0.2	95.0	0.6
22:1n-11	96.2 ^a	0.7	95.2 ^a	1.7	91.1 ^b	1.9
22:1n-9	96.2 ^a	0.7	94.9 ^{ab}	2.4	91.5 ^b	1.2
Total monoenes	97.9 ^a	0.4	96.4 ^{ab}	1.2	94.6 ^b	0.6
18:2n-6	97.8	0.4	97.3	0.5	97.2	0.5
20:2n-6	90.6 ^b	3.4	97.4 ^a	0.9	92.5 ^{ab}	1.4
20:4n-6	97.2 ^{ab}	0.2	95.0 ^b	0.0	99.2 ^a	1.1
Total n-6 PUFA	97.7	0.4	97.2	0.5	97.5	0.5
18:3n-3	98.7	0.3	98.8	0.3	98.3	0.3
18:4n-3	98.8	0.4	97.5	0.9	99.1	0.3
20:3n-3	85.7 ^b	5.1	97.5 ^a	1.0	97.0 ^a	0.3
20:4n-3	96.1 ^{ab}	1.6	91.7 ^b	2.5	98.4 ^a	0.5
20:5n-3	98.3 ^a	0.3	97.0 ^b	0.4	98.5 ^a	0.3
22:5n-3	95.4 ^b	1.0	92.7 ^c	1.1	98.4 ^a	0.4
22:6n-3	95.6 ^a	0.5	92.2 ^b	0.7	96.2 ^a	0.8
Total n-3 PUFA	97.7	0.4	97.9	0.4	97.9	0.4
Total PUFA	98.3	1.3	97.6	0.4	97.7	0.5

798 COM, fish oil/rapeseed oil feed; WCO, wild-type camelina oil feed; TCO, transgenic
799 camelina oil feed; LC-PUFA, long-chain polyunsaturated fatty acids (sum of 20:4n-3, 20:5n-
800 3, 22:5n-3, 22:6n-3). Different superscript letters denote statistically significant differences
801 between the treatments as determined by one-way ANOVA with Tukey's and Duncan
802 comparison test (p < 0.05).

803

804 **Table 4.** Lipid contents (percentage of wet weight) and fatty acid compositions (percentage
805 of total fatty acids) of total lipid of whole body and flesh (muscle) of Atlantic salmon after
806 feeding the experimental diets for 12 weeks. Data are presented as means \pm SD (n = 3).

	COM		WCO		TCO	
	Mean	SD	Mean	SD	Mean	SD
<i>Whole body</i>						
Lipid content	11.7 ^a	0.5	10.9 ^a	0.3	9.8 ^b	0.2
16:0	10.6	0.3	10.9	0.1	11.0	0.1
Total saturated*	16.1 ^c	0.3	17.1 ^b	0.2	19.1 ^a	0.3
18:1n-9	38.6 ^a	0.7	22.9 ^b	0.3	18.5 ^c	0.9
Total monoenes [†]	52.3 ^a	0.6	40.3 ^b	0.2	31.2 ^c	0.8
18:2n-6	13.1 ^c	0.2	14.4 ^b	0.1	16.1 ^a	0.5
20:4n-6	0.3 ^b	0.0	0.3 ^b	0.0	1.2 ^a	0.2
Total n-6 PUFA [‡]	15.1 ^c	0.2	17.0 ^b	0.0	21.1 ^a	0.2
18:3n-3	4.9 ^c	0.1	13.2 ^a	0.2	6.5 ^b	0.1
20:5n-3	2.2 ^b	0.2	2.1 ^b	0.1	5.2 ^a	0.3
22:5n-3	0.9 ^b	0.1	0.8 ^b	0.0	3.1 ^a	0.1
22:6n-3	6.4 ^b	0.6	5.7 ^b	0.0	10.1 ^a	0.3
Total n-3 PUFA [‡]	16.2 ^c	0.9	25.3 ^b	0.0	28.3 ^a	0.7
EPA + DHA	8.5 ^b	0.8	7.8 ^b	0.1	15.3 ^a	0.6
Total n-3 LC-PUFA	8.7	1.2	8.7	1.6	11.2	1.5
<i>Flesh</i>						
Lipid content	3.9 ^a	1.5	2.6 ^{ab}	0.5	2.5 ^b	0.6
16:0	11.7	0.8	11.7	0.3	12.1	0.5
Total saturated*	17.2 ^b	1.0	17.6 ^b	0.2	19.7 ^a	0.4
18:1n-9	34.6 ^a	1.9	19.1 ^b	1.3	14.7 ^c	2.0
Total monoenes [†]	46.0 ^a	2.5	33.4 ^b	1.8	24.6 ^c	3.3
18:2n-6	12.2 ^b	0.6	12.8 ^b	0.3	13.7 ^a	0.4
20:4n-6	0.5 ^b	0.1	0.5 ^b	0.1	1.7 ^a	0.2
Total n-6 PUFA [‡]	14.2 ^b	0.6	15.3 ^b	0.2	18.2 ^a	0.5
18:3n-3	5.0 ^c	0.2	12.7 ^a	0.4	6.3 ^b	0.4
20:5n-3	2.9 ^b	0.5	3.3 ^b	0.4	6.5 ^a	0.8
22:5n-3	1.1 ^b	0.1	1.1 ^b	0.1	3.5 ^a	0.3
22:6n-3	10.2 ^b	0.6	12.4 ^b	0.8	17.7 ^a	2.0
Total n-3 PUFA [‡]	22.6 ^b	2.2	33.8 ^a	1.9	37.6 ^a	3.0
EPA + DHA	13.1 ^b	2.2	15.6 ^b	1.6	24.2 ^a	2.6
Total n-3 LC-PUFA	16.5 ^b	0.8	18.7 ^b	0.6	29.3 ^a	0.8

832 COM, fish oil/rapeseed oil feed; WCO, wild-type camelina oil feed; TCO, transgenic
833 camelina oil feed; LC- PUFA, long-chain polyunsaturated fatty acids (sum of 20:4n-3, 20:5n-
834 3 22:5n-3 and 22:6n-3). Different superscript letters within a row denote significant
835 differences among diets as determined by one-way ANOVA with Tukey's comparison test (p
836 < 0.05). *Contains 14:0, 15:0, 18:0, 20:0 and 22:0; [†]Contains 16:1n-7, 18:1n-7, 20:1n-9,
837 22:1n-11, 22:1n-9 and 24:1; [‡]Contains 20:2n-6 and 20:3n-6; [‡]Contains 18:4n-3, 20:3n-3 and
838 20:4n-3.

839 **Table 5.** Lipid contents (percentage of wet weight) and fatty acid compositions (percentage
 840 of total fatty acids) of total lipid of liver and head kidney of Atlantic salmon after feeding the
 841 experimental diets for 12 weeks. Data presented as means \pm SD (n = 3).

	COM		WCO		TCO		
	Mean	SD	Mean	SD	Mean	SD	
842	<i>Liver</i>						
844	Lipid content	4.7	1.0	4.4	0.9	4.1	0.5
845	16:0	13.2	1.7	14.0	1.4	15.0	0.9
846	Total saturated*	19.3 ^b	1.9	20.4 ^{ab}	1.3	23.5 ^a	1.0
847	18:1n-9	24.9 ^a	5.5	15.7 ^b	2.5	10.4 ^b	0.5
848	Total monoenes [†]	32.8 ^a	6.4	24.3 ^{ab}	3.8	16.1 ^b	1.0
849	18:2n-6	8.5	1.0	9.1	0.9	7.6	0.5
850	20:4n-6	2.5 ^b	0.5	2.3 ^b	0.3	6.3 ^a	0.0
851	Total n-6 PUFA [‡]	14.0 ^b	0.3	15.0 ^b	0.7	17.3 ^a	0.4
852	18:3n-3	2.6 ^b	0.2	6.7 ^a	1.1	3.1 ^b	0.5
853	20:5n-3	5.5 ^b	0.7	6.4 ^b	0.9	8.2 ^a	0.6
854	22:5n-3	1.6 ^b	0.2	1.6 ^b	0.3	3.5 ^a	0.4
855	22:6n-3	22.8	4.5	22.2	3.3	26.2	1.2
856	Total n-3 PUFA ^l	33.9 ^b	5.0	40.3 ^{ab}	2.9	43.1 ^a	0.6
857	EPA + DHA	28.4	5.1	28.5	3.7	34.4	1.0
858	Total n-3 LC-PUFA	30.8 ^b	2.1	31.8 ^{ab}	1.4	39.1 ^a	0.5
859	<i>Head kidney</i>						
860	Lipid content	5.6	1.1	4.2	0.4	4.1	1.0
861	16:0	12.6	0.4	14.0	1.0	14.4	1.6
862	Total saturated*	18.1 ^b	0.6	20.0 ^{ab}	1.1	22.3 ^a	2.1
863	18:1n-9	32.2 ^a	2.2	18.8 ^b	1.3	14.2 ^b	2.1
864	Total monoenes [†]	43.7 ^a	2.5	32.0 ^b	1.8	23.4 ^c	2.8
865	18:2n-6	11.3	0.4	11.8	1.0	11.9	1.6
866	20:4n-6	1.0 ^b	0.2	1.3 ^b	0.3	4.2 ^a	1.1
867	Total n-6 PUFA [‡]	13.7 ^b	0.4	14.9 ^b	1.1	18.4 ^a	0.6
868	18:3n-3	4.2 ^b	0.1	10.3 ^a	1.5	4.9 ^b	0.7
869	20:5n-3	4.0 ^b	0.5	4.8 ^b	1.3	7.1 ^a	0.5
870	22:5n-3	1.1 ^b	0.2	1.0 ^b	0.1	2.8 ^a	0.2
871	22:6n-3	11.6	1.5	13.2	2.0	16.5	2.7
872	Total n-3 PUFA ^l	22.8 ^b	2.1	32.6 ^a	1.8	34.1 ^a	2.2
873	EPA + DHA	15.7 ^b	2.0	18.1 ^{ab}	3.3	23.6 ^a	3.2
	Total n-3 LC-PUFA	17.5 ^b	0.6	20.2 ^b	1.0	27.7 ^a	1.2

867 COM, fish oil/rapeseed oil feed; WCO, wild-type camelina oil feed; TCO, transgenic
 868 camelina oil feed; LC- PUFA, long-chain polyunsaturated fatty acids (sum of 20:4n-3, 20:5n-
 869 3 22:5n-3 and 22:6n-3). Different superscript letters within a row denote significant
 870 differences among diets as determined by one-way ANOVA with Tukey's comparison test (p
 871 < 0.05). *Contains 14:0, 15:0, 18:0, 20:0 and 22:0; †Contains 16:1n-7, 18:1n-7, 20:1n-9,
 872 22:1n-11, 22:1n-9 and 24:1; ‡Contains 20:2n-6 and 20:3n-6; ^lContains 18:4n-3, 20:3n-3 and
 873 20:4n-3.

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Table 6. Summary of liver and pyloric caeca microarray analysis after removing duplicated probes

	COM/WCO	COM/TCO	TCO/WCO
<i>Liver</i>			
Total no. of probes		44000	
Total no. of DEG	249	312	506
Up-regulated genes	169	262	89
FC 1.3 – 1.5	58 (34.5 %)	75 (28.6 %)	25 (28.1 %)
FC 1.5 – 2.5	88 (52.4 %)	139 (53.1 %)	50 (56.2 %)
FC > 2.5	22 (13.1 %)	48 (18.3 %)	14 (15.7 %)
Down-regulated genes	80	50	417
FC 1.3 – 1.5	23 (28.7 %)	18 (36 %)	157 (37.6 %)
FC 1.5 – 2.5	48 (60 %)	28 (56 %)	211 (50.6 %)
FC > 2.5	9 (11.3 %)	4 (8 %)	49 (11.8 %)
<i>Pyloric caeca</i>			
Total no. of probes		44000	
Total no. of DEG	971	804	279
Up-regulated genes	918	729	182
FC 1.3 – 1.5	518 (56.4 %)	421 (57.8 %)	55 (30.1 %)
FC 1.5 – 2.5	263 (28.6 %)	207 (28.4 %)	62 (33.9 %)
FC > 2.5	137 (14.9 %)	101 (13.9 %)	66 (36.1 %)
Down-regulated genes	53	75	97
FC 1.3 – 1.5	5 (9.4 %)	14 (19.8 %)	20 (20.6 %)
FC 1.5 – 2.5	24 (45.3 %)	29 (39.2 %)	37 (38.1 %)
FC > 2.5	24 (45.3 %)	31 (41.9 %)	40 (41.2 %)

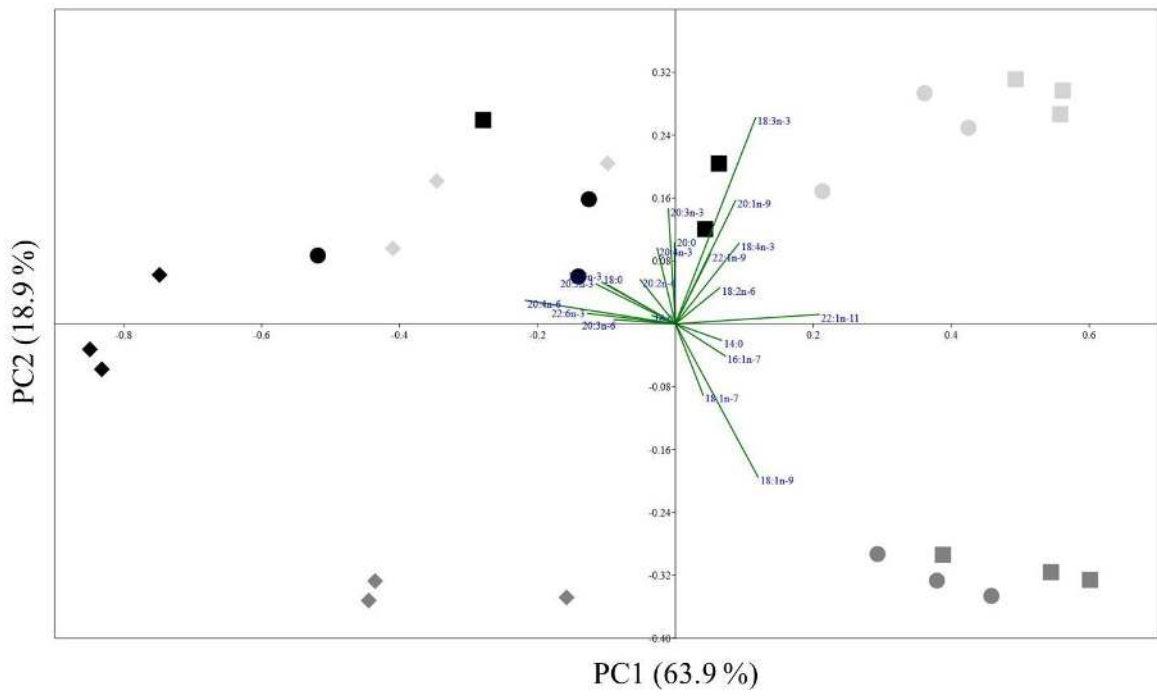
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COM, fish oil/rapeseed oil feed; WCO, wild-type camelina oil feed; TCO, transgenic camelina oil feed; DEG, differently expressed gene; FC, fold change.

884 **Table 7.** Respiratory burst (absorbance per 1×10^5 cells) and phagocytic activity of
 885 macrophages isolated from experimental fish after 12 weeks of feeding the experimental
 886 diets.
 887

	COM		WCO		TCO		P value
	Mean	SD	Mean	SD	Mean	SD	
Respiratory burst	0.16	0.04	0.18	0.04	0.15	0.03	0.057
Phagocytic activity	50.0	5.9	53.3	8.1	47.8	7.6	0.854

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 889 COM, fish oil/rapeseed oil feed; WCO, wild-type camelina oil feed; TCO, transgenic
 890 camelina oil feed.
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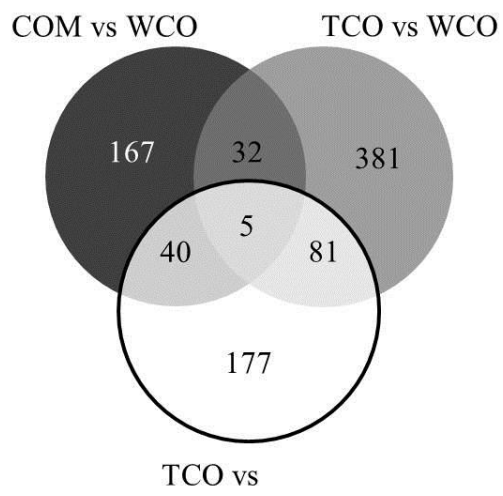
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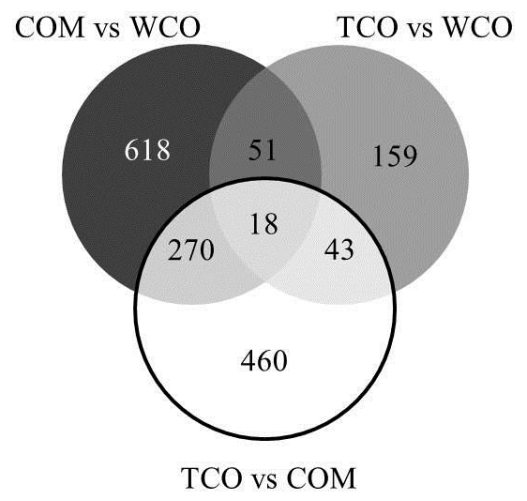
895 Figure 1.

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A.



B.

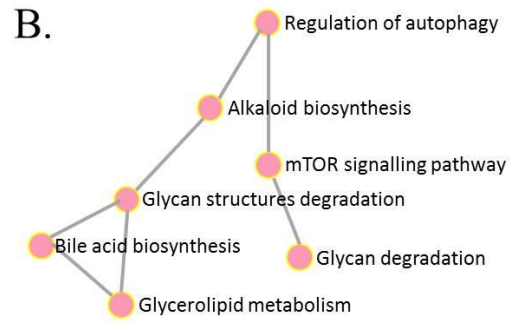
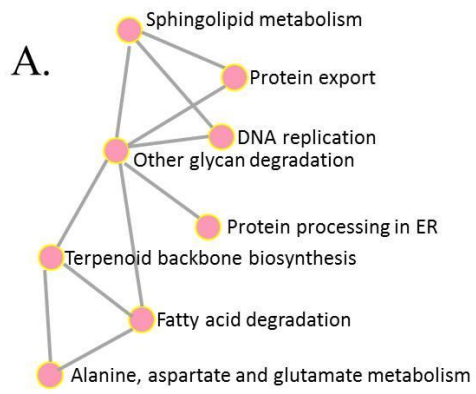


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899 Figure 2

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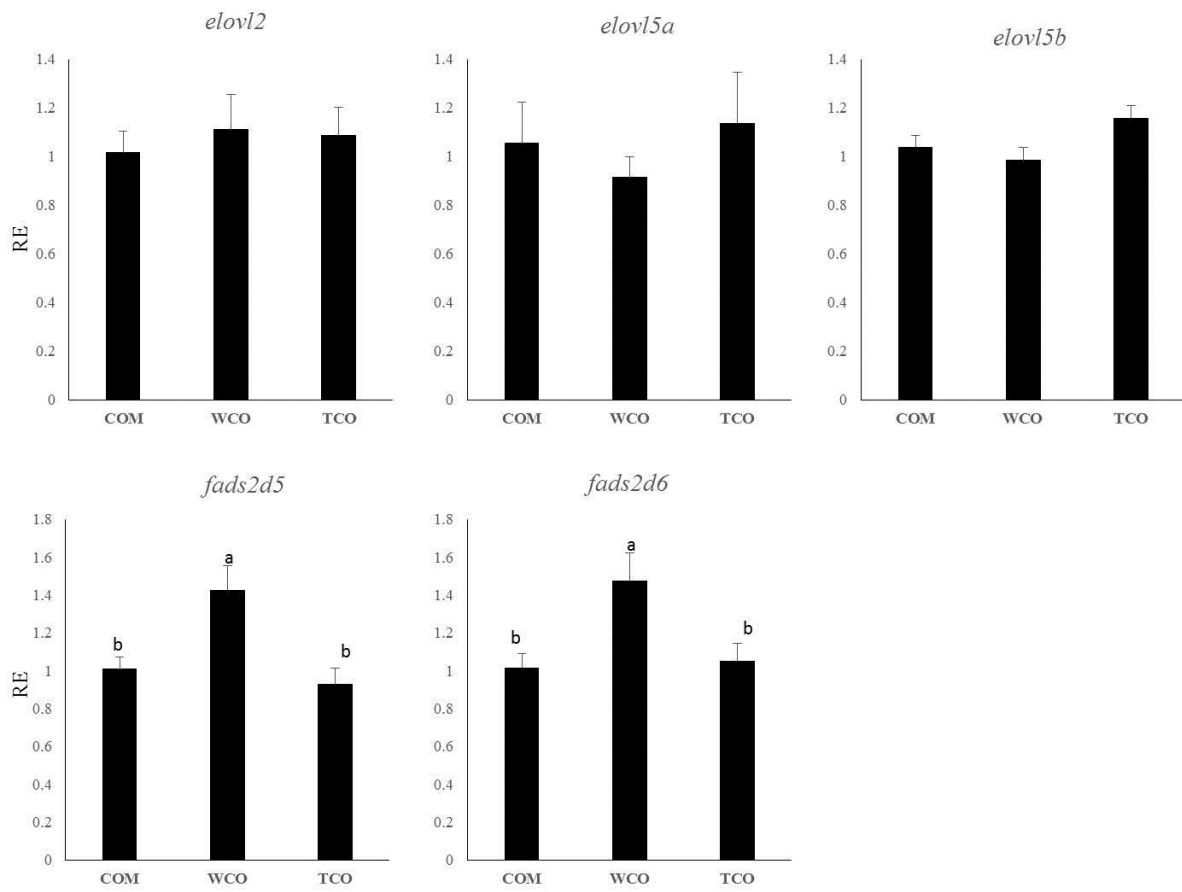


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903 Figure 3

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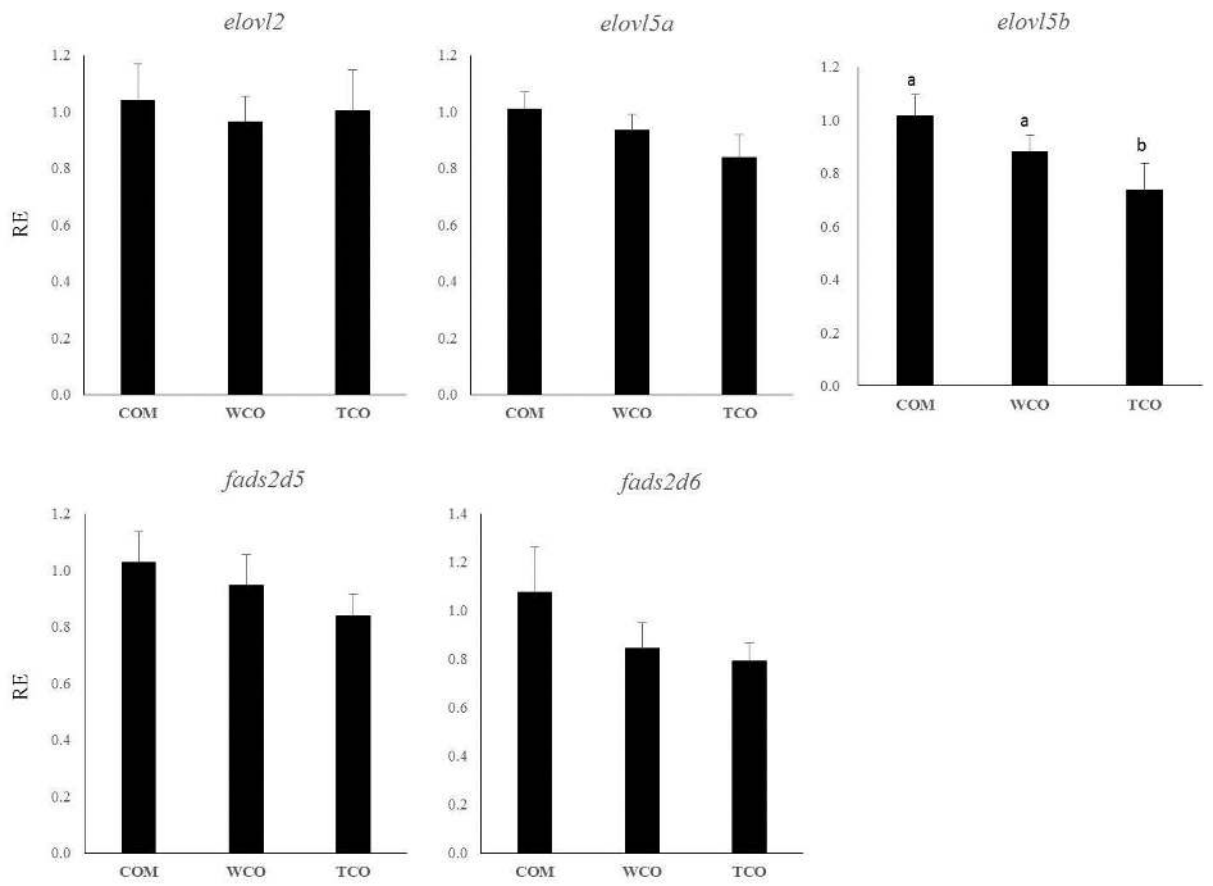


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907 Figure 4

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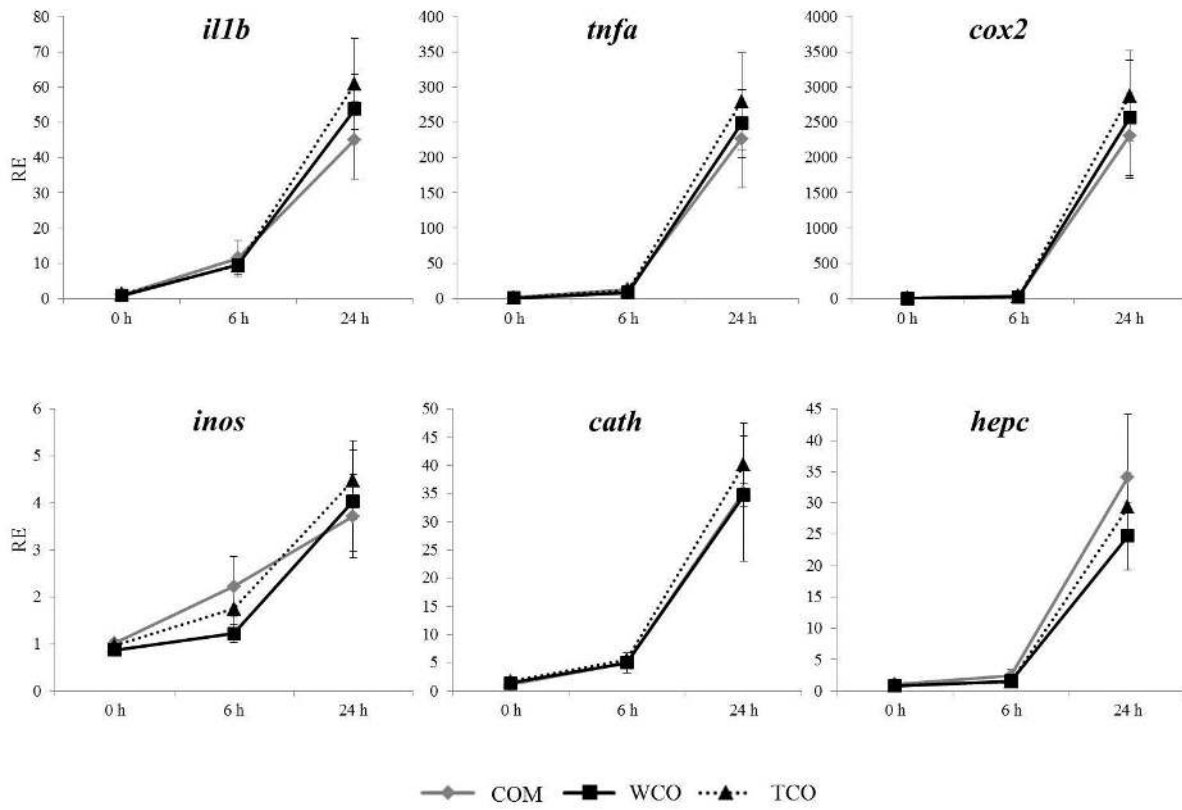
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912 Figure 5

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917 Figure 6