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

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Abstract

Domestication of animals imposes strong targeted selection for desired traits but can also result in unintended selection due to new domestic environments. Atlantic salmon (*Salmo salmar*) was domesticated in the 1970s and has subsequently been selected for faster growth in systematic breeding programmes. More recently, salmon aquaculture has replaced fish oils (FOs) with vegetable oils (VOs) in feed, radically changing the levels of essential long-chain polyunsaturated fatty acids (LC-PUFAs). Our aim here was to study the impact of domestication on metabolism and explore the hypothesis that the shift to VO diets has unintentionally selected for a domestication-specific lipid metabolism. We conducted a 96-day feeding trial of domesticated and wild salmon fed diets based on FOs, VOs or phospholipids, and compared transcriptomes and fatty acids in tissues involved in lipid absorption (pyloric caeca) and lipid turnover and synthesis (liver). Domesticated salmon had faster growth and higher gene expression in glucose and lipid metabolism compared to wild fish, possibly linked to differences in regulation of circadian rhythm pathways. Only the domesticated salmon increased expression of LC-PUFA synthesis genes when given VOs. This transcriptome response difference was mirrored at the physiological level, with domesticated salmon having higher LC-PUFA levels but lower 18:3n-3 and 18:2n-6 levels. In line with this, the VO diet decreased growth rate in wild but not domesticated salmon. Our study revealed a clear impact of domestication on transcriptomic regulation linked to metabolism and suggests that unintentional selection in the domestic environment has resulted in evolution of stronger compensatory mechanisms to a diet low in LC-PUFAs.

KEYWORDS
circadian regulation, domestication, long-chain polyunsaturated fatty acids, transcriptomics, vegetable oil, wild salmon

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1 | INTRODUCTION

The genetics and physiology of domesticated animals are heavily influenced by the initial domestication process, the captive environment, followed by persistent targeted selection for desirable animal production traits such as faster growth and delayed sexual maturation (Mueller & Diamond, 2001; Zeder, 2015). In addition, domesticated animals evolve "domestication syndromes" linked to unintended selection due to the new domestic environments (Zeder, 2015). One such collateral variable that changes dramatically with domestication is feed and feeding regimes. Unlike wild animals which rely on opportunistic hunting and foraging for different foods, domesticated animals often receive standard artificial diets with balanced nutritional levels and regular feeding intervals. This dietary change has probably influenced standard metabolism in domesticated animals (Bicskei, Bron, Glover, & Taggart, 2014; López et al., 2019).

Atlantic salmon (*Salmo salmar*) was domesticated in 1971 and is considered a pioneer aquaculture species (Harache, 2002). Since its initial domestication, systematic breeding programmes have aimed to improve traits such as faster growth, delayed sex maturation, higher feed conversion rate, as well as many other traits important for animal production (Gjedrem, Gjøen, & Gjerde, 1991; Powell, White, Guy, & Brotherstone, 2008; Quinton, McMillan, & Glebe, 2005). And as with most domesticated animals, unintentional selection is hypothesized to have shaped the physiology of domesticated salmon, especially related to adaptations to new feed composition and feeding regimes.

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 (Hansen & Quinn, 1998; Renkawitz & Sheehan, 2011). Their natural prey, in both freshwater and seawater, often contain substantial amounts of long-chain polyunsaturated fatty acids (LC-PUFAs) including docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) or arachidonic acid (ARA, 20:4n-6) (Bell, Ghioni, & Sargent, 1994). Domesticated 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. Until the late 1990s this lipid source was mainly fish oils (FOs) from wild fisheries, which contained high levels of LC-PUFAs. However, in the last two decades the FOs have gradually been substituted with vegetable oils (VOs) naturally devoid of LC-PUFAs. The LC-PUFAs are important for fish because 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 (Sargent, Tocher, & Bell, 2002; Tocher & Glencross, 2015). This is also reflected in the ability of domesticated salmon to increase endogenous synthesis of LC-PUFAs when given VO-rich diets (Datsomor et al., 2019; Stubhaug, Tocher, Bell, Dick, & Torstensen, 2005; Zheng, Tocher, Dickson, Bell, & Teale, 2004). Another major difference between wild and domesticated diets is the levels of dietary phospholipids (PLs). PLs are important for growth and development of salmon especially for early developmental stages (Poston, 1990; Taylor et al., 2015), and dietary PLs are more efficient

at delivering LC-PUFAs into the circulatory system and ultimately the cells compared to neutral lipids such as triacylglycerols (Cahu et al., 2009; Olsen et al., 2014). The efficiency of utilizing dietary PLs could therefore also be different between wild and domesticated fish, although this has not previously been investigated.

In this study we use a comparative approach to study domestication-associated evolution of transcriptomic and lipid metabolism phenotypes in salmon. Specifically, we hypothesized that the shift to VO diets has selected for a domestication-specific lipid metabolism phenotype to compensate for dietary shortage of LC-PUFAa. We approach this question by feeding domesticated and wild salmon contrasting diets either rich in FOs, VOs or PLs, and then perform comparative analyses of transcriptomes and fatty acids in tissues involved in lipid uptake (pyloric caeca) and endogenous synthesis (liver). This experiment allows us to identify metabolic pathways that respond differently in domesticated compared to wild salmon and reveal novel lipid metabolism features in domesticated salmon putatively linked to unintentional selection and adaptation to a typical domestic VO diet with low LC-PUFA levels.

2 | MATERIALS AND METHODS

2.1 | Fish, diets and experimental plan

The domesticated salmon used in this study was a fast-growing strain (AquaGen AS) which have been selected for faster growth and delayed sexual maturation for 11 generations since 1971. The selective breeding of domesticated salmon has doubled the growth rate and reduced the production cycle for the fish by ~1.5 years compared with the wild origin fish (Thodesen, Grisdale-Helland, Helland, & Gjerde, 1999). The previous generations of domesticated salmon were always fed standard commercial diets available at the time. This means that the fish were given a freshwater diet with only marine ingredients at early developmental stages but have experienced a gradual switch in seawater diet from FOs to VOs since the 1990s. The wild salmon stain was purchased from Haukvik Smolt AS, a wild salmon bank used for the preservation of wild Norwegian Atlantic salmon located in Trødelag, Norway. Wild salmon were originally sourced from five independent lines caught in Lærdal river in Norway in 2011 and 2012. Eggs from these fish were grown in the hatchery facility for one or two generations. During this time the wild fish were kept in outdoor tanks with a transparent roof and water that had the same temperature as the river. The wild fish were fed a standard "Nutra Sprint" diet (Skretting AS) at fry and early juvenile stages (https://www.skretting.com/en/feeds-services/nutra-sprint/1585246) which satisfied their nutritional requirements. The juvenile the fish were then given a "Vitalis Røye" diet from Skretting AS (https://www.skretting.com/nb-NO/produkter/vitalis-r-ye/476027), which has an EPA + DHA content of 19%–20% of the fat, and 70% of the ingredients are of marine origin. Approximately 1,300 newly fertilized eggs of domesticated (AquaGen) and 1,300 of wild salmon (a mixture of the 2nd and 3rd generation from the

five independent lines) were transported to hatching tanks in Ervik hatchery (Frøya, Norway). The water temperature of hatching tanks for domesticated and wild eggs was slightly different to ensure that both strains hatched and start to feed at the same time.

When the yolk sac was depleted, the wild and domestic salmon strains were separated into 12 tanks (2 fish strains × 3 diet treatments × 2 replicate tanks) with 100 L water and 200 fish per tank. Feeding was initiated from the next day. The experimental tanks were randomly distributed in the hatchery and the fish of each tank were reared under the same temperature, continuous light and received 24 hr continuous feed every day. The fish were given three contrasting diets, either an FO diet high in LC-PUFAs, or a plant and VO-enriched diet low in LC-PUFAs, or a marine PL-enriched diet with medium level of LC-PUFAs but rich in PsL (Table 1). All three diets were given to the fish from the start of feeding up to 94 days. To ensure sufficient DHA and EPA levels the PL used to prepare the PL diet was a 50:50 mixture of krill oil (Aker BioMarine AS) and herring roe oil (kindly provided by Erik Løvaas from Marine BioExploitation AS). The diets were produced by Sparos AS. The composition of the diets is shown in Table S1. The FO diet had higher DHA and ARA than the PL diet, while the EPA composition was similar between the two diets (Table 1). The VO diet contains higher 18:3n-3 and 18:2n-6 but lower DHA, EPA and ARA compared to the other two diets. Other components except the lipid source were identical between the three diets (Table S1).

Fish weight (n ≥ 20 from each group) was measured at 0, 48, 65, 78 and 94 days post-initial feeding (dpf). The fish were killed by exposure to 200 mg ml^{-1} Benzoak vet. (ACD Pharmaceuticals AS) before measuring weight. Fish for gene expression and fatty acid measurements were sampled at 94 dpf, when domesticated fish reached an average weight of 4.5 g and wild salmon 2.6 g. Fish samples were immediately placed in sterile Petri dishes after weight measurement and dissected under a dissecting microscope. The pyloric caeca and liver tissues were immediately transferred to 2-ml 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 24 hr 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.

2.2 | RNA isolation and transcriptomic sequencing

Four individuals per group (n = 4, 2 fish per tank × 2 replicate tanks) were used for RNA isolation. The RNA extraction was performed with the RNeasy Plus Universal Kit (Qiagen), according to the manufacturer's instructions. The concentration and integrity of RNA were determined by using a Nanodrop 8000 (Thermo Fisher Scientific) and a 2100 Bioanalyzer (Agilent Technologies), respectively. All RNA samples had RNA integrity (RIN) values >8, which is sufficient for RNA sequencing. Sequencing libraries were prepared with a TruSeq Stranded mRNA Library Prep Kit (Illumina) according to the manufacturer's protocol. Libraries were sequenced using 100-bp single-end mRNA sequencing (RNA-seq) on an Illumina Hiseq 2500 (Illumina) at the Norwegian Sequencing Centre (Oslo, Norway).

The method for handling RNA-seq data has been described in detail in previous studies (Gillard et al., 2018; Jin et al., 2018). In brief, read sequences were quality trimmed using CUTADAPT (version 1.8.1) before being aligned to the salmon genome (ICSASG _version 2). Raw gene counts were generated using HTSEQ-COUNTS (version 0.6.1pl) and the NCBI salmon genome annotation (http://salmobase.org/Downloads/Salmo_salar-annotation.gff3).

2.3 | Lipid class separation and fatty acid analysis

Total lipid was extracted from two individual fish from each tank by using the method of Folch, Lees, and Stanley (1957). Extracted total lipid was then applied onto 10 × 10-cm silica plates (Merck) and separated by using methyl acetate/isopropanol/chloroform/methanol/0.25% KCl (25:25:25:10:9, by vol.) for polar lipids and hexane/diethyl ether/glacial acetic acid (80:20:2, by vol.) for neutral lipids (Olsen & Henderson, 1989). To avoid the oxidation of fatty acids, the plates were exposed to iodine vapour to visualize the lipid class for fatty acid analysis (Li & Olsen, 2017). Lipid bands of phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn) and triacylglycerol (TAG) were separately scraped out into 10-ml glass tubes. Fatty acid methyl esters (FAMEs) of each lipid class were prepared by acid-catalysed transesterification at 50°C for 16 hr (Christie, 1973) before being quantified by a Agilent 7890B gas chromatograph with flame ionization detector (Agilent Technologies).

2.4 | Data analysis

The analysis of RNA-seq data was performed in R (version 3.4.1) (Team, 2013). Only genes with a minimum count level of at least 1

TABLE 1 Percentage 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)

Fatty acid	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

Note: Data are shown in mean ± SD (n = 2).

count per million (CPM) in more than 25% of samples from each tissue were kept for differential expression analysis. Differential expression was tested separately on pyloric caeca and liver using R package EDGER (Robinson, McCarthy, & Smyth, 2010). A full interaction model described in the EDGER manual (Diet + Strain + Diet × Strain) was used in each tissue separately to find differentially expressed genes (DEGs) between wild and domesticated salmon under any dietary treatments. DEGs were determined if a gene had a q value (false discovery rate-adjusted p value) <.05 and absolute $\log_2$ fold change ($|Log2FC|$) > 1 between wild and domesticated salmon. KEGG ontology enrichment analysis (KOEA) was conducted using EDGER. Significant values ($p < .05$) were generated based on a 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 site (TFBS) motifs in the promoter regions (between −1,000 and 100 bp from the transcription start site) of salmon genes was done by using a hypergeometric test in the R package SALMOTIFDB, which interacts with a database of TFBSs for salmonids (https://salmobase.org/apps/SalMotifDB) (Mulugeta et al., 2019).

To further investigate diet-specific effects 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. The same cut-off was used ($q < 0.05$ and $|Log2FC| > 1$) to identify DEGs. To 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 acid composition was also performed in R. Two-way ANOVA with Tukey's HSD post-hoc test was used to test the effect of strain and diet on fish weight and fatty acid composition. Samples of different lipid class, tissue or sampling date were analysed separately. Differences were considered significant at $p < .05$.

2.5 | Ethical statement

All welfare and use of experimental animals was in accordance with the Norwegian Animal Welfare Act 2010. In addition, all personnel involved in rearing, handling and sampling the fish had undergone training approved by the Norwegian Food Safety Authority.

3 | RESULTS

3.1 | Growth and development

The domesticated salmon were significantly larger than wild salmon at all sampling times (Figure 1; Table S2). At the end of the trial (94 days), domesticated salmon reached an average weight of 4.5 g,
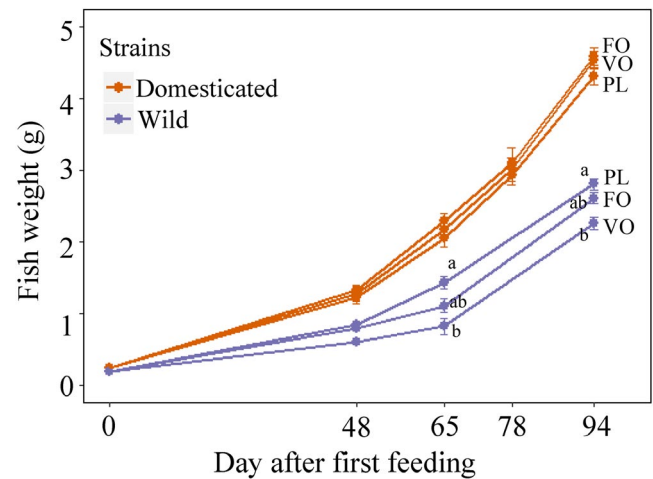

FIGURE 1 Weight of domesticated and wild salmon fed diets high in fish oil (FO), vegetable oil (VO) or phospholipid oil (PL) during early stages of development. Data are means ± SE ($n > 100$ per group at day 93, $n > 20$ at other days). Different letters indicate significant ($p < .05$) differences in fish weight between wild fish fed the FO, VO and PL diet at day 65 and 94 [Colour figure can be viewed at wileyonlinelibrary.com]

while wild salmon had a mean weight of 2.6 g. 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 ($p = .03$) and day 94 ($p = .02$). FO-fed wild salmon showed intermediate weights, but their weights were not significantly different from fish fed either FO or PL diets.

3.2 | Transcriptomic differences between domesticated and wild salmon

On average 20 million reads were generated from each sample (min.: 12 million reads, max.: 32 million reads), with ~85% of the reads mapping to the salmon genome. Out of 81,597 annotated loci, 28,980 and 24,119 genes passed this filtering criterion in pyloric caeca and liver, respectively. Principal components analysis (PCA) on $\log_2$ CPM of the top 1,000 most variable genes revealed a clear separation of domesticated and wild salmon in both pyloric caeca and liver (Figure 2).

Differential expression analysis revealed 187 DEGs in pyloric caeca and 379 DEGs in liver between wild and domesticated salmon (Table S3). By mapping DEGs to the KEGG database of metabolic pathways, we identified 17 pathways that were significantly enriched ($p < .05$) in pyloric caeca, while 11 pathways were enriched in liver (Figure 3a). The DEGs in pyloric caeca were enriched in pathways for glycerophospholipid, glycosphingolipid and glycosaminoglycan metabolism, compounds that are known to be major components of the cell membrane (Figure 3a). A number of cell-signalling pathways were also enriched, including the phosphatidylinositol signalling, calcium signalling, apelin signalling, C-type lectin receptor signalling and GnRH signalling pathways. In liver, the
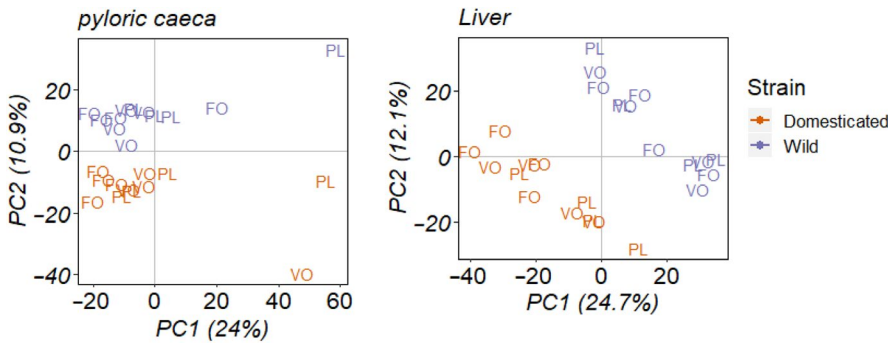
FIGURE 2 Score plot of PCA on log$_2$ count per million (CPM) of the top 1,000 most variant genes across all samples (4 replicates × 2 strains × 3 diets). Two salmon strains (domesticated and wild) were fed diets rich in either fish oil (FO), vegetable oil (VO) or phospholipid (PL) from initial feeding. Pyloric caeca and liver samples were taken after 94 days of feeding [Colour figure can be viewed at wileyonlinelibrary.com]

DEGs were enriched in metabolic pathways including the linoleic acid, glycolysis and gluconeogenesis, fructose and mannose, cysteine and methionine, and retinol metabolism pathways (Figure 3a).

TFBS motif enrichment analysis on promoters (–1,000 to 200 bp from the transcription start site) of DEGs for each tissue resulted in 16 significantly ($p < .005$) enriched motifs in pyloric caeca and 128 enriched motifs in liver (Table S4). The most enriched motif in pyloric caeca was BHLHB2, which is known to be involved in circadian regulation (Figure 3b) (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) and 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, the 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 3c; Figure S1). A systematic difference in circadian clock gene expression was observed between livers of wild and domesticated salmon (Figure 3c; Figure S1), although not all genes were significantly regulated at $q < 0.05$. Nevertheless, the regulators (*cry2-c*, *nr1d1-a*, *per1-a*, *bhlhb2-d* and *nr1d1-a* genes) acting as suppressors of the master regulators of circadian rhythm (CLOCK/BMAL) were consistently expressed at lower levels in domesticated salmon compared to wild salmon (Figure 3c). However, similar expression levels of *clock* (logFC = 0.2, $q = 0.7$) and *bmal1* (logFC = −0.1, $q = 0.8$) genes, which encode master regulators, were found between domesticated and wild salmon (Figure 3c; Figure S1). No difference in circadian clock gene expression was observed between pyloric caeca of wild and domesticated salmon.

3.3 | Differential regulation of lipid metabolism genes between domesticated and wild salmon

To better understand the effect of diets on gene expression differences between domesticated and wild salmon, we compared gene expression separately between domesticated and wild salmon under each diet. In pyloric caeca, a total of 230 DEGs were identified between domesticated and wild salmon with the FO diet, 164 DEGs were found with the VO diet and 689 DEGs were found with the PL diet (Table S5). Of these DEGs, only eight genes were involved in lipid metabolism pathways. This includes the *ptdss2* gene of phosphatidylserine synthesis, which was significantly ($q < 0.05$ and |Log2FC| > 1) more highly expressed in domesticated salmon regardless of the dietary treatment (Figure 4). Two phosphatidylethanolamine synthesis genes, *pcyt2c-a* and *pcyt2c-b*, were both expressed at higher levels in domesticated than wild salmon when fed the FO or VO diet, while no difference in gene expression was found when the fish were given the PL diet. On the other hand, the *etnk2-a* gene involved in phosphatidylethanolamine synthesis was significantly more highly expressed in domesticated compared to wild salmon only when the fish were given the PL diet. Feeding with VOs induced key genes in the LC-PUFA synthesis pathway (*fads2d5* and *fads2d6a*, see Figure 4) in both domesticated and wild salmon. However, no expression difference was observed for these two genes, or any other LC-PUFA synthesis genes, between domesticated and wild salmon for any dietary treatment (Figure 4; Table S5).

The number of DEGs between liver of domesticated and wild salmon under each diet was 591 (FO), 179 (VO) and 243 (PL) (Table S5). Liver had more DEGs involved in lipid metabolism (28) compared to pyloric caeca (eight). Four DEGs in liver had significantly ($q < 0.05$ and |Log2FC| > 1) higher expression in domesticated compared to wild salmon under the VO diet, but not under the FO or PL diet (Figure 5b). This includes genes with key functions in LC-PUFA synthesis (*fads2d5*, Log2FC = 1 and $q = 0.02$), in acyl-CoA synthesis (*acsbg2b-b*, Log2FC = 1.4 and $q = 0.03$) and fatty acid transport (*fabp7b*, Log2FC = 3.6 and $q = 0.02$). Although not significant, domesticated salmon fed the VO diet also had higher expression of *fads2d6a* (Log2FC = 0.7 and $q = 0.2$) and *srebp1d* (Log2FC = 0.8 and $q = 0.2$) compared to wild salmon fed the same diet, while the difference in expression of the two genes was negligible when the fish were given the FO or PL diet (Figure 5a,b). A key gene involved in conversion of lipids to energy, *cpt1aa*, was expressed at lower levels (Log2FC = −1.2 and $q = 0.01$) in domesticated salmon when fed the VO diet. The regulator of fatty acid metabolism *pparg-b* was consistently more highly expressed in domesticated compared to wild salmon under all diets, but this was only significantly different for salmon fed the FO diet (Figure 5a).
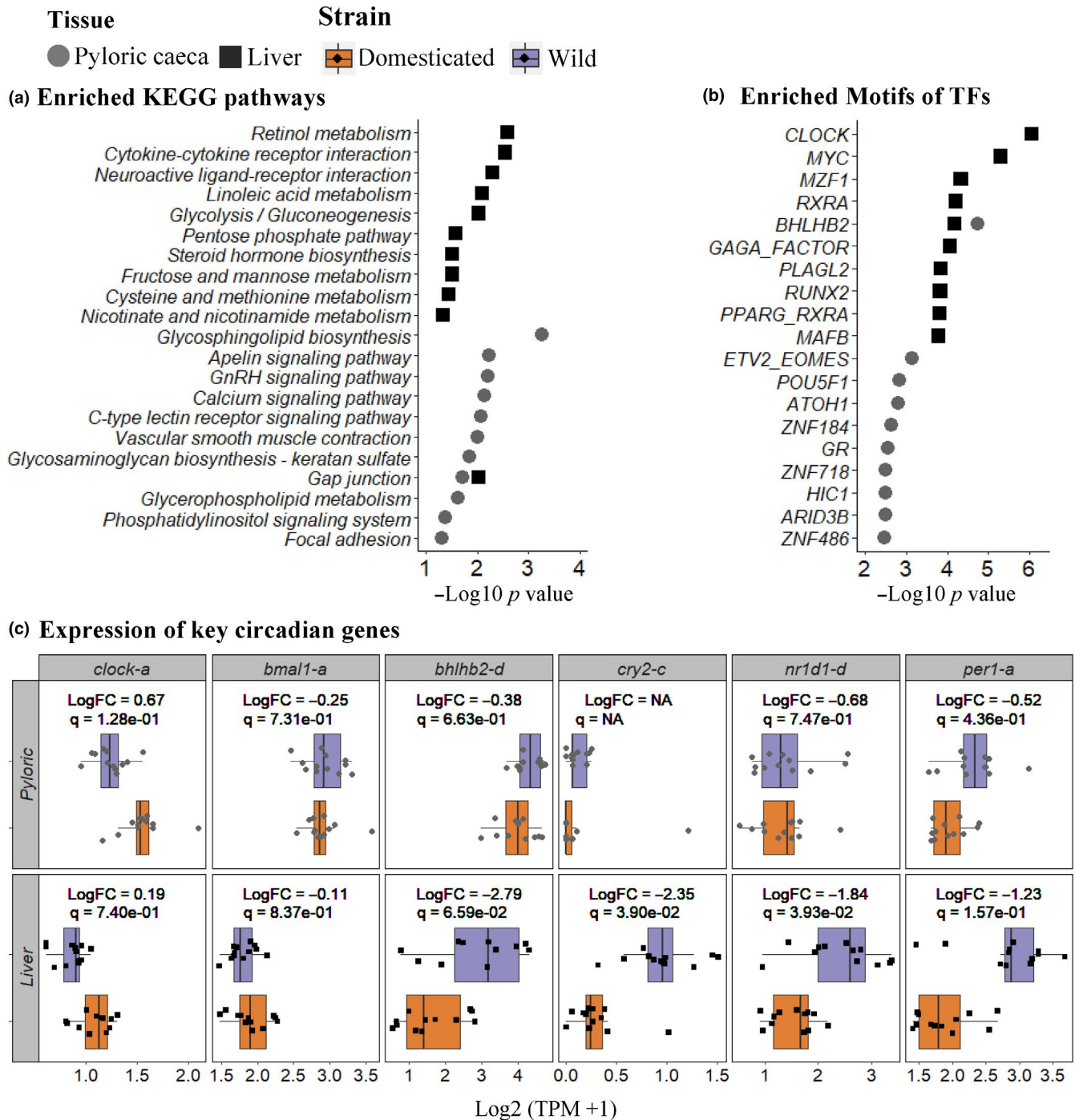
Tissue ● Pyloric caeca ■ Liver **Strain** ◆ Domesticated ◆ Wild

(a) Enriched KEGG pathways

(b) Enriched Motifs of TFs

(c) Expression of key circadian genes

FIGURE 3 Differentially expressed genes (DEGs) between domesticated and wild salmon. (a) KEGG enrichment shows significant (*p* < .05) enriched pathway, and proportion (%) of up/down-regulated DEGs in each pathway. (b) Motif enrichment analysis shows top 10 most significantly (*p* < .005) enriched motifs of transcription factors in promoter regions (−1,000 to 200 bp from the transcription start site) of DEGs as compared to all expressed genes in pyloric caeca and liver. A 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/SalMotifDBwileyonlinelibrary.com]). (c) Expression of key circadian genes in pyloric caeca and liver of domesticated and wild salmon. Gene expression is shown as log₂ transcript per million plus one (TPM + 1). No statistics are shown for the *cry2-c* gene in pyloric caeca, as gene expression was too low (CPM < 1) to be used for differential expression analysis [Colour figure can be viewed at wileyonlinelibrary.com]

In addition to the DEGs of fatty acid metabolism, five DEGs involved in phospholipid, cholesterol and triacylglycerol metabolism were found between domesticated and wild salmon (Figure 5c).

This included the *apoa1-b* gene involved in lipoprotein synthesis and lipid transport, which was strongly more highly expressed in domesticated salmon than wild salmon, regardless of dietary treatment
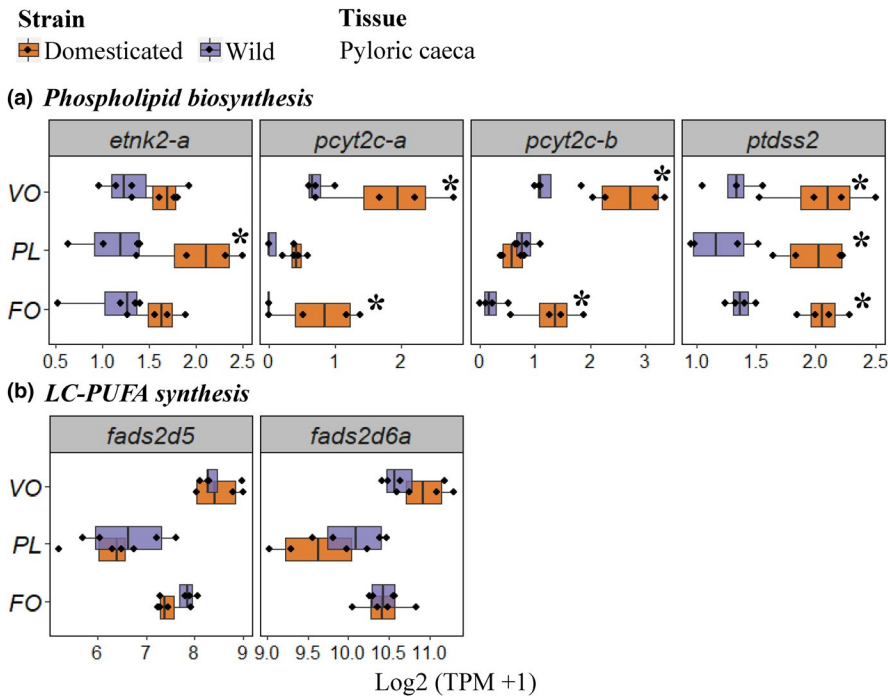
Strain
🔶 Domesticated 🟣 Wild

Tissue
Pyloric caeca

(a) *Phospholipid biosynthesis*

(b) *LC-PUFA synthesis*

Log2 (TPM +1)

FIGURE 4 Expression of six 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. (a) Expression of genes involved in phospholipid metabolism; (b) Expression of genes involved in LC-PUFA synthesis pathway. Gene expression is shown as $\log_2$ transcript per million plus one (TPM + 1) which was normalized by library size and mRNA length. An asterisk indicates differential expressed genes (DEGs, $q < 0.05$ and $|\log2FC| > 1$) between domesticated and wild salmon under each dietary treatment [Colour figure can be viewed at wileyonlinelibrary.com]

($\text{Log2FC} > 3$ and $q < 0.001$, see Figure 5c; Table S5). A key gene involved in the synthesis of bile acid (*cyp7a1-a*), which is responsible for removal of cholesterol in liver, was highly expressed in domesticated salmon when given the PL diet. The gene *ptdss2* involved in the synthesis of phosphatidylserine, which is a major phospholipid in salmon, was more highly expressed in domesticated salmon than wild salmon fed the VO diet ($\text{Log2FC} = 1.9$, $q = 0.0008$), although a similar trend was also found when the fish were given the FO ($\text{Log2FC} = 1$, $q = 0.09$) or PL diet ($\text{Log2FC} = 0.9$, $q = 0.2$). Expression of the *hsl* gene involved in hydrolysing triacylglycerol (stored fat) to diacylglycerol, and diacylglycerol to monoacylglycerol was generally expressed at higher levels in domesticated salmon than wild salmon. On the other hand, the expression of *mgll* involved in hydrolysing monoacylglycerol into free fatty acids was lower in domesticated salmon (Figure 5c). In conclusion, direct comparison of the transcriptomes of domesticated and wild salmon suggests that domestic salmon have boosted expression of genes involved in many aspects of lipid metabolism such as transport, endogenous synthesis and conversion of lipids and fatty acids in both gut and liver (Table S5).

To further investigate differences in the plasticity of fatty acid metabolism between domesticated and wild salmon, we analysed differences in putative compensatory shifts in gene regulation under diets with low (VO) vs. high (FO) levels of LC-PUFAs for wild and domesticated salmon separately. These analyses identified 38 DEGs in domesticated and two DEGs in wild salmon (Table S5). However, only DEGs in domesticated salmon (nine genes) were linked to lipid metabolism, specifically involved in fatty acyl-CoA synthesis (two genes), LC-PUFA synthesis (two genes), lipogenesis (two genes) and transcriptional regulation of lipid metabolism (two genes) (Table 2).

3.4 | Comparison of fatty acid composition between domesticated and wild salmon

The variation in fatty acid composition was generally more driven by diet than by strain. About 85% of the fatty acid content in liver and pyloric caeca differed between diets, but only 32% of the fatty acids differed in levels between wild and domesticated salmon ($p < .05$; Table S6). Both wild and domesticated salmon given the VO diet showed higher levels of 18:3n-3 and 18:2n-6 in both liver and pyloric caeca but lower contents of the longer chain fatty acids (ARA, EPA and DHA) compared to both fish given the FO and PL diets (Figure 6). This pattern was consistent for all three lipid classes analysed (PtdCho, PtdEtn and TAG). Although the differences in fatty acid content were generally small between wild and domesticated salmon fed the same diet, wild fish contained higher contents of 18:2n6 (9.1% in wild vs. 7.3% in domesticated fish, $p = .06$) and 18:3n3 (2.3% vs. 1.5%, $p = .006$) in PtdEtn of liver when fed the VO diet. Wild salmon also had higher contents of 18:3n3 (2.1% vs. 1.8%, $p = .04$) in PtdCho of liver when fed the VO diet. On the other hand, wild salmon had a significantly lower content of ARA in both PtdCho (1.6% vs. 2.1%, $p = .02$) and PtdEtn (3.1% vs. 4.3%, $p = .02$) of liver than wild fish when fed the VO diet. Wild salmon also contained lower levels of 18:4n-3, 18:3n-6 and 18:4n-6, but higher 20:3n-3 levels when fed the VO diet (Table S6). No significant differences in DHA and EPA contents were found between domesticated and wild salmon fed the same diets.

4 | DISCUSSION

Atlantic salmon provides a unique opportunity to study domestication-related evolution because the wild populations that gave
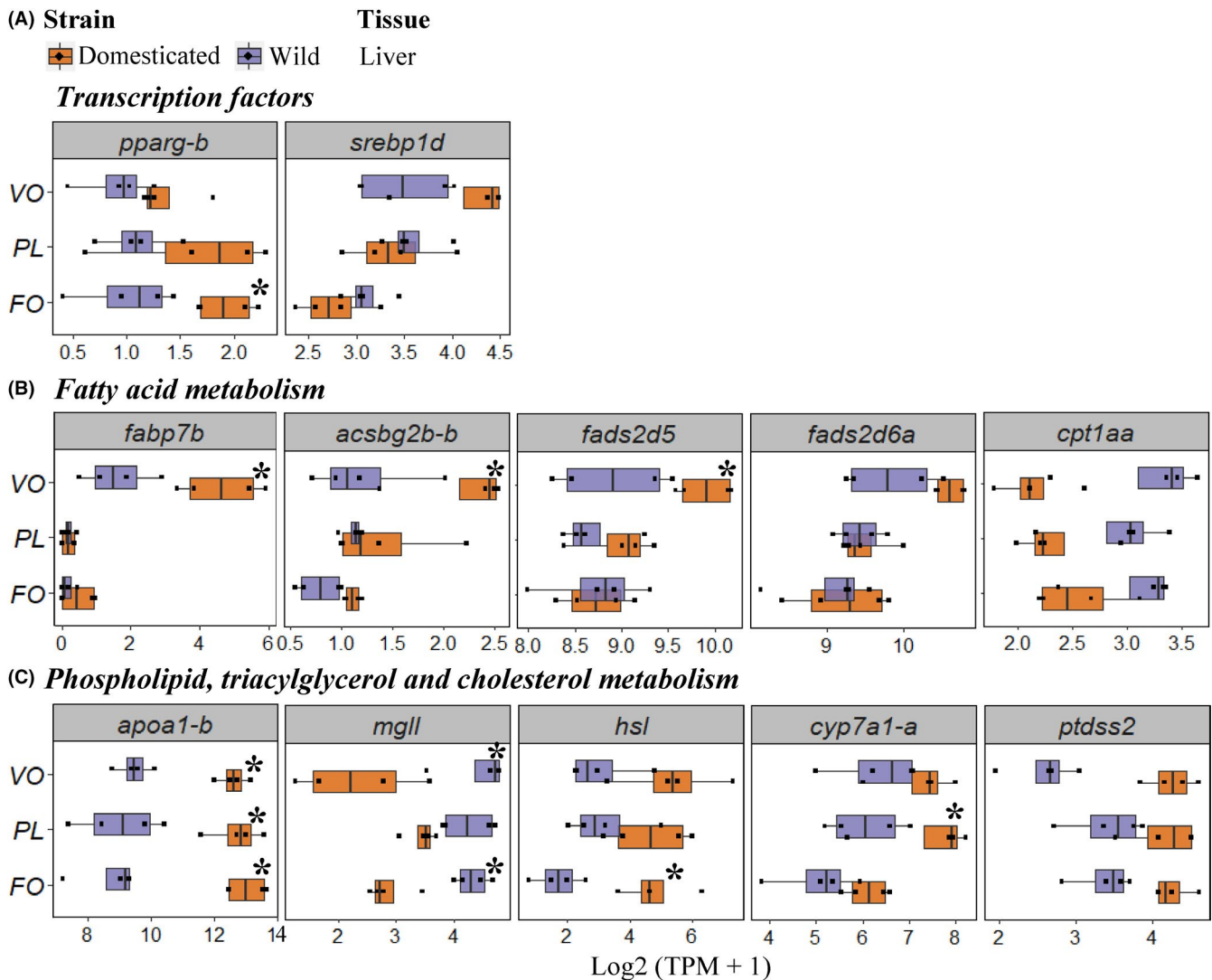
FIGURE 5 Expression of 14 genes involved in lipid metabolism in liver of wild and domesticated salmon at day 94 after feeding either fish oil (FO), vegetable oil (VO) or phospholipid (PL) diets. (a) Gene expression of key transcription factors involved in lipid metablism; (b) Expression of genes involved in fatty acid metabolism; (c) Expression of genes involved in phospholipid, triacylglycerol and cholesterol meatabolism. Gene expression is shown as $\log_2$ transcript per million plus one (TPM + 1) which was normalized by library size and mRNA length. *Significant ($q < 0.05$ and |Log2FC| > 1) difference in gene expression between domesticated and wild salmon under each dietary treatment separately [Colour figure can be viewed at wileyonlinelibrary.com]

rise to domesticated salmon are well preserved and also accessible in live gene banks in Norway (O'Reilly & Doyle, 2007). Here we took advantage of this and performed a comparative study of domesticated and wild salmon metabolism to test for signatures of unintended selection on lipid metabolism traits in domesticated salmon.

4.1 | Linking evolution of the domesticated metabolic syndrome with the circadian clock pathway

As demonstrated in other studies, we found that domesticated salmon grew faster than wild salmon (Bicskei et al., 2014; Reid, Armstrong, & Metcalfe, 2012). This reflect 50 years of targeted breeding for fast growth, which has resulted in higher standard metabolic

rate, higher feed intake and improved feed conversion (Thodesen et al., 1999), referred to as the "domesticated metabolic syndrome" (Bicskei et al., 2014; Tymchuk, Sakhrani, & Devlin, 2009). In line with this, gene expression differences in liver suggest that energy assimilation and expenditure is higher in domesticate salmon (Figure 3a), similar to what is found in domesticated pigs (Li et al., 2013), chicken (Jackson & Diamond, 1996) and rat (Zeng et al., 2017). The differences in pyloric caeca gene expression between domesticated and wild salmon were associated with regulatory networks controlling intestinal development and cell differentiation (Jedlicka & Gutierrez-Hartmann, 2008; Kanki et al., 2017; Lebenthal & Lebenthal, 1999) (Figure 3b), which could be linked to higher growth rates and/or feed intake in domesticated fish (Thodesen et al., 1999).

Our results strongly suggest a functional link between evolution of the domesticated metabolic syndrome (i.e., faster growth and

higher energy turnover) and regulation of genes through the circadian clock pathway (Figure 3). This is interesting as top regulators (CLOCK/BMAL) are known to impact (directly or indirectly) a multitude of downstream processes including metabolism (Lowrey & Takahashi, 2000; Preitner et al., 2002; Takahashi, 2015). Moreover, the *CLOCK* gene has also been under selection during domestication of rats (Zeng et al., 2017) and is associated with key features of the

domestic metabolic syndrome, such as regulation of feed intake, metabolic rates, and glucose and lipid metabolism in both mammals and fish (Esther, Nuria, Ana, Ángel, & María, 2017; Paschos, 2015; Rudic et al., 2004). Finally, we found that predicted TFBSs of the PPAR-RXR heterodimer, a key regulator of glucose (Jones et al., 2005) and lipid (Kliewer et al., 1997) homeostasis, were enriched in promoters of DEGs between wild and domesticated salmon (Figure 3b), and that the *pparg* gene was consistently more highly expressed in domesticated salmon (Figure 5). This also links to the circadian clock as the *pparg* gene is known to be under circadian rhythmicity in salmon (Betancor et al., 2014).

Unfortunately, our study was not designed to investigate the connection between differences in circadian oscillations between wild and domestic salmon. However, we are confident that sampling bias related to daily rhythms has not impacted our results. First, all samples used for the gene expression were sampled between morning and noon over a 2 hr time period. Second, all fish were raised under constant light and continuous feeding in this study. Such rearing conditions are known to abolish daily rhythmicity for both *nr1d1* (Betancor et al., 2014) and *cry-2* (Huang, Ruoff, & Fjelldal, 2010), but nevertheless these genes were still expressed at lower level in domesticated salmon regardless of fish size and age (Figure S1).

In conclusion, our results support strong links between the salmon "domestic metabolic syndrome" and evolution of novel

TABLE 2 Log$_2$ fold change and adjusted *p* value (*q*) of lipid gene expression in liver of domesticated/wild salmon fed vegetable oil (VO) diet compared to fish oil (FO)

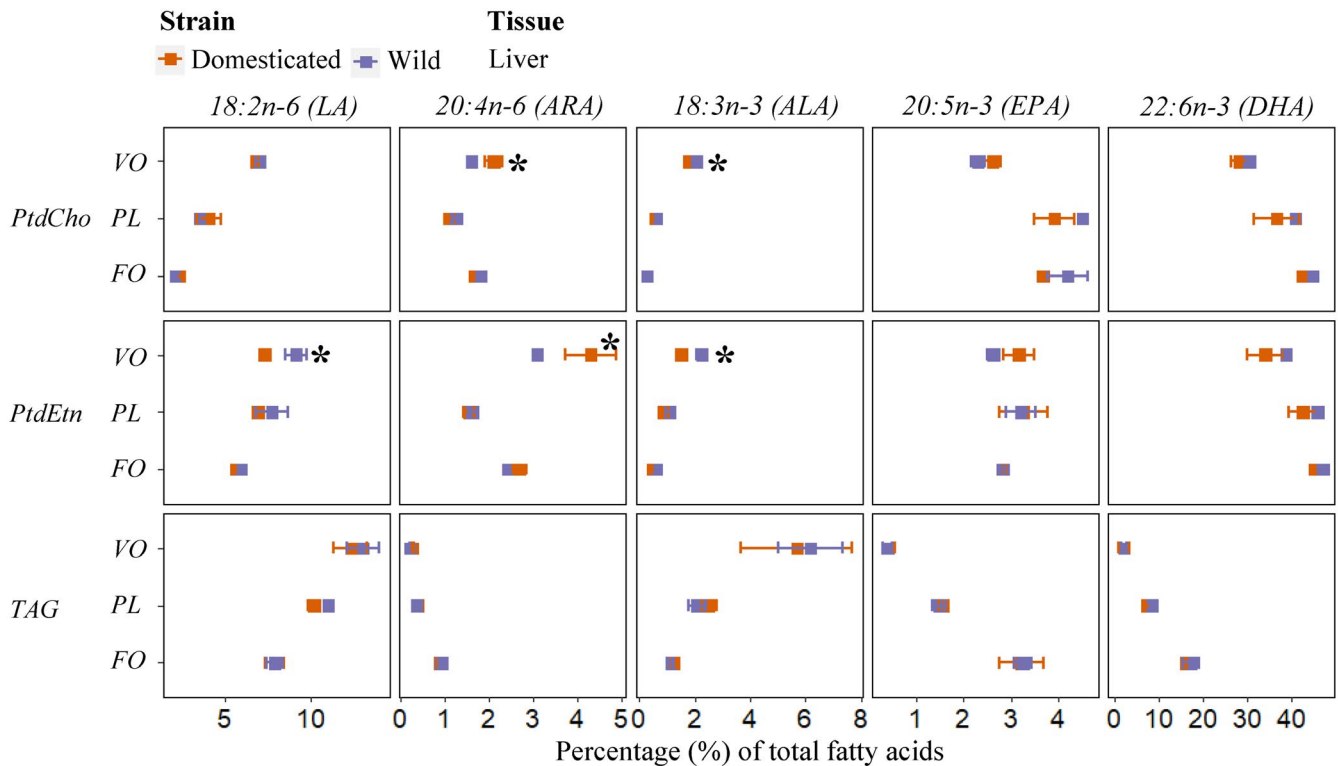| Gene name	Farm VO vs. FO		Wild VO vs. FO	
	logFC	q	logFC	q
acsbg2b-b	1.7	0.01	1.0	0.64
acsl1a-a	1.6	0.02	1.0	0.64
agpat3a-b	1.4	0.002	1.2	0.09
agpat3b-a	1.1	0.01	0.5	0.72
fabp7b	6.0	0.001	4.2	0.13
fads2d6a	1.3	0.008	0.8	0.54
fads2d5	1.2	0.01	0.2	1.00
srebp1c	1.4	0.03	0.9	0.62
srebp1d	1.6	0.003	0.5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

FIGURE 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) or 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. *Significant (*p* < .05) difference in fatty acid content between domesticated and wild salmon for a certain day and dietary treatment. The composition of other fatty acids and their ANOVA test are shown in Supporting Information 7 [Colour figure can be viewed at wileyonlinelibrary.com]

regulation of the circadian clock pathway. We therefore hypothesize that the strong selection on "fast growers" with high energy metabolism and high nutrient requirements target genetic variation linked to regulation of the circadian clock pathway.

4.2 | Lipid metabolism in domesticated salmon show signatures of unintended selection in the domestic environment

A main aim of this study was to explore the hypothesis that domesticated salmon had undergone unintended selection on lipid metabolism as a response to low levels of LC-PUFAs in the domesticated environment. In line with this, we showed that the growth of wild but not domesticated salmon was affected by low LC-PUFA availability in the feed. This suggests that domesticated salmon have evolved more effective lipid absorption and lipid transport, and/or better ability for compensatory endogenous conversion and synthesis of lipids under shortage of essential fatty acids.

In-depth analyses of both transcriptomic and lipid composition data support the notion that all these processes differ between wild and domesticated salmon. First, domesticated fish display higher *apoa1_2* gene transcription, encoding a major component of high-density lipoprotein (HDL) which plays a key role in lipid transport and regulation of cellular cholesterol levels (Otis et al., 2015; Toth et al., 2013). Second, the hormone-sensitive lipase gene (*hsl*) was also expressed at higher levels in liver of domesticated salmon compared to wild salmon. This suggests that domesticated salmon had greater ability to hydrolyse triacylglycerol, diacylglycerol and cholesterol ester into monoacylglycerol and free fatty acids (Kraemer & Shen, 2002; Quiroga & Lehner, 2012) which is used for energy production or lipid synthesis. The fact that genes responsible for hydrolysing monoacylglycerol (*mgll*) and transporting fatty acids into the mitochondria for β-oxidation (*cpt*) were expressed at lower levels in domesticated salmon compared to wild salmon supports the latter. Third, the growth of wild (but not domesticated) salmon was impacted positively by dietary PL supplementation, which is known to promote absorption and transport of dietary lipids, especially LC-PUFAs (Olsen, Tore Dragnes, Myklebust, & Ringø, 2003; Olsen et al., 2014; Tocher, Bendiksen, Campbell, & Bell, 2008). This points towards a higher ability for LC-PUFA absorption and transport in domesticated salmon due to more effective de-novo synthesis of PLs (Figure 5).

Finally, under dietary shortage of LC-PUFAs, domesticated salmon respond with a compensatory increase in gene expression of *fads2d5* and *fads2d6a*, which encode rate-limiting enzymes for endogenous synthesis of LC-PUFAs (Figure 5). Parallel to this finding, marine stickleback that colonize freshwater environments with lower levels of available dietary DHA evolve a greater endogenous LC-PUFA synthesis ability through increased copy number of the same gene (*fads2*) (Ishikawa et al., 2019). As the ability to perform endogenous synthesis of LC-PUFAs is heritable in salmon (Horn, Ruyter, Meuwissen, Hillestad, & Sonesson, 2018), it is likely that the VO-based diets in the domestic environment have unintendedly

selected for improved ability of LC-PUFA synthesis in domesticated salmon. The high LC-PUFA levels in domesticated salmon ensures the essential requirement for normal growth (Bou et al., 2017), while growth of wild salmon is stunted when fed VO diets due to insufficient synthesis of LC-PUFAs. Sterol regulatory binding protein 1 (SREBP-1) transcription factor is probably the key regulator for the differential expression of the *fads2* gene (Figure 5) (Datsomor et al., 2019), but other mechanisms such as epigenetic changes may also contribute to the regulation of gene expression (Clarkson et al., 2017; Vera et al., 2017).

5 | CONCLUSION

The present study provides evidence for domestication-associated evolution of metabolism in Atlantic salmon, both as a consequence of targeted breeding for fast growth, and as an unintended consequence of adapting to modern aquaculture feed. To further understand causal links between genotype and regulation of metabolism in domesticated salmon, future studies should integrate analyses that shed light on the genomic signatures of domestication selection.

AUTHOR CONTRIBUTIONS

Y.J., Y.O., R.E.O., S.R.S. and O.V. designed and performed the research. Y.J. and T.N.H. performed the transcriptomic analysis. Y.J. and K.L. performed the lipid and fatty acid analysis. J.O.V. and S.R.S. guided the transcriptomic analysis and revised the manuscript. Y.O. and R.E.O. guided the lipid analysis and revised the manuscript.

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