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Assessment of a land-locked Atlantic salmon (*Salmo salar* L.) population as a potential genetic resource with a focus on long-chain polyunsaturated fatty acid biosynthesis

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Abstract

The natural food for Atlantic salmon (*Salmo salar*) in freshwater has relatively lower levels of omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA) than found in prey for post-smolt salmon in seawater. Land-locked salmon such as the Gullspång population feed exclusively on freshwater type lipids during its entire life cycle, a successful adaptation derived from divergent evolution. Studying land-locked populations may provide insights into the molecular and genetic control mechanisms that determine and regulate n-3 LC-PUFA biosynthesis and retention in Atlantic salmon. A two factorial study was performed comparing land-locked and farmed salmon parr fed diets formulated with fish or rapeseed oil for 8 weeks. The land-locked parr had higher capacity to synthesise n-3 LC-PUFA as indicated by higher expression and activity of desaturase and elongase enzymes. The data suggested that the land-locked salmon had reduced sensitivity to dietary fatty acid composition and that dietary docosahexaenoic acid (DHA) did not appear to suppress expression of LC-PUFA biosynthetic genes or activity of the biosynthesis pathway, probably an evolutionary adaptation to a natural diet lower in DHA. Increased biosynthetic activity did not translate to enhanced n-3 LC-PUFA contents in the flesh and diet was the only factor affecting this parameter. Additionally, high lipogenic and glycolytic potentials were found in land-locked salmon, together with decreased lipolysis which in turn could indicate increased use of carbohydrates as an energy source and a sparing of lipid.

Keywords: omega-3 long chain polyunsaturated fatty acids, Atlantic salmon, land-locked, desaturases, elongases

1. Introduction

Omega 3 (n-3) long chain polyunsaturated fatty acids (LC-PUFA), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), are essential dietary nutrients with well-known health benefits in humans [1]. Fish and seafood is the main dietary source of n-3 LC-PUFA for humans, with aquaculture providing close to half (47 %) of all the products on the market [2], although almost all commercially available Atlantic salmon (*Salmo salar* L.) is farmed. Feeds for farmed fish have traditionally relied on the use of fishmeal and fish oil derived from marine fisheries as the main protein and lipid sources. The resulting demand for marine resources is such that requirements for aquaculture feeds now exceed global supplies of fish oil [3]. Continued expansion of aquaculture to supply the global demand for fish is only possible by replacing fish oil and, currently the main sustainable alternatives are vegetable oils, which can be rich in C_{18} PUFA such as linoleic (LNA; 18:2n-6) and α-linolenic (ALA; 18:3n-3) acids, but devoid of EPA and DHA [4]. Thus, fish fed diets formulated with vegetable oil are characterised by increased levels of C_{18} PUFA and decreased levels of n-3 LC-PUFA compared to fish fed fish oil, reducing their nutritional value to human consumers [5]. Therefore, flesh n-3 LC-PUFA content and composition is a key trait determining the nutritional quality of farmed salmon [6].

A recent study comparing 50 families from a salmon breeding programme fed vegetable oil showed high variability in flesh n-3 LC-PUFA levels, with the trait showing a high level of heritability estimated at $h^2 = 0.77$ [7], indicating that the trait is affected by environment to only a very limited degree and that differences observed between populations have most likely a genetic origin. This demonstrated the potential of selective breeding to improve this trait, and has prompted a focus on the genetic architecture of LC-PUFA biosynthesis and metabolism in salmon. Recent studies have indicated that tissue fatty acid compositions are determined by the relative activities of a range of metabolic pathways including transport and uptake, oxidation and deposition, and endogenous biosynthesis [8-11]. However, precisely what the most critical pathways and genes in determining tissue LC-PUFA compositions are, and how diet interacts with and affects these molecular pathways are poorly understood in all animals, including salmon.

Atlantic salmon start life in freshwater followed by smoltification and migration to the sea before they return to their native river to spawn [12]. However, after the last glacial period, some Atlantic salmon stocks were isolated from the sea in both North America and Europe

and these land-locked populations complete their life-cycle in freshwater, spending their adult life mainly in large lakes instead of the sea. This means that land-locked salmon are exposed to lower levels of EPA and DHA, during their lifespan than their anadromous counterparts, which asks the question of whether land-locked populations may have potentially higher capacity for LC-PUFA biosynthesis than anadromous populations [5,13]. In an earlier study, the fatty acid compositions of land-locked and farmed Atlantic salmon fed diets formulated with vegetable oils were compared [14]. The authors interpreted the data to suggest that land-locked salmon may have a higher conversion capacity for n-3 and n-6 PUFA than anadromous counterparts, and that there was a genetic influence on DHA content in phospholipids [14]. This suggested that these wild populations could provide a highly valuable genetic resource for enhancing farmed Atlantic salmon stocks but, as few studies have assessed farmed and land-locked salmon populations in detail, they represent an under-exploited resource.

In this context, the overarching aim of this work was to characterise the n-3 LC-PUFA trait in land-locked salmon and to determine the molecular and biochemical basis for any differences observed in the trait between farmed and land-locked Atlantic salmon populations. To achieve this, a 2 x 2 factorial nutritional trial employing two feeds containing fish oil or vegetable oil as the added lipid were tested over 8 weeks in two populations of Atlantic salmon, the Norwegian national farmed stock of the Aquagen strain (F) and the Swedish Gullspång land-locked stock (W). Effects of dietary fatty acid composition on gene expression in liver by microarray (transcriptome) analysis, hepatocyte fatty n-3 LC-PUFA biosynthesis activities by incubation with radiolabelled 18:3n-3, and resultant muscle (flesh) fatty acid compositions were determined.

2. Materials and methods

2.1 Ethics statement

All procedures and protocols were performed in accordance with Local (Institute of Marine Research), Norwegian national, and European (EU) Regulations for the use of animals in scientific experimentation (Directive 2010/63/EU).

2.2 Dietary trial and sampling

Eggs were obtained from a land-locked Atlantic salmon population (Lake Vanern/Gullspång Swedish stock) and incubated and hatched in April 2013 at the facilities of the Institute of Marine Research (IMR) (Matre, Norway). Two isonitrogenous and isoenergetic feeds, formulated to satisfy the nutritional requirements of salmonid fish (NRC, 2011), were manufactured by the Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima Fôrteknologisenteret, Fana, Norway) by vacuum coating identical dry basal extruded pellets with either fish oil (FO) or rapeseed oil (RO) and were named according to the oils used (Table 1). The dietary fatty acid profiles reflected the oil source with the FO diet containing high levels of LC-PUFA (17.3 % vs. 7.8 % in RO), whereas the levels of 18:3n-3 were higher in the RO diet than in the FO diet (6.0 % vs. 1.0 %) (Table 1). A classic factorial 2 trial was performed comparing land-locked (termed W) and farmed (termed F, Aquagen strain) parr fed the two diets to produce four experimental treatments (WFO, WRO, FFO and FRO). Table 1 here

Twelve 400 L tanks (1x1 m) were stocked with 50 parr (initial weight ~ 25 g) with 6 tanks of each population and the two experimental feeds were fed to triplicate tanks for 8 weeks until at least a doubling of weight (~ 60 g). At the end of the feeding trial, twelve fish per treatment (4 fish per replicate tank) were sampled and flesh (white muscle) collected in liquid nitrogen. In addition, liver of 12 fish was removed and divided into two portions. One portion (~100 mg) was stabilised in RNALater® for subsequent gene expression analyses, whereas the remaining portion was used to determine the hepatocyte fatty acyl desaturation and elongation activities. All fish were weighed at the end of the experimental period and specific growth rate (SGR) calculated as follows: SGR = 100 * (lnWf – lnWo)/t, where Wo = initial weight (g) and Wf = final weight (g) at time t (days).

2.3 Determination of hepatocyte fatty acyl desaturation/elongation activities

Each pool (per tank) of liver was chopped, incubated with 1 % collagenase, and dissociated cells sieved through 100 μm nylon gauze and isolated as described in detail previously [16]. One hundred μl of each cell preparation was taken for protein determination [17] following incubation with 1 M NaOH/0.25 % (w/v) SDS for 1 h at 60 °C. Five ml of each cell preparation were dispensed into 25 cm^2 tissue culture flasks and incubated at 18 °C for 1 h with 0.25 μCi (final fatty acid concentration, 2 μM) of [1-^{14}C]18:3n-3 added as a complex with fatty acid free-bovine serum albumin [18]. After incubation, cells were harvested, washed and cell total lipid extracted as described in detail previously [16]. Fatty acid methyl

esters were prepared as described below, separated by argentation chromatography on silver nitrate-impregnated TLC plates, subjected to autoradiography, and radioactivity in individual fatty acids determined by liquid scintillation counting, all as described in detail previously [16].

2.4 Total lipid extraction

Lipid was extracted from flesh samples using a modified method of Folch et al. [19]. Briefly, samples were homogenised in 16 ml of chloroform/methanol (2:1, v/v) using an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK). Non-lipid impurities were removed by washing with 4 ml of 0.88 % aqueous KCl (w/v) and the upper aqueous layer removed by aspiration, and the lower solvent layer containing the lipid extract dried under oxygen-free nitrogen. Total lipid content was determined gravimetrically after overnight desiccation *in vacuo*.

2.5 Fatty acid composition

Fatty acid methyl esters were prepared from 10 mg of total lipid by acid-catalysed transmethylation according to the method of Christie [19]. FAME were separated and quantified by gas-liquid chromatography using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30 m × 0.32 mm i.d. × 0.25 μm ZB-wax column (Phenomenex, Cheshire, UK), on-column injector and a flame ionisation detector. Hydrogen was used as the carrier gas in constant flow mode at 2.5 ml min^{-1}, with an initial thermal gradient from 50 °C to 150 °C at 40 °C min^{-1} and to a final temperature of 230 °C at 2 °C min^{-1}. Data were collected and processed using Chromcard for Windows (version 2.01; Thermoquest Italia S.p.A., Milan, Italy). Individual FAME were identified by comparison to known standards and published data [21-22]. Selected FAME were confirmed by gas chromatography-mass spectrometry (GC-MS) using a gas chromatograph (GC8000) coupled to a MD800 mass spectrometer (ThermoFisher Scientific, Hemel Hempstead, UK).

2.6 RNA extraction

Liver from twelve individual fish per dietary treatment were homogenised in 1 ml of TriReagent® (Sigma-Aldrich, Dorset, UK) RNA extraction buffer using a bead tissue disruptor (Bio Spec, Bartlesville, Oklahoma, USA). Total RNA was isolated following manufacturer's instructions, purified using a commercial kit (RNeasy Mini Kit, Quiagen,

Manchester, UK) and quantity and quality determined by spectrophotometry using a Nanodrop ND-1000 (Labtech Int., East Sussex, UK) and electrophoresis using 500 ng of total RNA in a 1 % agarose gel. To perform the RNA amplifications, 2500 ng from two fish belonging from the same tank were pooled to obtain six samples per treatment and stock.

2.7 Microarray hybridizations and image analysis

Transcriptome analysis was performed in liver using an Atlantic salmon custom-made oligoarray with 44k features per array on a four-array-per-slide format (Agilent Technologies UK Ltd., Wokingham, UK), ArrayExpress accession number A-MEXP-2065. The probes were co-designed by researchers at the Institute of Aquaculture (University of Stirling, UK) and the Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima, Tromsø, Norway). A dual-label experimental design was employed for the microarray hybridisations with Cy3-labelled test samples competitively hybridised to a common Cy5-labelled pooled-reference per array. A total of 24 arrays were utilised. The common reference was a pool of equal amounts of amplified RNA from all test samples.

Indirect labelling methodology was employed in preparing the microarray targets. Amplified antisense RNA (aRNA) was produced from each RNA sample using TargetAmpTM 1-Round Aminoallyl-aRNA Amplification Kit 101 (Epicentre, Madison, Wisconsin, USA), as per manufacturer's methodology, followed by Cy3 or Cy5 fluor incorporation through a dye-coupling reaction. Briefly, 250 ng of total RNA per sample were amplified and column purified (RNeasy Mini Kit, Qiagen, Manchester, UK) according to manufacturer's instructions. Resultant aRNA quantity and quality was assessed by spectrophotometry and electrophoresis as above. In addition, amino-allyl incorporation (ratio A289/A260) was determined. Experimental and the pooled reference samples were labelled with Cy3 and Cy5 dye suspension respectively, (GE HealthCare, Little Chalfont, UK). To attach the Cy dyes, 2.5 µg from each aRNA sample were suspended in 10.5 µl nuclease-free water and heated to 75 ˚C for 5 min. When cooled, 3 µl of coupling buffer (0.5M $NaOHCO_3$; pH 9.0) and 1.5 µl of Cy3 or 0.8 µl of Cy5 dye suspension were added as appropriate followed by incubation for 1 h at 25 ˚C in the dark. Unincorporated dye was removed by column purification (Illustra AutoSeq G-50 spin columns, GE Healthcare). Dye incorporation and aRNA yield were quantified by spectrophotometry and further quality controlled by separating 0.4 µl of the sample through a 1 % agarose gel and products visualised on a fluorescence scanner (Typhoon Trio, GE Healthcare).

Microarray hybridisations were performed in SureHyb hybridisation chambers in a DNA Microarray Hybridisation Oven (Agilent Technologies). For each hybridisation, 825 ng of Cy3-labelled experimental biological replicate and Cy5-labelled reference pool were combined and total volume made up to 35 µl with nuclease-free water. A fragmentation master mix was prepared containing per reaction, 11 µl 10X Blocking agent, 2 µl 25X fragmentation buffer and 7 µl nuclease-free water and added to the Cy-dyes mix. After incubating in the dark at 60 ˚C for 30 min, 57 µl 2X GE Hybridisation buffer (pre-heated at 37˚C) was added, contents gently mix, centrifuged at 20000 g for 1 min and kept on ice until loaded onto the microarray slides. Hybridisation was carried out in an oven rotator (Agilent Technologies) at 65 ˚C and 10 rpm for 17 h. Post-hybridisation washes were carried out in EasyDip™ Slide staining containers (Canemco Inc., Quebec, Canada). After disassembling the array-gasket sandwiches submersed in wash buffer 1 at room temperature, the microarray slides were transferred to an EasyDip™ container and incubated in wash buffer 1 for 1 min at 150 rpm, and then a further 1 min at 31 ˚C at 150 rpm in wash buffer 2. A final dip in wash buffer 2 at room temperature was performed, after which the slides were dried and kept in the dark until scanned the same day. Scanning was performed at 5 µm resolution using an Axon GenePix 4200AL Scanner (MDS Analytical Technologies, Wokingham, Berkshire, UK). Laser power was kept constant (80 %) and PMT adjusted for each channel such than less than 0.1 % features were saturated and that the mean intensity ratio of the Cy3 and Cy5 signals was close to one.

2.8 Quantitative real time PCR

Expression of candidate genes of interest (*fatty acyl desaturases 5* and *6* and *fatty acyl elongases 2*, *5a* and *5b*) as well as genes for microarray validation (Supplementary Table 1) was determined by quantitative PCR (qPCR) in liver from fish of all treatments. Results were normalised using reference genes, *elongation factor 1-α* (*ef1a*) and *cofilin-2* (*cfl2*), which were chosen as the most stable according to GeNorm. cDNA was synthesised using 2 µg of total RNA and random primers in 20 µl reactions and the High capacity reverse transcription kit without RNase inhibiter according to the manufacturer's protocol (Applied Biosystems, Warrington, UK). The resulting cDNA was diluted 20-fold with milliQ water. Primers were designed using Primer 3 [23] in regions that included the microarray probes and efficiency evaluated by serial dilutions to ensure that it was close to 100 %. qPCR was performed using a Biometra TOptical Thermocycler (Analytik Jena, Goettingen, Germany) in 96-well plates

in duplicate 20 µl reaction volumes containing 10 µl of Luminaris Color HiGreen qPCR Master Mix (Thermo Scientific), 1 µl of the primer corresponding to the analysed gene (10 pmol), 3 µl of molecular biology grade water and 5 µl of cDNA, with the exception of the reference genes, which were determined using 2 µl of cDNA. In addition amplifications were carried out with a systematic negative control (NTC-no template control) containing no cDNA. Standard amplification parameters contained an UDG pre-treatment at 50 °C for 2 min, an initial activation step at 95 °C for 10 min, followed by 35 cycles: 15 s at 95 °C, 30 s at the annealing Tm and 30 s at 72 °C.

2.9 Statistical analysis

All data are means ± S.E. (n = 3) unless otherwise specified. Percentage data were subjected to arcsin square-root transformation prior to statistical analyses. Data were tested for normality and homogeneity of variances with Levene's test prior to one-way analysis of variance followed by a Tukey-Kramer HSD multiple comparisons of means. All statistical analyses were performed using SPSS software (IBM SPSS Statistics 19; SPSS Inc., Chicago, IL, USA). Microarray hybridisation data were analysed in GeneSpring GX version 12.6.1 (Agilent Technologies, Wokingham, Berkshire, UK) by two-way analysis of variance (ANOVA), which examined the explanatory power of the variables diet and population as well as "diet x population" interaction. No multiple test correction was employed as previous analyses indicated that they were over-conservative for these nutritional data [24]. Data were submitted to the Kyoto Encyclopedia of Genes and Genomes (KEGG) [25] for biological function analysis. Gene expression results were analysed using the relative expression software tool (REST 2009; http://www.gene-quantification.info/), which employs a pairwise fixed reallocation randomisation test (10,000 randomisations) with efficiency correction [26] to determine the statistical significance of expression ratios (gene expression fold changes) between two treatments.

The relationship (if any) between hepatic expression of LC-PUFA biosynthesis-related genes and flesh PUFA profiles was explored by applying multivariate principal component analysis (PCA) using SPSS. Data included in the PCA analysis were $\log_2$ fold change of gene transcripts and the percentages of n-6 and n-3 PUFA. After performing PCA, components scores were clustered according to the Ward's method followed by K-means cluster in order to quantitatively define the relationships observed in the PCA.

3. Results

3.1 Fish performance

Fish doubled their weight by the end of the dietary trial and survival was 100 % throughout the experimental period. No differences were observed in fish final weight among the dietary treatments or populations (Table 2). However, as the farmed stock fish had a higher initial weight this meant that the Gullspång land-locked population had a significantly higher specific growth rate (SGR) (Table 2). Two-way ANOVA highlighted that SGR was significantly affected by the population factor ($p<0.001$), and there was no interaction between population and dietary factors. The land-locked population also had a higher K-factor at the end of trial (> 1.4) compared to the farmed strain (ca 1.3)

Table 2 here

3.2 Flesh lipid content and fatty acid profile

Salmon fed the RO diet tended to display higher lipid deposition in muscle tissue, although no statistically significant differences were observed among the populations or dietary treatments ($p = 0.174$; Table 3). In general, fatty acid composition only varied according to the diet with population having no effect on the flesh fatty acid profile. Therefore, fish fed the FO diet reflected the dietary fatty acid composition with higher levels of n-3 LC-PUFA, particularly EPA, 22:5n-3 and DHA. Conversely, the RO-fed fish displayed a typical terrestrial-type fatty acid composition, with higher levels of total monoenes and n-6 PUFA. No differences were observed in flesh fatty acid compositions between land-locked and farmed salmon for each of the dietary treatments. Nevertheless, significant interactions between diet and population were observed for 20:4n-3 and 22:5n-3 levels in flesh.

Table 3 here

3.3. Activity of LC-PUFA biosynthesis pathway in hepatocytes

The fatty acyl desaturation/elongation activities determined in hepatocytes at the end of the dietary trial using [1-^{14}C]18:3n-3 as substrate showed significant differences between the populations and dietary treatments (Table 4). Thus, recovery of radioactivity in desaturated products was higher in land-locked salmon regardless of dietary treatment. The rank order for overall pathway activity (Total) and recovery of radioactivity in EPA and 22:5n-3 was WRO > WFO >FRO > FFO. Recovery of radioactivity in DHA was also highest in hepatocytes from WRO and over two-fold higher than the recovery of radioactivity in DHA in FRO. The recovery of radioactivity in the 20:3n-3 elongation product showed a reciprocal response to desaturated products with the rank order being FRO > FFO > WFO >WRO. Population had a more profound effect on desaturation activity than dietary treatment, being significant for all

the desaturated products (Table 4). Diet only significantly affected recovery of radioactivity in EPA and DHA and, similarly, these were the fatty acids where there was no interaction between population and diet.

Table 4 here

3.4 Expression of genes of LC-PUFA biosynthesis in liver

Quantification of expression of fatty acyl desaturase and elongase genes involved in the biosynthesis of LC-PUFA showed there was a clear effect of population in fish fed the FO diet with higher expression of all genes, other than *elovl5b*, in land-locked salmon compared to the farmed stock (Fig.1). In contrast, there was no difference between the populations in the expression of the LC-PUFA genes when fed the RO diet, other than lower expression of *elovl5b* in the land-locked population. Diet had a clear effect on the expression of the LC-PUFA biosynthesis genes in the farmed population with higher expression of all genes, significantly so in the case of the *fads* genes and *elovl5b*, in fish fed RO compared to fish fed FO (Fig.1). However, the opposite trend was apparent in the land-locked population with generally lower expression in fish fed the RO diet compared top fish fed the FO diet albeit this was only significant with *elovl5b*.

Fig. 1 here

3.5 Principal component analysis

The application of PCA and algorithm clustering resulted in the segregation of three clear clusters in both the farmed and land-locked stocks (Fig. 2). PCA explained around 80 % of the total variation present in the data matrix in both populations with PC1 accounting for about 69 % and 62 % in farmed and land-locked populations, respectively. However, how the fatty acids and genes clustered was very different between the two salmon populations. In the farmed stock, PC1 clearly separated the n-6 PUFA, other than arachidonic acid (ARA, 20:4n-6), from the n-3 PUFA with the main pathway products (ARA, EPA and DHA) also in a distinct cluster (Fig. 2A). All the *fads* and *elovl* genes clustered with the n-6 PUFA. In the land-locked population, PC1 still generally separated the n-6 and n-3 PUFA but in the opposite direction to that observed in the farmed population (Fig. 2B). Most genes of LC-PUFA biosynthesis clustered with the main n-3 PUFA group other than *fads2d6* that clustered separately with the main products in the n-6 and n-3 pathways, ARA and DHA (Fig. 2B).

Fig. 2 here

3.6 Responses of liver transcriptome

A first approach to the analysis of the liver transcriptome dataset showed that that the normalised data grouped into two well differentiated clusters, with population (F and W) being the condition influencing the hierarchical clustering (Fig.3). Each population separated into subclusters reflecting dietary regime with two obvious clusters representing the FO and RO fed groups for the land-locked (W) salmon although this was not as clear in farmed (F) salmon where there was some overlap between the two dietary groups. Fig. 3 here

Two-way ANOVA of the cDNA microarray dataset to identify differentially expressed genes and classify the observed expression patterns returned a high number of features showing evidence of differential expression ($p < 0.05$) for each factor. A total of 6889 probes were affected by population with a subset 3924 that were exclusively affected by population and not diet (Fig. 4A). Similarly, 6063 probes exhibited differential expression in response to diet, with 3089 probes only affected by diet, 2371 probes affected by both population and diet and 445 affected by diet and the interaction of diet and population (Fig. 4A). The lists for each comparison were subjected to further analysis by assigning KEGG orthology (KO) numbers and mapping them to a known compendium of categories (KEGG; excluding 39-40 % non–annotated features). The functional categories most affected were the same when analysing effects of population, diet and interaction, with those being metabolism (mainly carbohydrate, amino acid, lipid and energy metabolism) followed by signalling and immune responses (Figs. 4B-D). Fig. 4 here

To elucidate which pathways were affected by the genetic background of the fish, the list of genes exclusively affected by population (3924 probes) were KEGG-mapped and differentially expressed pathways evaluated. Figure 5 represents a chart where each pathway is represented by a bubble that is colour coded for functional category with the size of the bubble representing the number of differentially expressed genes (DEG) in each pathway. The main functional category affected was that of Environmental Information Processing, displaying 9 significantly affected pathways with a variable number of genes affected in each pathway (19-45 differentially expressed genes, DEG). Genetic Information displayed the highest number of DEG, particularly in ribosome (80 DEG), which also showed one of the lowest p values ($p = 0.007$). Similarly, the highest absolute fold change (FC) was found in two pathways belonging to the Genetic Information category (ubiquitin mediated proteolysis and protein processing in endoplasmic reticulum; FC = 2.02 and 1.95, respectively). Organismal System was also affected, displaying 8 different pathways with over 18 DEG in each. Within this category, two pathways intimately related to metabolism, insulin and

glucagon signalling, were affected. Regarding metabolism, only three pathways displayed over 18 DEG. Nucleotide metabolism was affected by population with purine and pyrimidine metabolism showing similar p values (0.013 and 0.014 respectively) albeit a higher absolute FC was found for pyrimidine (1.52 *vs.* 1.41). Fig. 5 here

The metabolic category most affected by population was carbohydrate metabolism (157 DEG) followed by lipid and amino acid metabolism (104 and 102 DEG, respectively; Fig. 6A). In lipid metabolism, glycerophospholipid and ARA metabolism were highly affected, with highest up-regulated FC in *phosphatidate phosphatase* (*ppap2*, +5.30) and *lysophosphatidic acid acyl transferase* (*agpat*, +2.63; Supplementary Table 2). In addition, there was high representation of genes of fatty acid oxidation including *carnitine palmitoyl transferase 2* (*cpt2*), *3-hydroxyacyl-CoA dehydrogenase* (*hoad*) and *acyl-coA oxidase* (*aco*) that were all down-regulated in land-locked salmon (Supplementary Table 2). In contrast, lipogenic pathway genes including *fatty acid synthase* (*fas*), *glucose 6-phosphate dehydrogenase* (*g6pd*) and *peroxisome proliferator-activated receptor gamma* (*pparg*) were up-regulated in land-locked salmon regardless of diet (Supplementary Table 2). The top 10 metabolic pathways, according to the number of DEG, showed that amino acid metabolism was the most represented category (4 pathways), followed by lipid and carbohydrate (three pathways each; Fig.6B). A positive correlation (R^2 = 0.9715) was found between the metabolic functional categories, where high FC was correlated with high –log10 P values, indicating that highly significant genes also displayed high FC. This was particularly evident for the metabolic categories of energy, carbohydrate and lipid. Fig. 6 here

Listing the top 100 genes according to significance, an overrepresentation of metabolic pathways was found (33 %) with carbohydrate and lipid metabolism being the most represented (8% each; Supplementary Table 3). Translation (18 %) and signal transduction (17 %) were the most affected categories after metabolism. Six percent of DEG belonged to amino acid metabolism with all being down-regulated in land-locked salmon regardless of dietary treatment. Two DEG in the lipid metabolism category belonged to ether lipid metabolism (*ectonucleotide pyrophosphatase* and *phospholipase D3/4*) although regulated in opposite directions, while *decaprenyl-diphosphate synthase subunit 2*, involved in terpenoid backbone biosynthesis displayed the highest negative FC (-4.19) within lipid metabolism (Supplementary Table 3). The top 100 most significant genes included those involved in glucose metabolism such as the glycolytic enzyme *6-phosphofructokinase 1* (*6pfk*) that was strongly up-regulated (+3.73) and the gluconeogenic enzyme *pyruvate carboxylase* (*pc*) that

was down-regulated (-1.69). Two pathways closely related to carbohydrate (insulin pathway) and lipid metabolism (PPAR signalling pathway) were present in the top 100 category list. *Protein phosphate 1 regulatory subunit 3A*, belonging to the insulin signalling pathway displayed a -1.67 FC down-regulation, whereas the two genes belonging to the PPAR signalling pathway showed divergent regulation, with *intestinal fatty acid binding protein* up-regulated (+2.06) and fatty acid transporter (*solute carrier family 27*) down-regulated (-1.50).

The microarray data were validated by qPCR by comparing the expression of 5 genes belonging to the LC-PUFA biosynthesis pathway (Supplementary Table 4). Good correspondence in terms of intensity (FC) of direction of change (up- or down-regulated) was observed among all the studied genes (100 %). The match was also consistent in terms of significance (p value) when comparing microarray and qPCR results (80 %; 4 out of 5).

4. Discussion

Finding alternative dietary sources to substitute for FO in aquafeeds is one priority in aquaculture nutrition. Although most research has focussed on testing alternative raw materials such as vegetable oils [10,24,27-28], microalgal lipids [29-30] or *de novo* sources of omega-3 [31-32], there has been less effort investigating the n-3 trait in fish. It is known that both fatty acid deposition and the activity of the bioconversion pathway are dependent on the genetic background of the fish [14,33-34] and the level of n-3 LC-PUFA in flesh was shown to be highly heritable trait in salmon [7]. However, it is also known that changes in dietary lipid source can alter the genetic potential, as well as the magnitude and direction of response between family groups [24,27]. In the present study, a two-factorial design nutritional trial was employed to elucidate the potential of parr from a land-locked Atlantic salmon population (only ever exposed to freshwater) compared to parr from a farmed anadromous stock (exposed to seawater every generation) when fed diets formulated with fish oil (FO) and rapeseed oil (RO).

A major finding in the present study was that there were differences in n-3 LC-PUFA metabolic trait between the two salmon populations with definite indications that the land-locked population may have higher potential for n-3 LC-PUFA biosynthesis than the farmed stock. Thus, a clear result was the higher expression of the LC-PUFA biosynthesis genes including *fads2d6, fads2d5, elovl2* and *elovl5a,* in land-locked salmon compared to the

427 farmed stock when fed FO. This was reflected in the activity of the LC-PUFA biosynthesis
428 pathway in hepatocytes with increased recovery of radioactivity from labelled 18:3n-3 being
429 found in all intermediates and products of the pathway in the WFO group compared to the
430 FFO group. However, the same situation was not found in fish fed the RO diet because,
431 although the activity of the LC-PUFA biosynthesis pathway was higher in the land-locked
432 salmon (WRO) than in the farmed stock (FRO), this was not a reflection of gene expression
433 as there was no difference between the populations in the expression of any of the LC-PUFA
434 biosynthetic genes when fed RO other than e*lovl5b*, which actually showed lower expression
435 in the WRO group. Therefore, the expression of the genes in the two populations was not
436 fully reflected in the activities of the enzymes. This variation with diet was unexpected and
437 the mechanism unclear from these data alone.

438 However, further insight was provided by comparing the effect of diet within the populations.
439 Thus, the farmed population showed a very clear response to diet with the expression of all
440 genes, especially the *fads*, being higher in fish fed the RO diet compared to fish fed the FO
441 diet. Similarly, the activity of the LC-PUFA biosynthetic pathway in hepatocytes was
442 significantly higher in the FRO group compared to the FFO group. This was entirely
443 consistent with many previous studies investigating the LC-PUFA biosynthesis pathway in
444 salmon from farmed stocks, with fish fed vegetable oil, including rapeseed, diets showing
445 higher expression of LC-PUFA genes (especially *fads*) [24,31,35-40] and higher enzymatic
446 activity of the pathway in hepatocytes [16,37,40-43] than fish fed fish oil diets. In contrast,
447 the land-locked population did not show the same response to the RO diet with no increased
448 expression of any of the genes involved in LC-PUFA biosynthesis and no increased activity
449 of the biosynthetic pathway in hepatocytes compared to fish fed FO diet.

450 Therefore, whereas the results for the farmed population were consistent with previous
451 studies, the land-locked population showed important differences. Firstly, land-locked salmon
452 displayed higher LC-PUFA biosynthetic capacity than farmed fish, irrespective of diet.
453 However, gene expression in liver of land-locked fish only paralleled biosynthetic activity in
454 hepatocytes in fish fed FO and not in fish fed RO and, furthermore, land-locked salmon did
455 not respond to the RO diet in the same way as farmed fish have been shown to. This gives a
456 clue to one of the genetic differences between these two populations. The many studies in
457 salmon using fish from farmed stock fed FO and VO diets have led to the conclusion that the
458 higher activity and gene expression in fish fed VO was due to dietary LC-PUFA in fish oil
459 suppressing expression of the genes of LC-PUFA biosynthesis, especially the *fads* [35-36,44-

46] but, to a lesser extent, perhaps also elongases [47]. It has been established more recently that the suppression is due specifically to DHA as EPA was not effective in suppressing LC-PUFA biosynthesis [40] or expression of the biosynthetic genes [32]. Taking all the data obtained together the present study has suggested that the land-locked salmon are perhaps less influenced by dietary fatty acid composition and, specifically, less sensitive to dietary DHA. Thus, the expression of the LC-PUFA biosynthetic genes, and consequent biosynthesis pathway activity, are not suppressed in fish fed the FO diet. The molecular mechanism for this is not clear but is possibly related to differences in the upstream regulatory UTR (promoter) region of the genes [48]. It is interesting to speculate on whether this is an evolutionary adaptation to a natural diet lower in DHA [44]. The argument being that upon moving to seawater, anadromous salmon start consuming a diet with high levels of DHA and as an adaptation to that, a mechanism evolved preventing unnecessary biosynthesis. In land-locked salmon this mechanism was lost due to the fact that DHA was no longer a major component of the diet and evolutionary pressure for the mechanism was relieved. However, domestication itself could potentially affect the regulation of LC-PUFA metabolism as it has been suggested that domestication may be sufficient to induce heritable alterations in transcription levels compared to wild populations [49-51].

However, the fact that, irrespective of diet, the activity of the biosynthetic pathway was higher in land-locked salmon compared to farmed salmon may suggest other differences in the *fads* and/or *elovl* genes, perhaps even in primary sequence. Studies in the human have demonstrated that cellular fatty acid compositions are influenced by SNPs in the FADS1 and FADS2 gene cluster [52-54]. Furthermore, it has been shown previously that there are potentially three functional *fads2d6* (Δ6) genes in Atlantic salmon and each appeared to show a different pattern of regulation in response to dietary VO in a farmed salmon stock [55]. A further interesting factor to consider in evaluating differences between farmed and land-locked populations is Δ8 desaturase activity, which is an inherent property of Δ6 desaturases, and that catalyses the conversion of 20:3n-3 to 20:4n-3 [56]. Traditionally 20:3n-3 had been considered a "dead-end" product in the biosynthesis of n-3 LC-PUFA as it was not regarded as an intermediate in the desaturation-elongation pathway [45]. In the present study, the amount of radioactivity recovered in 20:3n-3 (and 20:4n-3) was lower in the land-locked population compared to the farmed population whereas the recovery of radioactivity in 20:4n-3 was higher in the land-locked fish. However, there was also increased recovery of radioactivity in 18:4n-3 in land-locked fish (resulting from Δ6 desaturation of 18:3n-3) and so

the source of radioactivity in 20:4n-3 could be from either Δ6 or Δ8 activity. Studies on the Δ8 desaturase activity of several fish species showed that FADS2D6 of diadromous fish species such as the Atlantic salmon exhibited limited Δ8 desaturation activity, especially when compared with seawater teleost [56]. The lower proportion of radioactivity recovered in 20:3n-3 in the present study may suggest that land-locked salmon FADS2D6 might have an enhanced Δ8 activity compared to their farmed counterparts. Studies evaluating polymorphic variation in the salmon genome could be insightful as the previous studies on SNPs in the human FADS1-FADS2 desaturase gene cluster have indicated [52-54]. The molecular mechanism(s) of the differences in LC-PUFA biosynthetic capacity between the farmed and land-locked stocks are clearly of interest and worthy of further studies in the future.

There was no indication of any substantial LC-PUFA biosynthesis observed in the flesh of either population of salmon as both strains primarily reflected the dietary fatty acid compositions as also reported previously [14]. This was not surprising taking into account the role of muscle in salmon as a lipid storage tissue with limited metabolic activity [5]. Several studies have shown that, whereas salmon muscle (flesh) closely reflects dietary fatty acid composition [5,57-58], liver fatty acid composition reflects the higher metabolic activity, including LC-PUFA biosynthesis, in that tissue [31-32]. However, desaturase expression and activity in one tissue can affect fatty acid composition in another as demonstrated in poultry [59-60]. Therefore, in the present study, we focused on investigating whether flesh compositions reflected any potential genetic differences between the populations and, simply looking at overall compositions, that appeared not. However, the PCA analysis showed an interesting difference between the two populations in the correlation/association between flesh n-3 LC-PUFA levels and the expression of the LC-PUFA biosynthesis genes in the liver. Previously, this analyses had shown that Atlantic salmon fed camelina products displayed a link between 22:5n-3 and DHA levels and *elovl5a* transcript level [28]. In the present study, all the biosynthetic genes other than *fads2d6*, which clustered with the main end-point fatty acids ARA and DHA, clustered with n-3 PUFA in land-locked salmon whereas all the genes clustered with n-6 PUFA in farmed population. While this result demonstrates a clear difference between the populations in the relationship between flesh fatty acids and liver gene expression the precise mechanism underpinning the data is unclear.

Apart from enhanced n-3 LC-PUFA biosynthetic capacity, land-locked salmon from the Gullspång Swedish stock displayed higher growth in terms of SGR than the farmed stock independent of diet, indicating that overall fish performance was affected by population.

Similar results were reported in a previous study on another land-locked salmon population (Penobscott strain), which showed higher daily growth rate than a farmed stock [14]. Thus, it is likely that several physiological processes, rather than just LC-PUFA metabolism may vary between farmed and land-locked salmon populations that allow the latter fish to better utilise dietary nutrients to promote growth. It was in this context that liver transcriptome was analysed in the present study. This analysis indicated that metabolism was a highly affected category and, within metabolism, carbohydrate, lipid and energy metabolism showed a large number of genes whose expression varied depending on the genetic background. Lipid and glucose metabolism are principally regulated by insulin and previous studies in rainbow trout (*Oncorhynchus mykiss*) demonstrated diverging patterns of expression in glucose and lipid-related genes between two experimental lines developed for high (fat) or low (lean) muscle fat when insulin was administered [61]. Dietary glucose functions mainly as a fuel through the glycolytic pathway or, in excess, is converted to lipids for storage. Expression of *6pfk*, the most important regulatory enzyme in glycolysis, was up-regulated in land-locked salmon whereas *pk*, a key enzyme in the synthesis of glucose was down-regulated. This increased potential for glucose catabolism was accompanied by stimulation in lipogenic gene expression (*fas*, *g6pd*, and *pparg*) as well as an inhibition in lipolysis pathways (*cpt2*, *hoad* and *aco*). These results suggested that the land-locked salmon had a higher capacity for lipogenesis together with limited lipolysis as well as an enhanced utilization of glucose as a source of energy that could, in part, contribute to higher growth rate. This is contrary to the generally accepted view that carnivorous fish such as Atlantic salmon have a limited ability to use dietary carbohydrates efficiently [62]. However, previous studies in rainbow trout demonstrated enhanced glycolytic potential as well as decreased fatty acid oxidation in the liver of a selected fat line [63] although no differences in the expression of glycolytic or fatty acid oxidation genes were found among Atlantic salmon families with high or low fat content in muscle [24]. As mentioned above, it is also possible that differences in metabolism between populations could be influenced by domestication and the fact that food acquisition is not a threat to survival in farmed populations. In this respect, differential expression in several metabolic pathways was found between farmed and wild salmon populations at early life stages suggesting that domestication may influence transcriptomic responses (49-51).

The genetic background of the two populations also appeared to have an effect on the insulin pathway as up-regulation of *pi3k* and *raptor* was found in land-locked salmon. In mammals insulin activates *pi3k* that phosphorylates a number of downstream proteins such as *raptor*

559 [64]. Some of the effects of insulin are to promote the uptake of fatty acids, to increase
560 lipogenesis and inhibit fatty acid oxidation [61,65], which are the effects observed in the
561 land-locked salmon. Thus, our results may indicate that, in addition to enhanced glycolytic
562 and lipogenic potential and decreased fatty acid oxidation, the regulatory effect of insulin
563 might be stronger in land-locked than in farmed salmon. However, previous studies in fish
564 have not shown a correlation between enhanced lipid/carbohydrate metabolism and insulin
565 regulation [61] and thus more studies are required to elucidate the influence of insulin
566 regulation on metabolism of land-locked and farmed salmon populations.

567 The metabolism of glycerophospholipids (phospholipids) was one of the lipid metabolic
568 pathways that differed between the land-locked and farmed salmon, which agreed with a
569 previous study where this pathway was down-regulated in a lean Atlantic salmon line in
570 contrast to a fat line [24]. Phospholipids are the main components in cell membranes and play
571 a major role in maintaining the structure and functions of cell membranes as well as energy
572 production [66]. Two enzymes that act consecutively in the pathway, *agpat* and *ppap2*,
573 showed higher expression levels in land-locked salmon. *agpat* converts lysophosphatidic acid
574 into phosphatidic acid whereas *ppap2* catalyses the dephosphorylation of phosphatidic acid
575 yielding diacylglycerol [67]. Both phosphatidic acid and diacylglycerol can act as second
576 messengers [68-69] potentially leading to an alteration in the levels of lipid signalling
577 molecules which could in turn affect several key physiological processes influencing growth
578 performance. Similarly *agpat* was up-regulated in the fat Atlantic salmon line [24].
579 Additionally two phospholipases, secretory phospholipase A2 and phospholipase D4,
580 enzymes that hydrolyse the ester bond at the *sn-2* position of membrane phospholipids, also
581 showed higher expression in the liver of land-locked salmon. The released fatty acids
582 (particularly EPA and ARA) are substrates for the production of eicosanoids, a range of
583 highly bioactive derivatives implicated in many physiological processes [5]. The remaining
584 lysophospholipid also has important roles in several biological processes such as stimulation
585 of preadipocyte proliferation and differentiation [70-71]. Thus, this range of highly
586 biologically active products obtained from the actions of phospholipases may act as
587 mediators of metabolism contributing to the physiological differences observed between
588 land-locked and farmed salmon stocks.

589 In conclusion, the present study indicated a higher capacity to synthesise n-3 LC-PUFA in the
590 liver of land-locked salmon than in their farmed counterparts regardless of dietary fatty acid
591 composition. Increased expression of enzymes (desaturases and elongases) involved in this

pathway was generally observed in land-locked salmon compared to the farmed salmon stock regardless of dietary treatment and this was associated with higher activity in the biosynthetic pathway. Apart from an enhancement in desaturation/elongation activities, high lipogenic and glycolytic potential were found in land-locked salmon, together with decreased lipolysis which in turn could indicate increased use of carbohydrates as an energy source and a sparing of lipid. This hypothesis was reinforced by the up-regulation found in the insulin pathway. However, the fatty acid of flesh reproduced dietary contents and thus the enhanced LC-PUFA biosynthetic capability of Atlantic land-locked salmon was not reflected. It must be noted thought that firstly, the trial had a short duration (8 weeks) and maybe not enough time was given so that the dietary n-3 LC-PUFA could be deposited and stored by the fish muscle. Secondly, only total lipid fatty acids were analysed and triglycerides can dilute the total percentage of DHA incorporated into the phospholipids.

5. References

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828 **Legends to Figures**

829

830 Fig. 1. Expression of genes of the LC-PUFA biosynthesis pathway in Atlantic salmon liver
831 as determined by qPCR. Results are normalised expression ratios (means ± SEM; n = 6). F,
832 farmed salmon; W, land-locked salmon; FO, fish oil diet; RO, rapeseed oil diet. *fads2d6*,
833 delta-6 fatty acyl desaturase; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*, fatty acyl
834 elongase 2; *elovl5a*, fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase 5 isoform b.
835 Different superscript letters denote differences among treatments identified by one-way
836 ANOVA. The inset Table presents p values for the effect of diet, population and their
837 interaction on the relative gene expression.

838

839 Fig. 2. Principal component analysis of flesh fatty acid data (%) and expression of lipid
840 metabolism genes in liver (log2 FC). (A) Farmed salmon fed both dietary treatments. (B)
841 Land-locked salmon fed both fish oil and rapeseed oil diets. After performing PCA,
842 components scores were clustered according to the Ward's method followed by K-means
843 cluster in order to quantitatively define the relationships observed in the PCA

844

845 Fig. 3. Hierarchical cluster (10000 randomisations) showing the distribution of the
846 normalised data from the microarray analysis. F, farmed salmon; W, land-locked salmon;
847 FO, fish oil diet; RO, rapeseed oil diet.

848

849 Fig. 4 Functional categories of genes differentially expressed in farmed (F) or land-locked
850 (W) Atlantic salmon after 8 weeks of feeding fish oil (FO) or rapeseed oil (RO) diets. Genes
851 differentially expressed according to two-way ANOVA analysis ($p < 0.05$) between the two
852 diets (A), the population groups (B) as well as due to the interaction of both factors (C).
853 Non-annotated genes and features corresponding to the same gene are not represented.

854

855 Fig. 5 Bubble chart representing the top 30 categories with a highest number of DEG from
856 the list of genes exclusively regulated by population. The area of the circle is scaled to the
857 number of DEG within each pathways (17-80 DEG). Each colour represents a different
858 category; Genetic Information (blue), Environmental Information Processing (green),
859 Cellular Processes (yellow), Organismal System (red) and Metabolism (purple)

860

Fig. 6 Bubble chart representing the number of affected genes within each metabolism category. The area of the circle is scaled to the number of DEG within each pathways (32-157 DEG). (B) KEGG pathway analysis of genes belonging to category of metabolism that were exclusively regulated by population as indicated by two-way ANOVA analysis Each colour indicated a different nutrient metabolism. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 1- Formulation (% of the feed ingredients indicated) Formulations, proximate and fatty acid compositions (percentage of fatty acids) of the experimental feeds

	FO	**RO**
Fish meal	33.5	33.5
Soy protein concentrate	20.0	20.0
Fish oil	17.0	-
Rapeseed oil	-	17.0
Wheat	10.0	10.0
Wheat gluten	15.0	15.0
Vitamin mix	2.0	2.0
Mineral mix	0.5	0.5
Monosodium phosphate	2.0	2.0
Analysed composition		
Dry matter (%)	91.1	91.3
Protein (%)	50.0	49.4
Fat (%)	15.1	15.2
Ash	8.1	8.0
Fatty acid composition (%)		
Σ saturated[1]	22.4	14.2
Σ monounsaturated[2]	45.8	53.3
18:2n-6	7.0	16.6
20:2n-6	0.2	0.1
20:3n-6	0.0	0.1
20:4n-6	0.4	0.1
Σ n-6 PUFA[3]	7.9	17.1
18:3n-3	1.6	6.0
20:3n-3	0.15	0.05
20:4n-3	0.5	0.2
20:5n-3	7.0	2.9
22:5n-3	0.7	0.3
22:6n-3	9.6	4.6
Σ n-3 PUFA	22.6	15.1
Σ PUFA[4]	31.7	32.5
Total n-3 LC-PUFA	17.3	7.8

[1]contains 15:0, 22:0 and 24:0; [2]contains 16:1n-9, 20:1n-11, 20:1n-7, 22:1n-9 and 24:1n-9; [3]contains 22:4n-6 and 22:5n-6; [4]contains C16 PUFA. Fish and FO, fish oil and respective feed; LC- PUFA, long-chain polyunsaturated fatty acid (sum of 20:4n-3, 20:5n-3 22:5n-3 and 22:6n-3); n.d. not detected.

Table 2.- Growth performance, survival, feed utilisation and specific growth rate of Atlantic salmon from the two studied stocks after 8 weeks of feeding the experimental diets.

	FO		RO		p value two-way ANOVA		
	F	**W**	**F**	**W**	**POP**	**D**	**POP*D**
Initial weight (g)	31.2±0.9[a]	24.7±0.3[b]	30.5±0.8[a]	24.9±0.3[b]	**	n.s.	n.s.
Final weight (g)	58.4±1.7	57.4±2.9	57.8±2.5	57.8±3.2	n.s.	n.s.	n.s.
K factor	1.30±0.01[a]	1.43±0.04[b]	1.29±0.00[a]	1.44±0.003[b]	**	n.s.	n.s.
Survival (%)	100	100	100	100	n.s.	n.s.	n.s.
SGR	1.6±0.2[b]	2.1±0.1[a]	1.6±0.1[b]	2.1±0.1[a]	**	n.s.	n.s.

Data are means ± SD (n=3). Different superscript letters within a row denote significant differences among the diets and stocking conditions. SGR, specific growth rate , S, farmed salmon; W, land-locked salmon; FO, fish oil diet; RO, rapeseed oil diet; POP, population; D, diet. * $p<0.05$; ** $p<0.01$; n.s. not significant.

Table 3.- Lipid content (percentage of wet weight) and fatty acid compositions (percentage of total fatty acids) of total lipid from muscle tissues (flesh) of Atlantic salmon after 8 weeks of feeding the experimental feeds

	FO		RO		Two-way ANOVA p		
	F	**W**	**F**	**W**	**POP**	**D**	**POP*D**
Lipid content	2.8±1.3	2.4±0.7	3.0±1.0	3.6±1.7	n.s.	n.s.	n.s.
14:0	3.9±0.3^{a}	3.9±0.2^{a}	2.7±0.2^{b}	2.7±0.4^{b}	n.s.	**	n.s.
16:0	15.9±0.7^{a}	16.3±0.3^{a}	13.5±0.7^{b}	13.8±0.8^{b}	n.s.	**	n.s.
18:0	3.4±0.1	3.3±0.1	3.2±0.2	3.3±0.1	n.s.	*	n.s.
20:0	0.2±0.0^{bc}	0.1±0.0^{c}	0.2±0.1^{ab}	0.2±0.1^{a}	n.s.	**	n.s.
Σ saturated[1]	23.8±0.6^{a}	24.0±0.5^{a}	20.0±0.7^{b}	20.2±1.1^{b}	n.s.	**	n.s.
16:1n-7	4.3±0.4^{a}	4.5±0.3^{a}	3.0±0.3^{b}	3.0±0.5^{b}	n.s.	**	n.s.
18:1n-9	18.8±1.5^{b}	18.3±1.0^{b}	28.5±2.1^{a}	30.4±4.6^{a}	n.s.	**	n.s.
18:1n-7	2.8±0.2^{ab}	2.6±0.1^{b}	2.9±0.2^{a}	2.9±0.3^{a}	n.s.	**	n.s.
20:1n-11	0.9±0.1^{a}	0.9±0.1^{a}	0.4±0.0^{b}	0.5±0.1^{b}	n.s.	**	n.s.
20:1n-9	6.0±0.6^{a}	6.1±0.4^{a}	4.3±0.3^{b}	4.6±0.6^{b}	n.s.	**	n.s.
20:1n-7	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{b}	0.2±0.0^{b}	n.s.	**	n.s.
22:1n-11	6.4±0.8^{a}	6.6±0.6^{a}	3.6±0.3^{b}	3.8±0.7^{b}	n.s.	**	n.s.
22:1n-9	0.5±0.1^{a}	0.6±0.0^{a}	0.4±0.0^{b}	0.1±0.1^{b}	n.s.	**	n.s.
Σ monoenes[2]	41.1±3.2^{b}	40.9±2.3^{b}	44.3±3.1^{ab}	46.7±4.9^{a}	n.s.	**	n.s.
18:2n-6	6.3±0.6^{b}	5.7±0.3^{b}	9.9±0.8^{a}	10.3±1.7^{a}	n.s.	**	n.s.
18:3n-6	0.1±0.0^{b}	0.1±0.0^{b}	0.2±0.0^{a}	0.2±0.0^{a}	n.s.	**	n.s.
20:2n-6	0.6±0.1^{b}	0.5±0.0^{b}	0.9±0.1^{a}	0.9±0.1^{a}	n.s.	**	n.s.
20:3n-6	0.2±0.0^{b}	0.2±0.0^{b}	0.4±0.1^{a}	0.5±0.1^{a}	n.s.	**	n.s.
20:4n-6	0.5±0.1	0.5±0.1	0.5±0.1	0.4±0.1	n.s.	*	n.s.
Σ n-6 PUFA[3]	7.9±0.6^{b}	7.3±0f.3^{b}	12.1±0.8^{a}	12.5±1.8^{a}	n.s.	**	n.s.
18:3n-3	2.0±1.1^{b}	1.4±0.1^{b}	3.1±0.2^{a}	3.1±0.6^{a}	n.s.	**	n.s.
18:4n-3	1.5±0.1^{a}	1.4±0.1^{a}	1.1±0.1^{b}	1.0±0.1^{b}	n.s.	**	n.s.
20:3n-3	0.2±0.0^{b}	0.2±0.0^{b}	0.3±0.0^{a}	0.3±0.0^{a}	n.s.	**	n.s.
20:4n-3	0.9±0.0^{b}	0.8±0.0^{b}	0.7±0.0^{a}	0.6±0.1^{a}	n.s.	**	**
20:5n-3	4.1±0.5^{a}	3.9±0.6^{a}	2.7±0.3^{b}	2.4±0.7^{b}	n.s.	**	n.s.
22:5n-3	1.5±0.1^{a}	1.5±0.1^{a}	1.0±0.1^{b}	0.9±0.2^{b}	n.s.	**	*
22:6n-3	17.1±2.9^{a}	16.7±1.9^{a}	13.4±2.1^{b}	12.1±3.2^{b}	n.s.	**	n.s.
Σ n-3 PUFA	27.2±3.0^{a}	25.8±2.2^{a}	22.2±2.2^{b}	20.4±3.9^{b}	n.s.	**	n.s.
Σ PUFA[4]	35.6±2.6	33.7±2.1	34.9±1.6	33.3±2.7	n.s.	n.s.	n.s.
Σ n-3 LC-PUFA	22.7±3.4^{a}	22.1±2.2^{a}	17.2±2.4^{b}	15.4±4.4^{b}	n.s.	**	n.s.

Data expressed as means ± SD (n=12). Different superscript letters within a row denote significant differences among diets determined by one-way ANOVA with Tukey's comparison test ($p<0.05$). [1]contains 15:0, 22:0 and 24:0; [2]contains 16:1n-9 and 24:1n-9; [3]contains 22:4n-6 and 22:5n-6; [4]contains C16 PUFA. FO, fish oil feed; RO; rapeseed oil feed; F, farmed salmon stock; W, Gullspång strain. * $p<0.05$; ** $p<0.01$; n.s. not significant.

914 Table 4.- Elongation and desaturation (pmol/mg protein/h) of [1-^{14}C]18:3n-3 in landlocked
915 Atlantic salmon hepatocytes.

	Elongation	**Desaturation**					
	20:3n-3	**Total**	**18:4n-3**	**20:4n-3**	**20:5n-3**	**22:5n-3**	**22:6n-3**
FFO	5.5±0.7^{bc} (50.0%)	4.7±0.3^{c}	1.2±0.1^{c} (14.9%)	1.4±0.1^{c} (12.7%)	0.7±0.1^{c} (9.8%)	0.9±0.1^{c} (8.0%)	0.4±0.0^{b} (4.6%)
WFO	7.3±0.4^{ab} (45.7%)	8.9±0.4^{a}	2.0±0.1^{a} (15.9%)	2.6±0.2^{a} (12.5%)	1.7±0.2^{ab} (11.7%)	1.9±0.1^{a} (10.6%)	0.6±0.1^{b} (3.8%)
FRO	7.7±0.5^{a} (51.5%)	6.7±0.3^{b}	1.5±0.0^{b} (14.7%)	2.0±0.1^{bc} (10.5%)	1.2±0.1^{bc} (10.5%)	1.4±0.1^{b} (9.1%)	0.5±0.0^{b} (3.7%)
WRO	4.6±0.3^{c} (32.9%)	9.2±0.5^{a}	1.7±0.1^{ab} (17.0%)	2.3±0.1^{b} (12.0%)	1.7±0.1^{a} (17.6%)	2.4±0.2^{a} (12.2%)	1.1±0.2^{a} (8.3%)
Population	n.s.	**	**	**	**	**	**
Diet	n.s.	**	n.s.	n.s.	**	n.s.	**
Interaction	**	*	**	**	n.s.	*	n.s.

916

917 Results are means ± SE (n=12). F, farmed salmon; W, land-locked salmon; FO, fish oil diet; RO,
918 rapeseed oil diet. Different superscript letters within an individual column denote significant statistical
919 differences in fatty acid content according to one-way ANOVA. * $p<0.05$; ** $p<0.01$; n.s. not
920 significant.

921

922 Supplementary Table 1.- Primer used for qPCR analysis

Transcript	Primer sequence (5'→3')	Amplicon (bp)	Ta	Accession no
fads2d6	F: TCCTCTGGTGCGTACTTTGT	163	59˚C	NM_001123575.2[a]
	R: AAATCCCGTCCAGAGTCAGG			
fads2d5	F: GCCACTGGTTTGTATGGGTG	148	59˚C	NM_001123542.2[a]
	R: TTGAGGTGTCCACTGAACCA			
elovl2	F: GGTGCTGTGGTGGTACTACT	190	59˚C	NM_001136553.1[a]
	R: ACTGTTAAGAGTCGGCCCAA			
elovl5a	F: TGTTGCTTCATTGAATGGCCA	150	59˚C	GU238431.1[a]
	R: TCCCATCTCTCCTAGCGACA			
elovl5b	F: CTGTGCAGTCATTTGGCCAT	192	59˚C	NM_001136552.1[a]
	R: GGTGTCACCCCATTTGCATG			
cfl2	F: AGCCTATGACCAACCCACTG	224	60˚C	TC63899[b]
	R: TGTTCACAGCTCGTTTACCG			
ef1a	F: CTGCCCCTCCAGGACGTTTACAA	175	60˚C	AF321836[a]
	R: CACCGGGCATAGCCGATTCC			

923

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

925 [b] Atlantic salmon Gene Index (http://compbio.dfci.harvard.edu/tgi)

926 *fads2d6*, delta-6 fatty acyl desaturase; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*, fatty acyl
927 elongase 2; *elovl5a*, fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase isoform b;
928 *cfl2*, cofilin 2; *ef1a*, elongation fcator 1 alpha.

929

930

Supplementary Table 3. Liver transcripts corresponding to the top 100 most significant annotated features exhibiting common differential expression between land-locked and farmed salmon (W vs F). Features are arranged by functional categories and within them by increasing p value (assessed by two-way ANOVA). The percentages of genes distribution is represented after removing features belonging to the same gene.

KO no	Annotation	FC
Metabolism (33%)		
Carbohydrate (8%)		
K01069	Hydroxyacylglutathione hydrolase	+2.53
K00846	Ketohexokinase	-1.47
K00850	6-phosphofructokinase 1	+3.73
K01958	Pyruvate carboxylase	-1.69
K00780	Beta-galactoside alpha-2,3-sialyltransferase	-1.98
K01641	Hydroxymethylglutaryl-CoA synthase	+2.98
K13247	L-gulonate 3-dehydrogenase	+1.24
K00699	Glucuronosyl transferase	-1.30
Lipids (8%)		
K01122	Ectonucleotide pyrophosphatase	-1.65
K05929	Phosphoethanolamine N-methyltransferase	-1.37
K05309	Microsomal prostaglandin-E synthase 2	+1.54
K16860	Phospholipase D3/4	+2.06
K12505	Decaprenyl-diphosphate synthase subunit 2	-4.19
K07439	24-hydroxycholesterol 7 alpha-hydroxylase	-1.36
K07424	Cytochrome P450, family3, subfamily A	+1.32
K01047	Secretory phospholipase A2	+2.08
Aminoacids (6%)		
K00261	Glutamate dehydrogenase ($NAD(P)^+$)	+1.64
K01620	Threonine aldolase	+1.50
K00544	Betaine-homocysteine S-methyltransferase	+2.49
K00933	Creatine kinase	+1.27
K01581	Ornithine decarboxylase	+2.19
K00544	Betaine-homocysteineS-methyltransferase	+2.49
Energy (4%)		
K02263	Cytochrome c oxidase subunit 4	-1.75
K18246	Carbonic anhydrase 4	+6.43
K02260	Cytochrome c oxidase assembly protein subunit 17	-1.49
K17218	Sulfide:quinone oxidoreductase	-15.51
Nucleotide (4%)		
K00940	Nucleoside-diphosphate kinase	+1.80
K11540	Carbamoyl-phosphate synthase	-1.58
K00758	Thymidine phosphorylase	-1.37
K00939	Adenylate kinase	-1.17
Other (3%)		
K00275	Pyridoxamine 5'-phosphate oxidase	-1.20
K13624	Ceruloplasmin	+1.31
K10106	Vitamin K-dependent gamma-carboxylase	+1.22
Translation (18%)		
Ribosome (14%)		
K02940	Large subunit ribosomal protein L9e	-1.77
K02936	Large subunit ribosomal protein L7Ae	-1.40
K02978	Small subunit ribosomal protein S27e	-1.17
K02885	Large subunit ribosomal protein L19e	-1.23
K02898	Large subunit ribosomal protein L26e	-1.35
K02966	Small subunit ribosomal protein S19e	-1.17
K02876	Large subunit ribosomal protein L15	+1.33

K02883	Large subunit ribosomal protein L18e	-1.31
K02987	Small subunit ribosomal protein S4e	+2.41
K02930	Large subunit ribosomal protein L4e	-1.23
K02974	Small subunit ribosomal protein S24e	-1.15
K02989	Small subunit ribosomal protein S5e	-1.21
K02915	Large subunit ribosomal protein L34e	-1.52
K02877	Large subunit ribosomal protein L15e	-1.18
RNA transport (4%)		
K03262	Translation initiation factor 5	+2.92
K13130	Survival of motor neuron protein-interacting protein 1	+1.31
K12160	Small ubiquitin-related modifier	+1.52
Signal transduction (17%)		
mTOR signalling pathway (1%)		
K08269	Serine/threonine-protein kinase ULK/ATG1	+1.41
Jak-STAT signalling pathway (1%)		
K05069	Interleukin 2 receptor beta	-2.08
Cell adhesion molecules (2%)		
K06087	Claudin	+2.22
K06736	Cadherin 2, type 1, N-cadherin	+1.49
NF-kappaB signalling pathway (3%)		
K04725	E3 ubiquitin-protein ligase XIAP	-1.52
K07371	B-cell linker protein	-1.19
K10652	Tripartite motif-containing protein 25	-1.72
AMPK signalling pathway (2%)		
K00850	6-phosphofructokinase 1	+3.73
K07881	Ras-related protein Rab-14	+2.01
Ras signalling pathway (2%)		
K07836	Ras-related protein Rap-1B	+1.67
K02183	Calmodulin	+1.36
TNF signalling pathway (1%)		
K14625	C-C motif chemokine 20	+3.05
Rap1 signalling pathway (1%)		
K04294	Lysophosphatidic acid receptor 3	-2.63
FoxO signalling pathway (2%)		
K07199	5'-AMP-activated protein kinase	+1.18
K10628	V(D)J recombination-activating protein 1	-4.44
MAPK signalling pathway (1%)		
K04437	Filamin	+1.30
Transport and catabolism (8%)		
Lysosome (2%)		
K01371	Cathepsin K	+2.34
K12399	AP-3 complex subunit sigma	+1.29
Endocytosis (1%)		
K12484	Rab11 family-interacting protein 1/2/5	+1.88
Peroxisome (3%)		
K13279	Peroxiredoxin 1	-1.69
K01640	Hydroxymethylglutaryl-CoA lyase	-2.15
K12663	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase	-1.31
Phagosome (2%)		
K03990	Complement component 3	-1.56
K13813	Syntaxin 12/13	+1.81
Folding, sorting and degradation (8%)		
Ubiquitin mediated proteolysis (3%)		
K10610	DNA damage-binding protein 1	-1.77

K10615	E3 ubiquitin-protein ligase HERC4	-4.01
K10587	Ubiquitin-protein ligase E3 A	-4.73
Protein processing in ER (5%)		
K09554	Cyclic AMP-dependent transcription factor ATF-6 α	+1.81
K09555	BCL2-associated athano gene 1	-1.74
K13251	Translocon-associated protein subunit gamma	+13.64
K14018	Phospholipase A-2-activating protein	+1.49
K01367	calpain-1	+1.41
Immune system (6%)		
Complement and coagulation (6%)		
K03905	Fibrinogen gamma chain	-1.76
K01334	Component factor D	+2.37
K03904	Fibrinogen beta chain	+3.38
K03910	Alpha-2-macroglobulin	+1.37
K03909	Tissue factor pathway inhibitor	+1.52
K03990	Complement component 3	-1.56
Transcription (4%)		
Spliceosome (3%)		
K12627	U6 snRNA-associated Sm-like protein LSm8	-1.32
K12846	U4/U6.U5 tri-snRNP-associated protein 3	-1.40
K12733	Peptidyl-prolyl cis-trans isomerase-like 1	-2.38
DNA replication (2%)		
K02542	DNA replication licensing factor MCM6	-3.17
K02212	DNA replication licensing factor MCM4	-1.93
Endocrine system (3%)		
PPAR signalling pathway (2%)		
K08751	Intestinal fatty acid-binding protein	+2.06
K08745	Solute carrier family 27 (fatty acid transporter)	-1.50
Insulin signalling pathway (1%)		
K07189	Protein phosphatase 1 regulatory subunit 3A	-1.67
Cell death and motility (2%)		
Apoptosis (1%)		
K04724	Programmed cell death 8	+1.51
Regulation of actin cytoskeleton (1%)		
K05762	Radixin	+1.75

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936 Supplementary Table 4. Validation of microarray results by qPCR

	FFO vs WFO			
	Microarray		qPCR	
	p	FC	p	FC
fads2d6	0.003	-2.11	0.021	-1.93
fads2d5	0.002	-2.04	0.001	-1.38
elovl2	>0.05	-1.33	>0.05	-1.41
elovl5a	>0.05	-1.35	0.004	-1.67
elovl5b	>0.05	-1.20	>0.05	-1.17

937

938 Data shown are the fold-changes (FC) between expression levels in farmed salmon fed FO
939 diet (FFO) and land-locked salmon fed RO diet (WFO) and p value (assessed by REST
940 2009). *fads2d6*, delta-6 fatty acyl desaturase; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*,
941 fatty acyl elongase 2; *elovl5a*, fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase
942 isoform b

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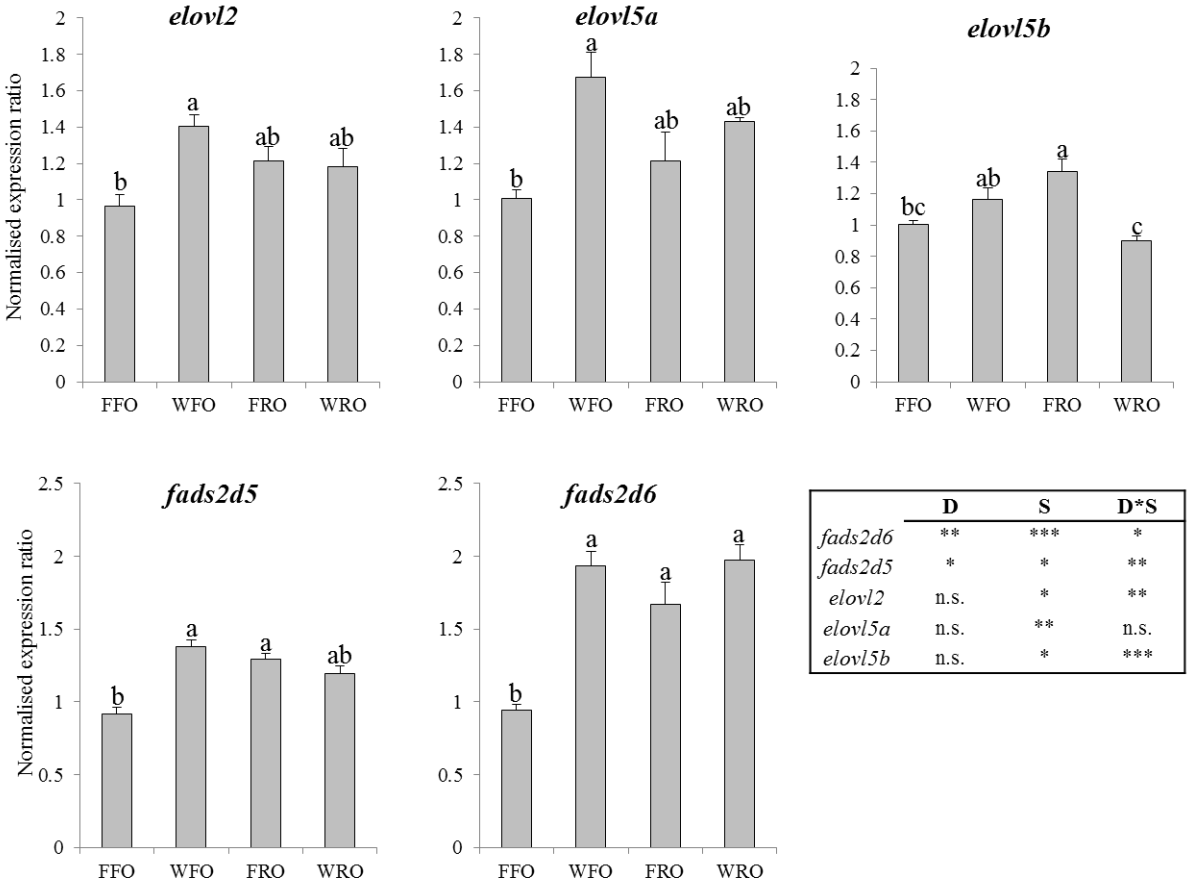

	D	S	D*S
fads2d6	**	***	*
fads2d5	*	*	**
elovl2	n.s.	*	**
elovl5a	n.s.	**	n.s.
elovl5b	n.s.	*	***

Figure 1.

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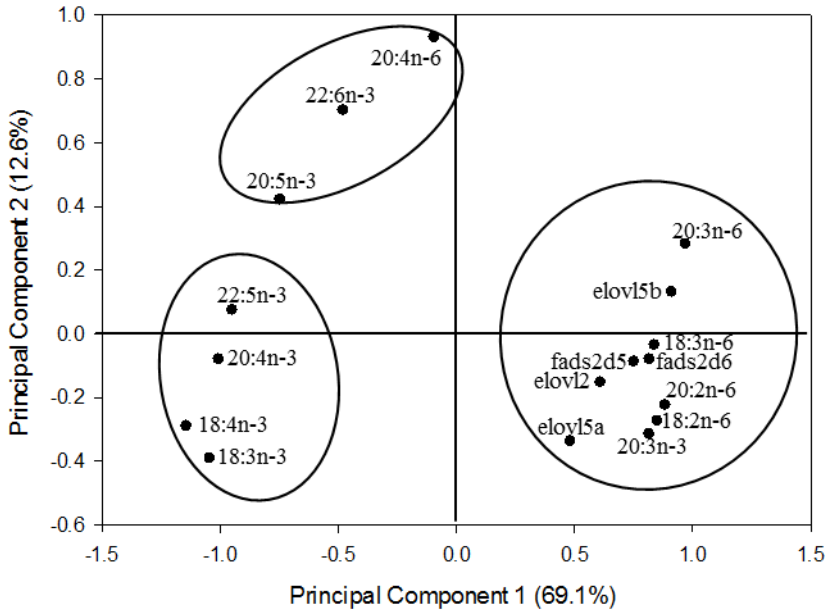
A



B

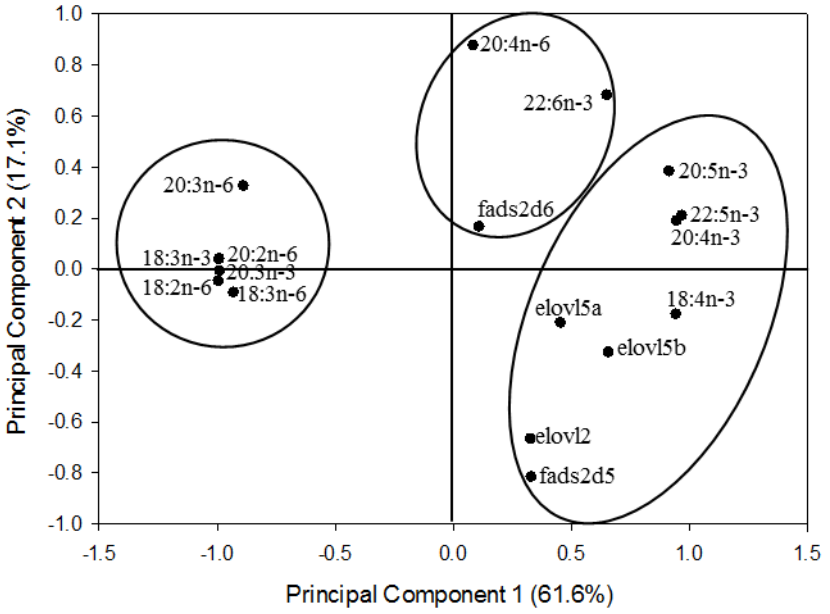


Figure 2.

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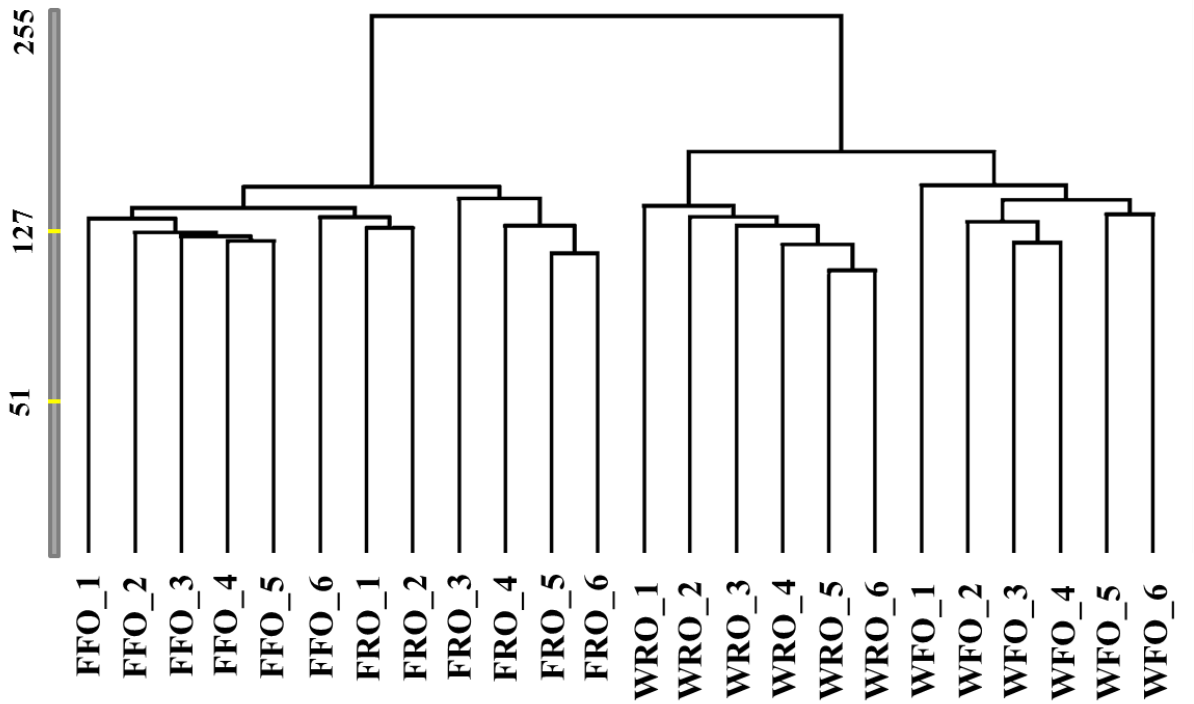

Figure 3.

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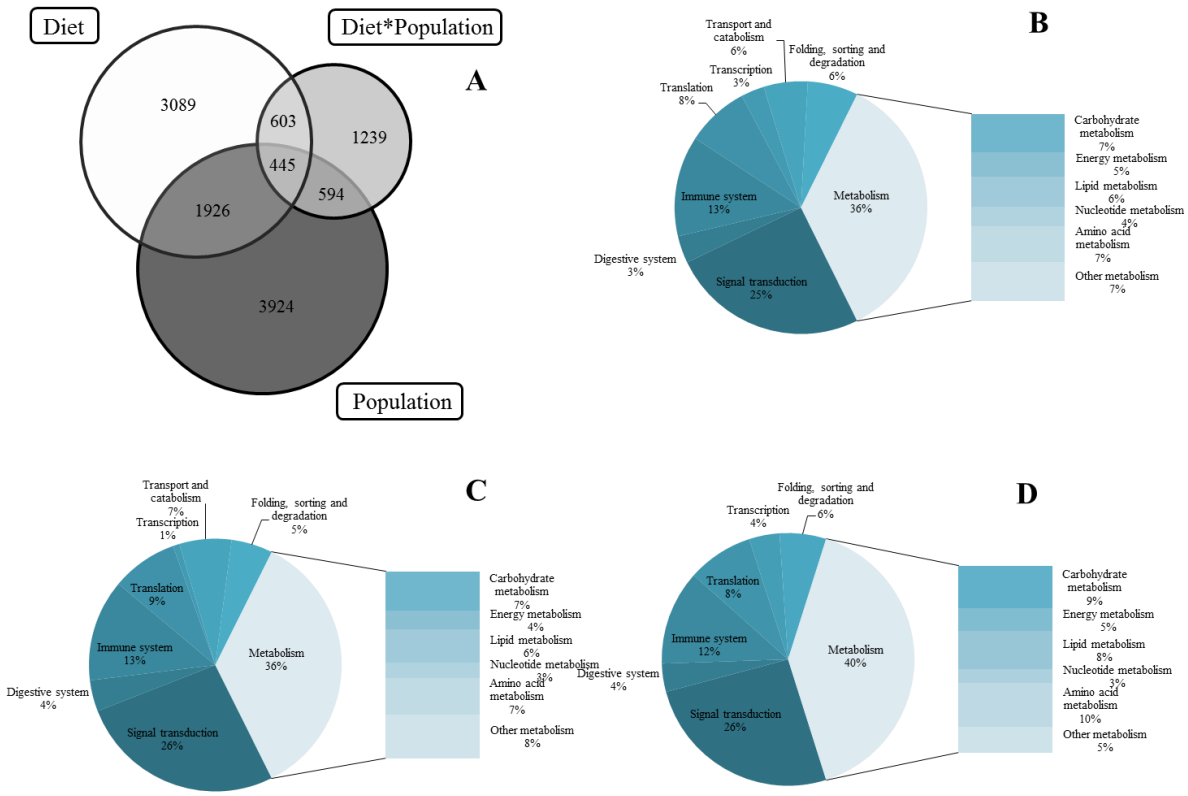

Figure 4.

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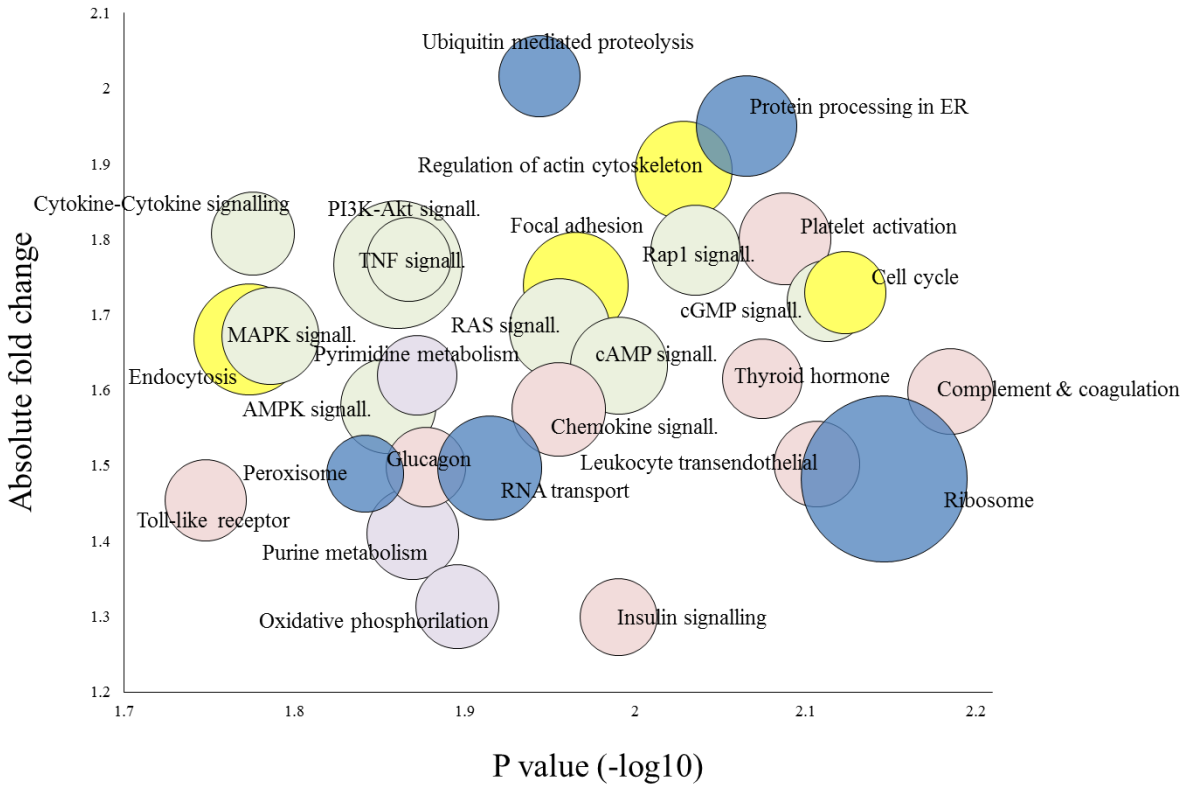

Figure 5.

953

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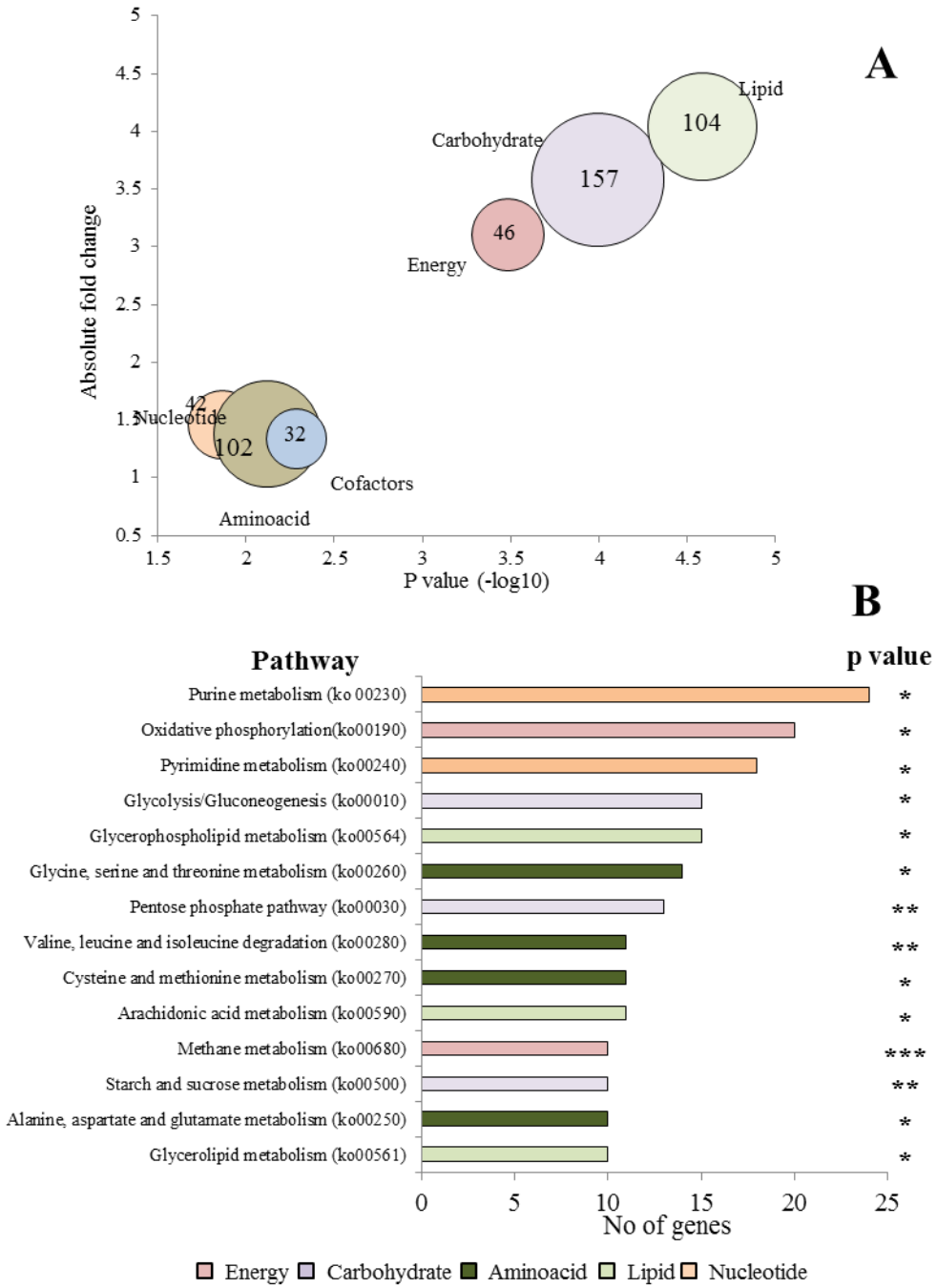
A
Absolute fold change
P value (-log10)
Lipid
104
Carbohydrate
157
46
Energy
42
Nucleotide
102
32
Cofactors
Aminoacid
B
Pathway
p value
Purine metabolism (ko 00230)
Oxidative phosphorylation(ko00190)
Pyrimidine metabolism (ko00240)
Glycolysis/Gluconeogenesis (ko00010)
Glycerophospholipid metabolism (ko00564)
Glycine, serine and threonine metabolism (ko00260)
Pentose phosphate pathway (ko00030)
Valine, leucine and isoleucine degradation (ko00280)
Cysteine and methionine metabolism (ko00270)
Arachidonic acid metabolism (ko00590)
Methane metabolism (ko00680)
Starch and sucrose metabolism (ko00500)
Alanine, aspartate and glutamate metabolism (ko00250)
Glycerolipid metabolism (ko00561)
No of genes
Energy
Carbohydrate
Aminoacid
Lipid
Nucleotide

Figure 6.