Comparative transcriptomics reveals domestication-associated features of Atlantic salmon lipid metabolism

Domestication and lipid metabolism in salmon

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Abstract

Domestication of *Salmo salar* has imposed strong selection for production traits since the 1970s. The domestication has also imposed a radical shift in diet. Whereas wild salmon eats invertebrates, crustaceans and fish, the dietary lipids in domestic feed has since 1990 gradually shifted from fish oil (FO) to vegetable oil (VO), causing a decrease intake of long-chain polyunsaturated fatty acids (LC-PUFA). We tested the hypothesis that this shift has induced domestication-specific features of lipid metabolism in a 96-day feeding trial of domesticated and wild salmon fed diets based on FO, VO or phospholipids (PL). We addressed this by sampling tissues central in fat uptake (pyloric caeca) and processing (liver) and quantifying RNA expression and fatty acid composition. Domesticated salmon grew faster than wild salmon, with higher gene expression in glucose and lipid metabolism pathways. The promoters of differentially expressed genes were enriched for transcription factors involved in circadian clock regulation. Domesticated salmon had lower expression of *cry2* and *nr1d1*, genes involved in negative regulation of circadian rhythm, with possible implications for the diurnal cycle of feed ingestion and basal metabolic rate. Only wild salmon showed a significant impact on growth of VO *versus* PL or FO feed, whereas domesticated but not wild salmon upregulated key LC-PUFA synthesis genes *fads2d5* and *fads2d6a* in response to VO diet. Domesticated salmon had higher LC-PUFA but lower 18:3n-3 and 18:2n-6 in liver when fed VO, suggesting

40 that domesticated salmon can better compensate for dietary shortage of LC-PUFA compared to
41 wild salmon.
42
43 **Keywords** Wild salmon; Domestication; Vegetable oil; Circadian regulation; Long-chain
44 polyunsaturated fatty acids; Transcriptomics
45
46 **1. Introduction**
47
48 Domestication of Atlantic salmon started in 1971 and since then they have undergone selection
49 in breeding programs for better growth and performance, later sex maturation, higher feed
50 conversion rate and many other measured traits (Gjedrem, Gjøen, & Gjerde, 1991). This has led
51 to genetic divergence, allowing domesticated salmon to grow twice as fast as wild salmon
52 (Fleming & Einum, 1997; C. Roberge, Normandeau, Einum, Guderley, & Bernatchez, 2008).
53 Compared to wild salmon, domesticated salmon are more aggressive during feeding in the tank
54 environment, and have higher feed intake, feed utilization, and increased metabolic efficiency
55 (Fleming & Einum, 1997; Christian Roberge, Einum, Guderley, & Bernatchez, 2006;
56 Thodesen, Grisdale-Helland, Helland, & Gjerde, 1999). In addition to the targeted selection on
57 production traits, domestication is also associated with unintentional selection on traits that are
58 linked to the new domestic environments (Fleming & Einum, 1997; Heath, Heath, Bryden,
59 Johnson, & Fox, 2003).

One environmental variable that changes dramatically with domestication is the feed composition and feeding regimes (Ytrestøyl, Aas, & Åsgård, 2015). In the wild, salmon is an opportunistic predator and its diet consists mostly of invertebrates in rivers, and crustaceans and small fish after they migrate to the sea. Domestic salmon on the other hand have 'unlimited' access to food and their diet is composed of proteins from fish and plant meal, as well as a lipid source. Up until the late 1990s this lipid source was mainly fish oil (FO) from wild fisheries. During the last two decades the FO have gradually been substituted with vegetable oils (VO) with a very different profile of long-chain polyunsaturated fatty acids (LC-PUFAs) compared to what salmon is exposed to in the wild. Lipids are not only major energy source for Atlantic salmon, but also play important roles in metabolic regulation and cell membrane function (Sargent, Tocher, & Bell, 2002). The LC-PUFAs docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6) are particularly important. They are key components of cell membranes, they regulate cell membrane fluidity, function as precursors for eicosanoid production, and are important components of neural tissues (Izquierdo, 1996; Sargent et al., 2002). It is therefore likely that modern VO based diets, with significantly reduced levels of DHA and EPA, have selected for a domestic features of lipid metabolism.

79 Feeding of VO-based diets naturally devoid of LC-PUFA is known to induce a compensatory
80 response of endogenous synthesis of LC-PUFA in salmon (Datsomor et al., 2019; Stubhaug et
81 al., 2005; Zheng, Tocher, Dickson, Bell, & Teale, 2004). Due to the past 20 years of including
82 VO as lipid sources in salmon feed, current generations of domesticated salmon are possibly
83 better adapted to dietary VO compared to earlier generations, but whether this involves higher
84 LC-PUFA synthesis abilities is unclear. Another major difference between wild and
85 domesticated diets is the levels of dietary phospholipids (PLs). PLs are important for growth
86 and development of salmon especially for early developmental stages (Poston, 1990; Taylor et
87 al., 2015), and dietary PL are more efficient at delivering LC-PUFA into the circulatory system
88 and ultimately the cells compared to neutral lipids such as triacylglycerols (Cahu et al., 2009; Y.
89 Olsen et al., 2014a). The efficiency of utilizing dietary PL could therefore also be shaped during
90 domestication in domesticated fish, however this has never been investigated.
91
92 In the present study, we aim to explore the idea that the diet of domesticated salmon has shaped
93 and selected for 'domestic features' of salmon lipid metabolism. We approach this question by
94 running a feeding trial with both domesticated and wild salmon that are fed contrasting diets
95 rich in either FO, VO or PL and then perform comparative transcriptomic and fatty acid
96 analyses of two tissues involved in lipid uptake (pyloric caeca) and endogenous synthesis of
97 LC-PUFAs (liver). Our study shows clear differences between metabolism in the wild and
98 domestic salmon genetic backgrounds and suggests that the regulation of genes involved in

99 circadian rhythm and lipid metabolism have been key targets during domestication related
100 selection.
101
102 **2. Material and methods**
103
104 2.1 Fish, diets and experimental plan
105
106 This experiment was approved by Norwegian Food Safety Authority (Case No. 16/10070). A
107 fast-growing strain of Atlantic salmon was kindly provided by the breeding company AquaGen
108 AS (Trondheim, Norway). The domesticated fish have been selected for good growth and
109 performance for 11 generations since 1971. The previous generations of domesticated salmon
110 were always fed standard commercial diets available at the time. This means that the fish were
111 given a freshwater diet with only marine ingredients at early developmental stages but have
112 experienced a gradual switch in seawater diet from FO to VO since the 1990s. A group of wild
113 salmon eggs was purchased from Haukvik Smolt AS, which is a live wild salmon gene bank
114 situated in Trøndelag, Norway. This gene bank is operating on behalf of the Norwegian
115 Environmental Agency to preserve genetic diversity of Norway's wild Atlantic salmon. The
116 parents of the wild salmon used in the present study was from the first generation of salmon
117 originally caught in 2008 in Vosso river of Norway. Wild salmon were kept in outdoor tanks
118 with a transparent roof, with waters that has same temperature as river. The wild fish was fed

119 "Vitalis Røye" diet from Skretting AS
120 (https://www.skretting.com/nb-NO/produkter/vitalis-r-ye/476027), which has an EPA + DHA
121 content of 19-20 % of the fat, and 70 % of the ingredients are of marine origin. Approximately
122 1300 newly fertilized eggs of both domesticated and wild salmon were transported to hatching
123 tanks in Ervik hatchery (Frøya, Norway). The water temperature of hatching tanks for
124 domesticated and wild eggs were slightly different to ensure that both strains hatched and
125 start-feed at the same time.
126
127 When the yolk sac was depleted, the wild and domestic salmon strains were separated into 12
128 tanks (2 fish strains x 3 diet treatments x 2 replicate tanks) with 100L water and 200 fish per
129 tank. Feeding was initiated from the next day. The experimental tanks were randomly
130 distributed in the hatchery and the fish of each tank were reared under same temperature,
131 continuous light and fed 24h continuously feed every day. The fish was given three contrasting
132 diets, either a fish oil (FO) diet high in LC-PUFA, or a plant and vegetable oil (VO) enriched
133 diet low in LC-PUFA, or a marine phospholipid (PL) enriched diet with medium level of
134 LC-PUFA but rich in PL (Table 1). All three diets were given to the fish from start feeding up to
135 94 days. To ensure sufficient DHA and EPA levels the PL used to prepare PL diet was a 50/50
136 mixture of krill oil (Aker BioMarine AS, Lysaker, Norway) and herring roe oil (kindly provided
137 by Erik Løvaas from Marine BioExploitation AS, Tromsø, Norway). The feeds were produced
138 by Sparos AS (Olhão, Portugal). The composition of the diets is shown in Supplementary Table

1. FO diets have higher DHA and ARA than PL diet, while the EPA composition was similar between the two diets (Table 1). VO diet contains higher 18:3n-3 and 18:2n-6 but lower DHA, EPA and ARA compared to the other two diets.

Fish was sampled after 94 days of feeding. The fish were sacrificed by exposure to 200 mg/ml Benzoak vet. (ACD Pharmaceuticals AS, Oslo, Norway), then immediately put in sterile pertri dishes and dissected under a dissecting microscope. The pyloric caeca and liver tissues were immediately transferred into 2mL Eppendorf tubes, and either filled with RNAlater and put on ice for RNA isolation, or frozen in dry ice for lipid extraction. Tissues for RNA isolation were kept at 4°C for 24h to allow sufficient penetration of the solution into the tissues, and then kept at -80 °C until RNA extraction. Tissues for lipid extraction were directly transferred to -80 °C after sampling.

2.2 RNA isolation and transcriptomic sequencing

Four individual fish from each tank were used for RNA isolation. The RNA extraction was performed with the RNeasy Plus Universal Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The concentration and integrity of RNA were determined by a Nanodrop 8000 (Thermo Fisher Scientific, Waltham, USA) and a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA), respectively. All RNA samples had RNA integrity (RIN)

159 values higher than 8, which is sufficient for RNA sequencing. Sequencing libraries were
160 prepared with a TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, USA)
161 according to the manufacturer's protocol. Libraries were sequenced using 100bp single-end
162 mRNA sequencing (RNA-seq) on Illumina Hiseq 2500 (Illumina, San Diego, CA, USA) at the
163 Norwegian Sequencing Centre (Oslo, Norway).
164
165 The method for handling RNA-sequencing (RNA-seq) data has been described in detail in
166 previous studies (Gillard et al., 2018; Jin et al., 2018). In brief, read sequences were quality
167 trimmed using Cutadapt (v1.8.1) before being aligned to the salmon genome (ICSASG_v2).
168 Raw genes counts were generated using HTSeq-counts (v0.6.1pl) and the NCBI salmon
169 genome annotation (available for download at
170 http://salmobase.org/Downloads/Salmo_salar-annotation.gff3).
171
172 2.3 Lipid class separation and fatty acid analysis
173
174 Total lipid was extracted from two individual fish from each tank by using the method of
175 Folch, Lees, and Stanley (1957). Extracted total lipid was then applied onto 10 x 10 cm silica
176 plates (Merck, Darmstadt, Germany) and separated by using methyl
177 acetate/isopropanol/chloroform/methanol/0.25% KCl (25:25:25:10:9, by vol) for polar lipids
178 and hexane/diethyl ether/glacial acetic acid (80:20:2, by vol) for neutral lipids (R. E. Olsen &

179 Henderson, 1989). To avoid the oxidation of fatty acids, the plates were exposed to iodine
180 vapor to visualize the lipid class for fatty acids analysis (Li & Olsen, 2017). Lipid bands of
181 phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn) and triacylglycerol (TAG)
182 were separately scrapped out into 10mL glass tubes. Fatty acid methyl esters (FAME) of each
183 lipid class were prepared by acid-catalyzed transesterification at 50˚C for 16 hours (Christie,
184 1973) before quantified by a Agilent 7890B gas chromatograph with flame ionization detector
185 (Agilent Technologies, Santa Clara, CA).
186
187 2.4 Data analysis
188
189 The analysis of RNA-seq data was performed in R (v3.4.1) (Team, 2013). Only genes with a
190 minimum counts level of at least 1 count per million (CPM) in more than 25% of samples from
191 each tissue were kept for differential expressional analysis. Differential expression was tested
192 separately on pyloric caeca and liver using R package edgeR (Robinson, McCarthy, & Smyth,
193 2010). A full interaction model (Diet + Strain + Diet x Strain) was used in each tissue
194 separately to find differential expressed genes (DEGs) between wild and domesticated salmon
195 under any dietary treatments. Genes with a false discovery rate (FDR), an adjusted *p* value (*q*)
196 <0.05 and absolute log2 fold change (|Log2FC|) > 1 were considered to be differentially
197 expressed genes (DEGs) between any test conditions. KEGG ontology enrichment analysis
198 (KOEA) was conducted using edgeR. Significant values ($p<0.05$) were generated based on

hypergeometric test where the number of DEGs was compared to total genes annotated to each KO term. A test for enrichments of transcription factor binding sites (TFBS) motifs in the promoter regions (between -1000bp and 100bp from transcription starting sties) of salmon genes was done by using a hypergeometric test in the R package SalMotifDB, which is interacting with a database of transcription factor binding sites for salmonids (https://salmobase.org/apps/SalMotifDB) (Mulugeta et al., 2019).

To further investigate diet specific effect on gene expression between wild and domesticated salmon, samples of different diet were separated to be used for testing differential expression of genes between wild and domesticated salmon under each diet. Same cut-off was used (q<0.05 & |Log2FC| > 1) to identify DEGs. For visualize expression levels between different genes and tissues, normalized counts in the form of transcripts per million (TPM) values were generated. Raw gene counts were first divided by their mRNA length in kilobases to normalize for transcript length, and then divided by the total number of counts from each library to normalize for sequencing depth (Jin et al., 2018).

Statistical analysis of fish weight and fatty acids composition was also performed in R. Two-way ANOVA with Tukey HSD post hoc test was used to test the effect of strain and diet on fish weight and fatty acids composition of each lipid class, tissue and sampling day. Differences were considered significant when $p < 0.05$.

3. Results

3.1 Growth and development

The domesticated salmon was significantly larger than wild salmon at all sampling times (Figure 1 and Supplementary Table 2). At the end of the trial (94 days), domesticated salmon reached an average 4.5g, while wild salmon had a mean size of 2.6g. There were no significant differences in weight between domesticated salmon fed FO, VO and PL enriched diets. The growth of wild fish appeared more sensitive to different diets, with VO-fed fish being smaller than PL-fed fish at day 65 (p = 0.03) and day 94 (p = 0.02). FO-fed wild salmon showed intermediate weights, but their weights were not significantly different from either FO or PL diets. A complete table of fish weight and statistics from all time points is found in Supplementary Table 2.

3.2 Transcriptomic differences between domesticated and wild salmon

On average 20 million reads were generated from each sample (min: 12M reads, max:32M reads), with ~85% of the reads mapping to the salmon genome. Out of 81597 annotated loci, 28980 and 24119 genes passed this filtering criteria in pyloric caeca and liver, respectively.

239 Principle component analysis (PCA) on Log2 CPM of the top 1000 most variable genes,
240 identified a clear separation of domesticated and wild salmon in both pyloric caeca and liver
241 (Figure 2).
242
243 A full interaction model design in edgeR (Chen, McCarthy, Robinson, & Smyth, 2014) was
244 applied to test differences in gene expression between domesticated and wild salmon across
245 all dietary treatments on day 94. This resulted in identification of 187 differential expressed
246 genes (DEGs) in pyloric caeca and 379 DEGs in liver between wild and domesticated salmon.
247 The complete list of DEGs in pyloric caeca and liver is shown in Supplementary Table 3. By
248 mapping DEGs to the KEGG database of metabolic pathways, we have identified 17
249 pathways that were significantly enriched (p<0.05) in pyloric caeca, while 11 pathways were
250 enriched in liver (Figure 3 A and Supplementary Table 4). The DEGs in pyloric caeca were
251 enriched in pathways for glycerophospholipid, glycosphingolipid and glycosaminoglycan
252 metabolism, which are known to be major component of cell membrane (Figure 3 A). A
253 number of cell-signalling pathways were also enriched, including phosphatidylinositol
254 signalling, calcium signalling, apelin signalling, C-type lectin receptor signalling and GnRH
255 signalling pathways. In liver, the DEGs were enriched in metabolic pathways including
256 linoleic acid, glycolysis and gluconeogenesis, fructose and mannose, cysteine and methionine,
257 retinol metabolism pathways (Figure 3 A). Enrich genes involved in intercellular interactions

included cytokine-cytokine receptor interaction, neuroactive ligand-receptor interaction and gap junction pathways.

Transcription factor binding site (TFBS) motif enrichment analysis on promoters (-1000bp to 200bp from TSS) of DEGs for each tissue resulted in 16 significant (p<0.005) enriched motifs in pyloric caeca and 128 enriched motifs in liver (Supplementary Table 4). The most enriched motif in pyloric caeca was BHLHB2, which is known to be involved in circadian regulation (Figure 3 B) (Dunlap, 1999). Several other enriched motifs are associated with intestinal development and cell differentiation, including ETV2 (Jedlicka & Gutierrez-Hartmann, 2008), ATOH1 (Shroyer et al., 2007), GR (Lebenthal & Lebenthal, 1999), GATA-1 (Kanki et al., 2017). The most enriched TFBS motif in liver was a CLOCK motif, which is a predicted binding motif for the master regulator of the circadian clock (Dunlap, 1999). Similar to pyloric caeca, BHLHB2 motif was also identified in the top 10 most enriched TFBS motifs in liver. In addition, three lipid metabolism related motifs (RXRA, PPARG, PLAGL2) populated the top-10 enriched TFBS list (Tontonoz, Hu, & Spiegelman, 1994; Van Dyck et al., 2007).

To further investigate differences in expression of genes linked to circadian rhythm between wild and domesticated salmon, we compared the expression of key genes encoding circadian clock related transcription factors *clock*, *nr1d1*, *bmal1*, *bhlhb2*, *per*, and *cry* (Figure 3 C and Supplementary Figure 1). A systematic difference in circadian clock gene expression was

278 observed between livers of wild and domesticated salmon (Figure 3 C and Supplementary
279 Figure 1), although not all genes were significant regulated at $q<0.05$. Nevertheless, the
280 regulators (*cry2-c*, *nr1d1-a, per1-a, bhlhb2-d* and *nr1d1-a* genes) acting as suppressors of the
281 master regulators of circadian rhythm (CLOCK/BMAL) were consistently lower expressed in
282 domesticated salmon compare to wild (Figure 3 C). However, similar expression levels of
283 *clock* (logFC=0.2, $q=0.7$) and *bmal1* (logFC=-0.1, $q=0.8$) genes which encodes master
284 regulator were found between domesticated and wild salmon (Figure 3 C and Supplementary
285 Figure 1). No difference in circadian clock gene expression was observed between pyloric
286 caeca of wild and domesticated salmon.
287
288 **3.4 Differential regulation of lipid metabolism genes between domesticated and wild**
289 **salmon**
290
291 To better understand the effect of diets on gene expression differences between domesticated
292 and wild salmon, we compared gene expression separately between domesticated and wild
293 under each diet. In pyloric caeca, a total number of 230 DEGs were identified between
294 domesticated and wild salmon with FO diet, 164 DEGs were found with VO diet and 689
295 DEGs were found with PL diet (Supplementary Table 5). Out of these DEGs, only 8 genes
296 were involved in lipid metabolism pathways. This includes *ptdss2* genes of phosphatidylserine
297 synthesis, which was significantly ($q<0.05$ & $|Log2FC|>1$) higher expressed in domesticated

298 salmon regardless of the dietary treatment (Figure 4). Two phosphatidylethanolamine
299 synthesis genes, *pcyt2c-a* and *pcyt2c-b* were both higher expressed in domesticated than wild
300 salmon when fed FO or VO diet, while no difference in gene expression was found when the
301 fish was given PL diet. On the other hand, *etnk2-a* gene involved in
302 phosphatidylethanolamine synthesis, was significantly higher expressed in domesticated
303 compared to wild salmon only when the fish was given PL diet. Feeding with VO induced key
304 genes in LC-PUFA synthesis pathway (*fads2d5* and *fads2d6a*, see Figure 4*)* in both
305 domesticated and wild salmon. However, no expression difference was observed for these two
306 genes, or any other LC-PUFA synthesis genes between domesticated and wild salmon for any
307 dietary treatment (Figure 4 and Supplementary Table 5).
308
309 The number of DEGs between liver of domesticated and wild salmon under each diet was 591
310 (FO), 179 (VO) and 243 (PL) (Supplementary Table 5). Liver had more DEGs involved in
311 lipid metabolism (28) compared to pyloric caeca (8). Four DEGs in liver had significantly
312 (q<0.05 & |Log2FC|>1) higher expression in domesticated compared to wild salmon under
313 VO diet, but not under FO or PL diet (Figure 5 B). This includes genes with key functions in
314 LC-PUFA synthesis (*fads2d5*, Log2FC = 1 & q = 0.02), gene involved in acyl-CoA synthesis
315 (*acsbg2b-b*, Log2FC = 1.4 & q = 0.03*)*, and fatty acid transport (*fabp7b*, Log2FC = 3.6 & q =
316 0.02). Although not significant, domesticated salmon fed VO diet also had higher expression
317 of *fads2d6a* (Log2FC = 0.7 & q = 0.2) and *srebp1d* (Log2FC = 0.8 & q = 0.2) compared to

318 wild salmon fed same diet, while the expression difference of the two genes was negligible
319 when the fish was under FO or PL diet (Figure 5 A & B). A key gene involved in conversion
320 of lipids to energy, *cpt1aa* was lower expressed (Log2FC = -1.2 & q = 0.01) in domesticated
321 salmon when fed VO diet. The regulator of fatty acid metabolism *pparg-b* was consistently
322 higher expressed in domesticated compared to wild salmon under all diets, but only
323 significantly different for salmon fed FO diet (Figure 5 A).
324
325 In addition to the DEGs of fatty acid metabolism, 5 DEGs involved in phospholipid,
326 cholesterol and triacylglycerol metabolism were found between domesticated and wild salmon
327 (Figure 5 C). This included the *apoa1-b* gene involved in lipoprotein synthesis and lipid
328 transport, which was strongly higher expressed in domesticated salmon than wild, regardless
329 of dietary treatment (Log2 FC > 3 & q<0.001, see Figure 5 C and Supplementary Table 5). A
330 key gene involved in synthesis of bile acid (*cyp7a1-a*), which is responsible for removal of
331 cholesterol in liver, was higher expressed in domesticated salmon when given PL diet. Gene
332 *ptdss2* involved in synthesis of phosphatidylserine, which is a major phospholipid in salmon,
333 was higher expressed in domesticated salmon than wild fed VO diet (Log2 FC = 1.9, q =
334 0.0008), though similar trend was also found when the fish was given FO (Log2 FC = 1, q =
335 0.09) or PL diet (Log2 FC = 0.9, q = 0.2). The expression of *hsl* gene involved in hydrolysing
336 triacylglycerol (stored fat) to diacylglycerol, and diacylglycerol to monoacylglycerol was
337 generally higher expressed in domesticated salmon than wild. On the other hand, the

338 expression of *mgll* involved in hydrolysing monoacylglycerol into free fatty acids was lower
339 expressed in domesticated salmon. (Figure 5 C). In conclusion, the direct comparison of the
340 transcriptomes of domesticated and wild salmon suggests that domestic salmon have boosted
341 expression of genes involved in many aspects of lipid metabolism such as transport,
342 endogenous synthesis and conversion of lipids and fatty acids in both gut and liver
343 (Supplementary table 5).
344
345 To further investigate differences in the plasticity of fatty acid metabolism between
346 domesticated and wild salmon, we analysed differences in putative compensatory shifts in gene
347 regulation under diets with low (VO) vs high (FO) levels of LC-PUFA for wild and
348 domesticated salmon separately. These analyses identified 38 DEGs in domesticated and 2
349 DEGs in wild salmon (Supplementary Table 5). However, only DEGs in domestic salmon (9
350 genes) were linked to lipid metabolism, specifically involved in fatty acyl-CoA synthesis (2
351 genes), LC-PUFA synthesis (2 genes), lipogenesis (2 genes), and transcriptional regulation of
352 lipid metabolism (2 genes) (Table 2).
353
354 3.5 Comparison of fatty acid composition between domesticated and wild salmon
355
356 To assess the differences between fish at the metabolite levels, we also measured fatty acid
357 content in liver and pyloric caeca of wild and domesticated salmon at day 94 (Supplementary

358 Table 6). The results showed that variation in fatty acids composition was generally more
359 driven by diet than strain. About 85% of the fatty acid content in liver and pyloric caeca differed
360 between diets, but only 32% of the fatty acids differed in levels between wild and domesticated
361 salmon (p<0.05, Supplementary Table 6). Both wild and domesticated salmon given the VO
362 diet showed higher levels of 18:3n-3 and 18:2n-6 contents in both liver and pyloric caeca but
363 lower contents of the longer chain fatty acids (ARA, EPA & DHA) compared to both FO and
364 PL diets (Figure 6). This pattern was consistent for all three lipid classes analysed (PtdCho,
365 PtdEtn, and TAG). Although the differences in fatty acids content were generally small
366 between wild and domesticated salmon fed the same diet, wild fish contained higher content of
367 18:2n6 (9.1% in wild *versus* 7.3% in domesticated fish, p = 0.06) and 18:3n3 (2.3% *versus*
368 1.5%, p = 0.006) in PtdEtn of liver when fed VO diet. Wild salmon also had higher content of
369 18:3n3 (2.1% *versus* 1.8%, p = 0.04) in PtdCho of liver when fed VO diet. On the other hand,
370 wild salmon had significantly lower content of ARA in both PtdCho (1.6 % *versus* 2.1%, p =
371 0.02) and PtdEtn (3.1% *versus* 4.3%, p = 0.02) of liver than wild fish when fed VO diet. No
372 significant differences in DHA and EPA contents were found between domesticated and wild
373 salmon fed the same diets.
374
375 **4 Discussion**
376

377 The present study aimed to improve our understanding of the differences in metabolism of
378 domesticated and wild salmon, with an emphasis on lipid metabolism. We approached this
379 question using comparative analyses of growth, liver and gut transcriptomes, and lipid
380 composition between domesticated and non-domesticated wild salmon given diets with three
381 different lipid composition.
382
383 4.1 The domestic metabolic syndrome and the link to the circadian clock pathway
384
385 As expected, domesticated salmon grew faster, both before and after start feeding, likely
386 reflecting a higher basal metabolic rate (Bicskei, Bron, Glover, & Taggart, 2014) and higher
387 feed intake and feed conversion efficiency (Thodesen et al., 1999) in domestic salmon. In line
388 with this we found a clear difference in expression of genes involved in basal metabolism in
389 liver between domesticated and wild salmon (Figure 3 A). In pyloric caeca, gene expression
390 between wild and domesticated fish was associated with several signaling pathways and
391 glycolipid synthesis (glycosphingolipids and glycophospholipid) which are main components
392 of cell membranes. The promoters of these genes were enriched in TFBS motifs (ETV2,
393 ATOH1, GR GATA-1) known to be involved in intestinal development and cell differentiation
394 (Jedlicka & Gutierrez-Hartmann, 2008; Kanki et al., 2017; Lebenthal & Lebenthal, 1999)
395 (Figure 3 B). One likely explanation for these transcriptomic differences in gut tissue is the

396 difference in growth rates and/or feed intake between domestic and wild fish domesticated
397 (Thodesen et al., 1999).
398
399 The enrichment of CLOCK and BHLHB2 motifs in promoters of DEGs (Figure 3) suggests a
400 link between the circadian clock pathway and gene expression differences observed between
401 wild and domesticated salmon (Dunlap, 1999). This finding is interesting as many studies on
402 mammals suggest a functional link between the circadian clock and regulation of feed intake,
403 metabolic rates, and glucose and lipid metabolism (Paschos, 2015; Rudic et al., 2004). The top
404 regulators of circadian oscillations are thought to be CLOCK and BMAL1 transcription factors
405 which form a heterodimer and activate expression of downstream target (Lowrey & Takahashi,
406 2000). CLOCK/BMAL are involved in regulating (directly or indirectly) a multitude of
407 downstream processes, including genes of metabolism as well as genes that maintain the
408 circadian oscillation of CLOCK/BMAL through negative feedback loops (Lowrey &
409 Takahashi, 2000; Preitner et al., 2002; Takahashi, 2015). Our study does unfortunately not
410 include samples that can shed light on differences between wild and domestic salmon circadian
411 oscillations. However, we find that domesticated salmon generally has lower expression of
412 genes encoding negative regulators of the CLOCK/BMAL (Figure 3 C) (Lowrey & Takahashi,
413 2000; Preitner et al., 2002; Takahashi, 2015). In this context it is also interesting to note that the
414 PPAR-RXR heterodimer, a major regulator of glucose (Jones et al., 2005) and lipid (Kliewer et
415 al., 1997) homeostasis, is known to be under circadian rhythmicity in salmon (Betancor et al.,

2014). The *pparg* gene is consistently higher expressed in domesticated compared to wild salmon (Figure 5) and both PPAR and RXR are predicted to be enriched in promoters of genes differentially expressed in wild and domestic salmon (Figure 3 B). This result is in agreement with the tendency of higher clock gene expression in domesticated fish. It is important to note that all samples used for the gene expression were sampled between morning and noon within a 2h time period. Moreover, fish were raised under constant light and continuous feeding which is known to abolish daily rhythmicity for both *nr1d1* (Betancor et al., 2014) and *cry-2* (Huang, Ruoff, & Fjelldal, 2010). We are thus confident that sampling bias related to daily rhythms has not impacted our results.

In conclusion, we speculate that the 'domestic metabolic syndrome' in salmon, characterized by increased basal metabolism and high growth (Bicskei et al., 2014; Tymchuk, Sakhrani, & Devlin, 2009), is partially caused by novel regulation or function of the circadian clock pathway. An interesting hypothesis is that strong selection on 'fast growers' during salmon domestication have selected for salmon genotypes that have impacted the circadian clock regulation and thereby induced changes in appetite and metabolism.

4.2 Differences in regulation of lipid metabolism between domesticated and wild salmon

435 In the present study we fed the fish three diets with different fatty acid composition to explore
436 the idea that domestication has changed regulation of lipid metabolism in salmon. We have
437 demonstrated that growth of domesticated salmon was virtually unaffected (Figure 1) by dietary
438 fatty acid composition in the diet, while wild salmon was. This suggest that domesticated
439 salmon have more effective lipid absorption, lipid transport, and better ability to conduct
440 endogenous conversion and synthesis of lipids to compensate for dietary shortage of essential
441 fatty acids. In line with this idea, our study found that *apoa1_2* gene was strikingly higher
442 expressed in liver in domesticated compared to wild salmon, regardless of dietary lipid
443 composition (Figure 5). Although the existence of lipoproteins and their apolipoprotein
444 composition is not well described in fish, it is suggested that they should be similar to mammals
445 (Jin et al., 2018). The higher expression levels of *apoa1_2* transcripts could therefore be one
446 factor that contributes to fast growth of domesticated salmon, as this gene is key to the
447 lipoprotein assembly and thereby transport of lipids between liver and other tissues such as
448 muscle and adipose tissue (Xu et al., 2013). We also observed higher expression of hormone
449 sensitive lipase gene (*hsl*) in liver of domesticated salmon than in wild. This suggests that
450 domesticated salmon has higher ability of hydrolysing triacylglycerol, diacylglycerol and
451 cholesterol ester into monoacylglycerol and free fatty acids (Kraemer & Shen, 2002; Quiroga &
452 Lehner, 2012). The hydrolysed monoacylglycerol and fatty acids were more likely to be used in
453 re-synthesis of lipids rather than further degraded to produce energy, because key genes
454 involved in fatty acid degradation, the *mgll* gene involved in hydrolysing monoacylglycerol and

455 *cpt* genes involved in transporting fatty acids into the mitochondria for *β*-oxidation, were lower
456 expressed in domesticated salmon than wild.

457 Wild fish fed the PL diet had the fastest growth rate, which supports previous findings that
458 dietary inclusion of PL provides juvenile salmon better growth and development (Poston, 1990;
459 Taylor et al., 2015). This is likely a consequence of PL promoting the absorption and transport
460 of dietary lipid especially LC-PUFA (Carmona-Antonanzas, Taylor, Martinez-Rubio, &
461 Tocher, 2015; Rolf Erik Olsen, Tore Dragnes, Myklebust, & Ringø, 2003; Y. Olsen et al.,
462 2014a; D. R. Tocher, E. Å. Bendiksen, P. J. Campbell, & J. G. Bell, 2008). Young salmon have
463 low capacity of *de-novo* synthesis of PL and may therefore struggle to maintain sufficient lipid
464 levels for high growth (Carmona-Antonanzas et al., 2015; Jin et al., 2018; Rolf Erik Olsen et al.,
465 2003; Y. Olsen et al., 2014b; D. R. Tocher, E. A. Bendiksen, P. J. Campbell, & J. G. Bell,
466 2008). In the present study, we identified higher expression of 2 phosphate cytidyltransferase
467 genes (*pcyt2c-a* and *pcyt2c-b*) in pyloric caeca of domesticated than wild salmon only when fish
468 was given FO or VO diets, while no difference of gene expression was found when the fish was
469 fed the PL diet. This suggests that domesticated salmon can increase the expression of genes
470 involved in PL biosynthesis to compensate for the dietary shortage of PL, while such ability is
471 limited in wild salmon at the same age. The expression of phosphatidylserine synthase gene
472 (*ptdss2*) was generally higher expressed in both pyloric caeca and liver of domesticated salmon
473 regardless of dietary treatment. The reason for the higher requirements of phosphatidylserine in
474 domesticated salmon is not clear. One of the reasons could be that phosphatidylserine is a

475 constituent of the cell membrane, which is required when there is a potential higher level of cell
476 proliferation in domesticated salmon.
477
478 Domesticated salmon are more adapted to the VO diet compared to wild strains. This was
479 clearly revealed in the growth data where dietary VO significantly affected growth and final
480 weight of wild salmon, but had no effect on the growth of domesticated salmon. It is generally
481 assumed that a VO enriched diet can induce the expression of LC-PUFA synthesis genes in
482 salmon (Stubhaug et al., 2005; Zheng et al., 2004). However, the key genes in the LC-PUFA
483 synthesis pathway, *fads2d5* and *fads2d6a* were up-regulated only in domesticated salmon when
484 fed the VO diet. This suggests that domesticated salmon is able to modify its fatty acid
485 metabolism to compensate for the shortage of essential LC-PUFA in the diet, while such ability
486 is very low in wild salmon. Although previous generations of domesticated salmon were fed
487 VO diet only after transition to seawater, we still observed shifted expression of lipid
488 metabolism domesticated but not wild salmon at freshwater stage. This suggests that the
489 adaption to VO diet in domesticated salmon is independent of life stages. The present study
490 showed higher contents of DHA, ARA and EPA and lower contents of 18:3n3 and 18:2n6 in
491 phospholipid of liver in domesticated salmon than wild only when they were given VO diet.
492 This was most likely an effect of higher LC-PUFA synthesis ability in domesticated salmon.
493 Sterol regulatory binding protein 1 (SREBP1) is believed to be the key regulator of fatty acid
494 metabolism and their expression is influenced by LC-PUFA level in the cell (Datsomor et al.,

2019). We observed an up-regulation of the *srebp1d* gene in domesticated salmon, but not in wild fish when fed VO diet. Acyl-CoA synthase (*acsbg2b-b*) genes were also up-regulated, which was believed to be targeted by SREBP1 transcription factor (Datsomor et al., 2019).

In conclusion, the present study has provided a broad overview on transcriptomic and fatty acids differences in wild and domesticated salmon fed either FO, VO or PL enriched diets during early stages of development. The higher growth and development of domesticated salmon was in agreement with a combination of various genetic advantages including better uptake, transport and endogenesis of lipids. This was associated with altered circadian clock regulation between domesticated and wild salmon, which could possibly explain the changes in appetite and metabolism. Moreover, domesticated salmon had higher plasticity on gene expression when fed a VO diet with less essential LC-PUFA, while this ability is very low in wild salmon as the growth of fish significantly dropped for the VO diet. However, further experiment on circadian oscillations is required to support the differential expression of circadian genes found in present study. Other layers of genetic and biochemical information are also needed to get an in-depth and complete understanding of divergence of salmon after domestication. We suggest that future studies should focus on comparing metabolites, lipidome and proteome between domesticated and wild salmon.

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Data Accessibility Statement

Raw sequences are publicly available on ArrayExpress under accession number E-MATB-8306.

Author Contributions

Yang Jin, Yngvar Olsen, Rolf Erik Olsen, Simen Rød Sandve and Olav Vadstein designed and performed the research. Yang Jin and Thomas Nelson Harvey performed the transcriptomic analysis. Yang Jin and Keshuai Li performed the lipid and fatty acid analysis. Jon Olav Vik and Simen Rød Sandve guided the transcriptomic analysis and revised the manuscript. Yngvar Olsen and Rolf Erik Olsen guided the lipid analysis and revised the manuscript. Mari-Ann Østensen and Nina Santi provided input on the experimental design, carried out the experiment and sampling and reviewed the manuscript. All authors participated in the revision of this paper by providing comments and editing.

Table 1 Percent of fatty acids in total fatty acids of three diets rich in fish oil (FO), vegetable and plant oil (VO), or vegetable and marine phospholipid oil (PL).

	FO	VO	PL
14:0	3.6±0.0	0.9±0.0	3.7±0.0
16:0	18±0.2	20±0.2	16±0.2
16:1n-7	4.3±0.0	0.8±0.0	3.1±0.0
18:0	4.4±0.1	3.7±0.1	3.5±0.5
18:1n-9	14±0.1	26±0.3	22±0.1
18:1n-7	2.5±0.0	7.1±0.2	3.2±0.0
18:2n-6	6.8±0.0	15±0.2	11±0.1
18:3n-3	1.2±0.0	11±0.2	3.1±0.0
20:1n-9	2.5±0.0	1.7±0.0	2.4±0.0
20:4n-6	1.3±0.0	0.3±0.0	0.7±0.0
20:5n-3	7.7±0.0	1.7±0.0	7.3±0.1
22:1n-9	2.2±0.0	1.2±0.0	1.8±0.0
22:6n-3	17±0.0	3.7±0.1	11±0.1

Data is shown in mean ± sd (n=2).

Table 2 Log2 fold change and adjusted *p* value (*q*) of lipid gene expression in liver of domesticated/wild salmon feeding vegetable oil (VO) diet compared to fish oil (FO).

	Farm VOvsFO		Wild VOvsFO	
genename	logFC	*q*	logFC	*q*
acsbg2b-b	1.7	0.01	1.0	0.64
acsl1a-a	1.6	0.02	1.0	0.64
agpat3a-b	1.4	0.002	1.2	0.09
agpat3b-a	1.1	0.01	0.5	0.72
fabp7b	6.0	0.001	4.2	0.13
fads2d6a	1.3	0.008	0.8	0.54
fads2d5	1.2	0.01	0.2	1.00
srebp1c	1.4	0.03	0.9	0.62
srebp1d	1.6	0.003	0.5	0.88

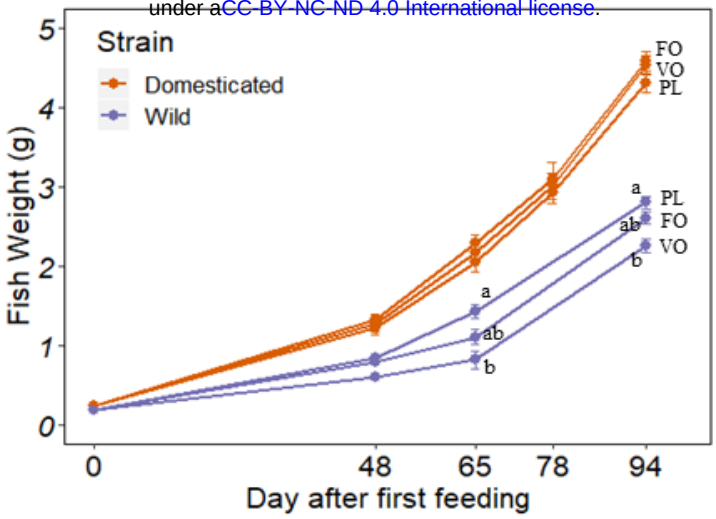

Figure 1 Weight of domesticated and wild salmon fed diets high in fish oil (FO), vegetable oil (VO) or phospholipid oil (PL) during early stages of development. Data are means ± SE (n>100 per group at day 93, n>20 at other days). Different letters indicate significant (p<0.05) different of fish weight between wild fish fed FO, VO and PL diet at day 65 and 94.

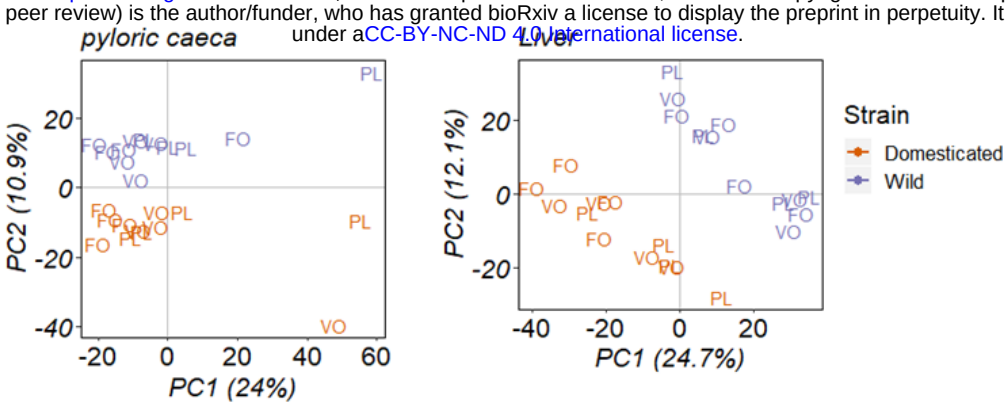

Figure 2 Score plot of PCA on log2 count per million (CPM) of the top 1000 most variant genes across all samples (4 replicates x 2 strains x 3 Diets). Two salmon strains (domesticated and wild) were fed either fish oil (FO), vegetable oil (VO) or phospholipid (PL) rich diets from initial feeding. Pyloric caeca and liver samples were taken after 94 days of feeding.

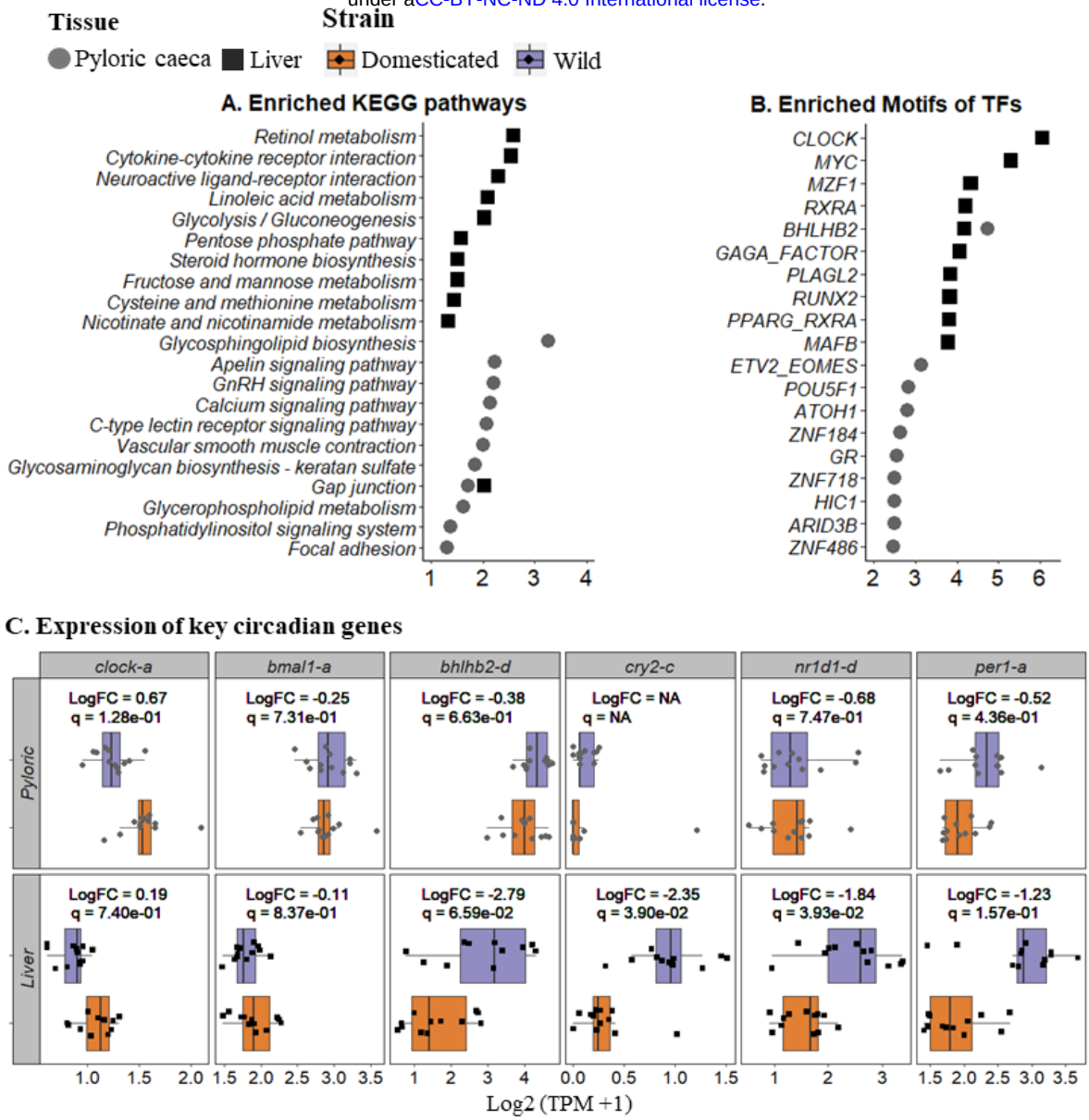

Figure 3 Differential expressed genes (DEGs) between domesticated and wild salmon. A) KEGG enrichment shows significant (p<0.05) enriched pathway, and proportion (%) of up/down regulated DEGs in each pathway. **B)** Motif enrichment analysis shows top 10 most significantly (p<0.005) enriched motifs of transcription factors in promoter regions (-1000bp to 200bp from TSS) of DEGs as compared to all expressed genes in pyloric caeca and liver. Hypergeometric test was applied on both KEGG and motif enrichment analyses, by comparing the number of DEGs to total genes annotated to each KEGG pathway or each motif. Motif enrichment analysis was done by using SalMotifDB (https://salmobase.org/apps/SalMotifDB). **C)** Expression of key circadian genes in pyloric caeca and liver of domesticated and wild salmon. Gene expression was shown in log2 transcript per million plus one (TPM +1). No statistics was shown for *cry2-c* gene in pyloric caeca, since the gene expression is too low (CPM <1) to be used for differential expression analysis.

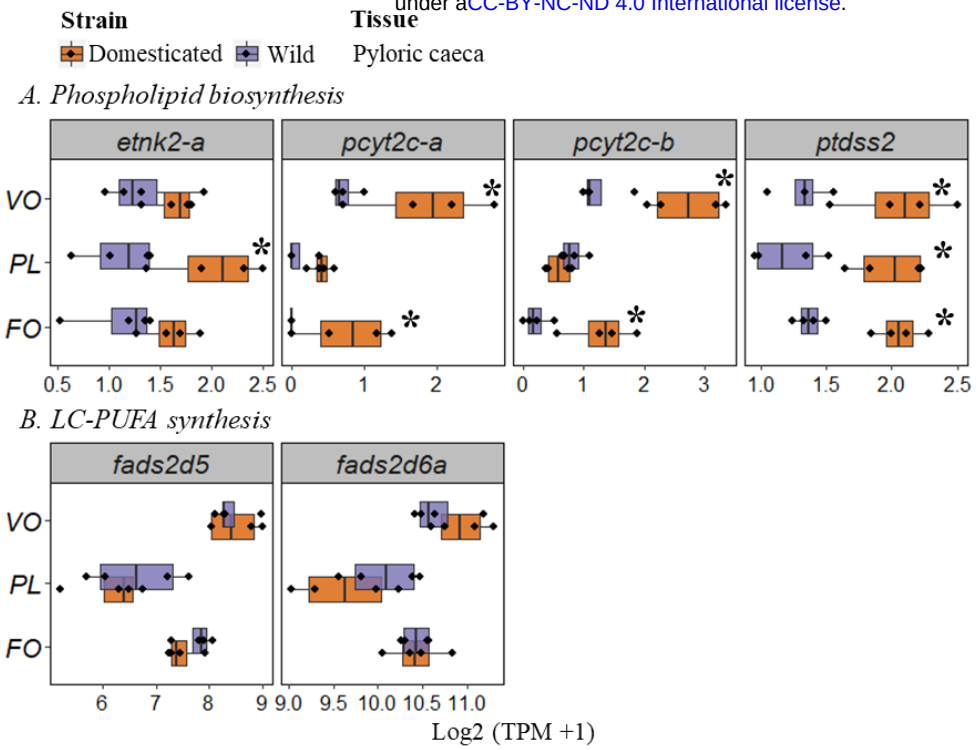

Figure 4 Expression of 6 genes involved in lipid metabolism in pyloric caeca of wild and domesticated salmon at day 94 after feeding either fish oil (FO), vegetable oil (VO) or phospholipid (PL) diets. Gene expression was shown as Log2 transcript per million plus one (TPM + 1) which was normalized by library size and mRNA length. Asterisk indicates differential expressed genes (DEGs, q<0.05 & |log2FC| >1) between domesticated and wild salmon under each dietary treatment.

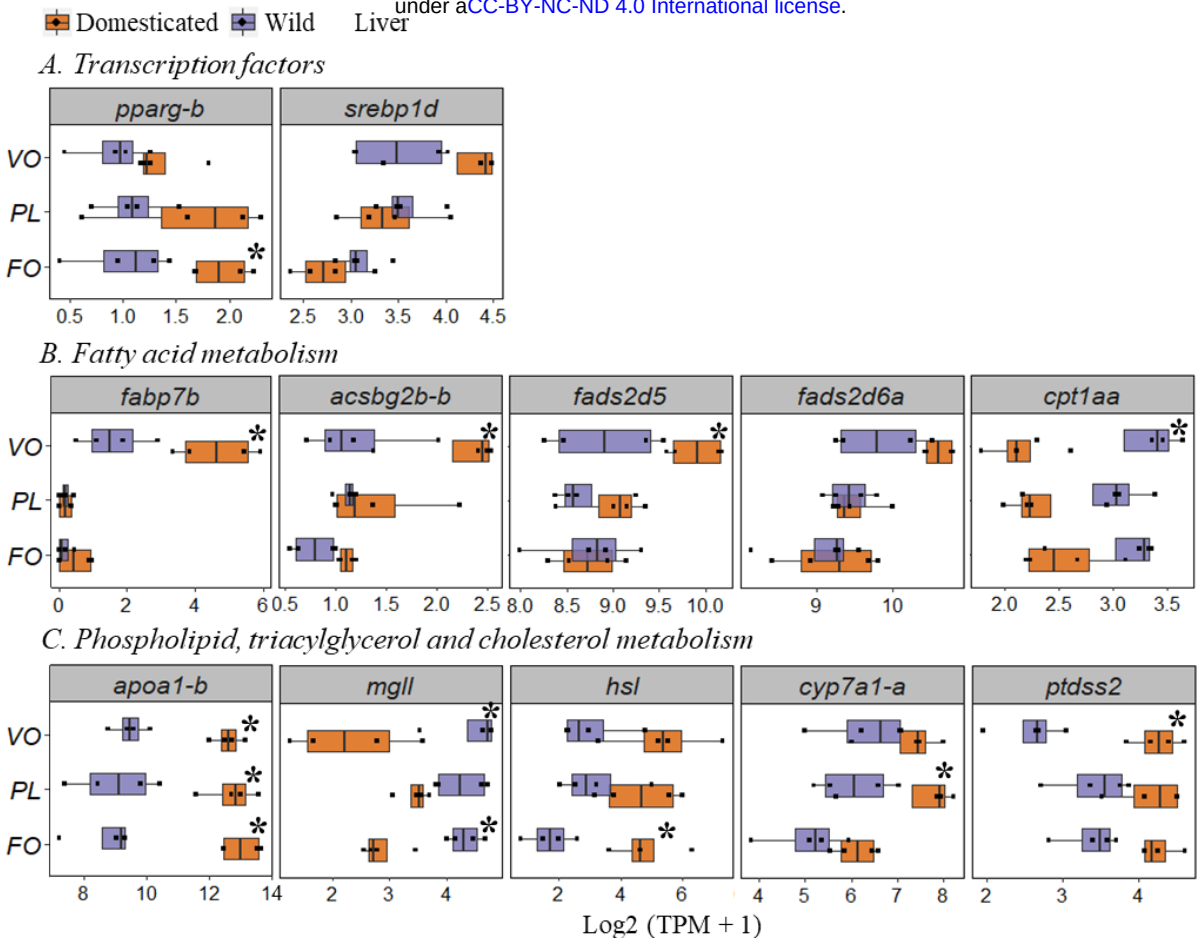

Figure 5 Expression of 14 genes involved in lipid metabolism in liver of wild and domesticated salmon at day 94 after feeding either fish oil (FO), vegetable oil (VO) or phospholipid (PL) diets. Gene expression was shown as Log2 transcript per million plus one (TPM + 1) which was normalized by library size and mRNA length. Asterisk indicates significant ($q<0.05$ & $|Log2FC|>1$) different of gene expression between domesticated and wild salmon under each dietary treatment separately.

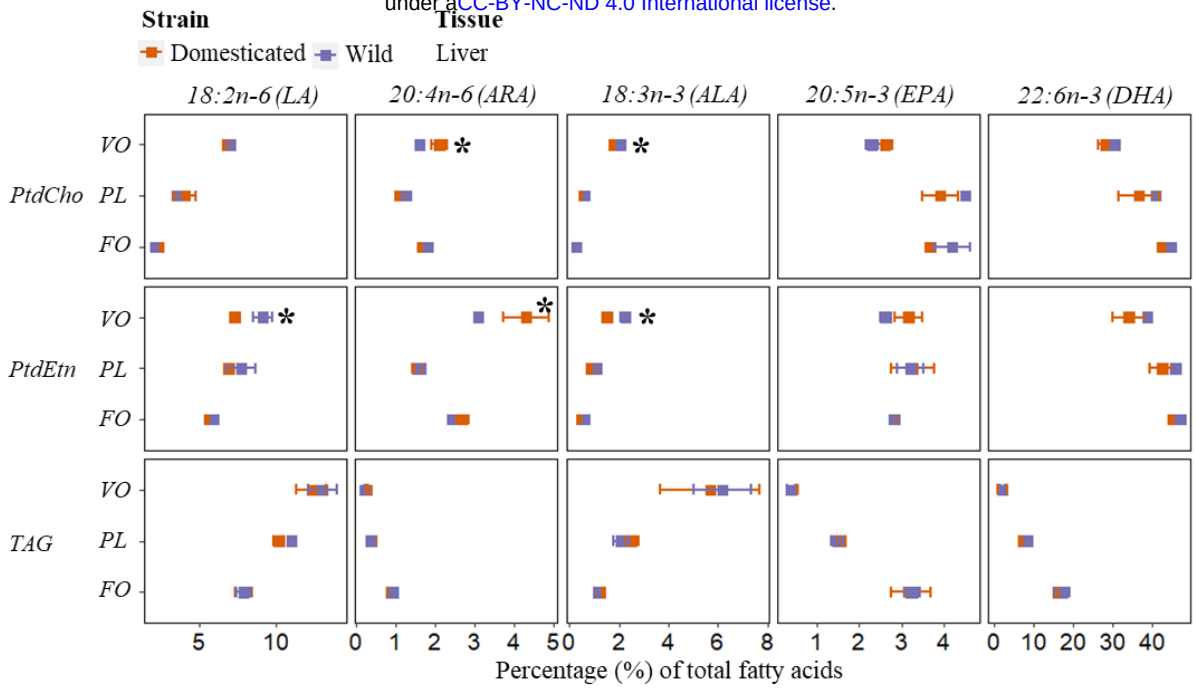

Figure 6 Percentage of liver fatty acid composition in triacylglycerol (TAG), phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn) of wild and domesticated salmon fed either fish oil (FO), vegetable oil (VO) and phospholipid (PL) rich diets at day 94. A two-way ANOVA was applied to test the fatty acid differences between fish strains and dietary treatment (strain*diet) separately in each lipid class. Tukey's HSD post-hoc test was then applied to test the fatty acid difference between each group. Asterix in the figure indicate significant (p<0.05) different of fatty acid between domesticated and wild salmon at certain day and dietary treatment. The composition of other fatty acids and their ANOVA test were shown in Supplementary 7.