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Does broodstock nutritional history affect the response of progeny to different first-feeding diets? A whole-body transcriptomic study of rainbow trout alevins

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Abstract :

The whole-body transcriptome of trout alevins was characterised to investigate the effects of long-term feeding of rainbow trout broodstock females a diet free of fishmeal and fish oil on the metabolic capacities of progeny. Effects were studied before first feeding and after 3 weeks of feeding diets containing different proportions of marine and plant ingredients. Feeding alevins plant-based diets resulted in lower fish body weight, irrespective of maternal nutritional history. No differences in whole-body lipids were found between treatments, and the tissue fatty acid profile strongly reflected that of the respective broodstock or first-feeding diets. We showed that the maternal diet history did not significantly affect expressions of any genes before the first feeding. Interestingly, we found an effect of maternal nutritional history on gene expression in alevins after 3 weeks of feeding. The major differences in the transcriptome of alevins from plant-based diet-fed females compared with those from commercial-fed females were as follows: (i) down-regulation of genes involved in muscle growth/contraction and (ii) up-regulation of genes involved in carbohydrate and energy metabolism related to the delay in growth/development observed with plant-based diets. Our findings also showed an effect of the first-feeding diets, irrespective of maternal nutritional history. Specifically, the introduction of plant ingredients resulted in the up-regulation of genes involved in amino acid/protein and cholesterol metabolism and in differences in the expressions of genes related to carbohydrate metabolism. Information gained through this study opens up avenues for further reduction of marine ingredients in trout diets, including the whole rearing cycle.

Keywords : Fish, Nutrition, Plant products, Fatty acids, Gene expression, Microarrays, Early stages

Introduction

Aquaculture is currently supplying increasing proportions of fish for global human consumption, resulting in an increasing demand for feeds for farmed fish. The use of fish meal (FM) and fish oil (FO) in fish nutrition, in particular for carnivorous species such as salmonids, has been common practice for years. This is due to the fact that FM and FO constitute excellent sources of essential amino acids and fatty acids, particularly highly unsatured fatty acids [1; 2; 3]. However, the current stagnation of FM and FO production from wild fisheries might limit the growth of aquaculture unless effective alternative ingredients are found.

Terrestrial plant-based products are thus nowadays increasingly used as substitutes for marine resources in feeds for farmed fish [3; 4]. The studies conducted with diets containing little or no FM and high levels of plant protein sources have shown lower growth performance in rainbow trout, possibly linked to reduced feed intake [5]. With regard to dietary FO replacement, several studies carried out in salmonids [6; 7] have shown that complete replacement of FO in the diet by vegetable oils does not affect growth or feed efficiency when the n-3 polyunsatured fatty acid (n-3 PUFA) requirements are met by lipids contained in FM. Indeed, one of the major consequences of the replacement of marine ingredients by plant products is the drastic modification of the fatty acids (FA) content of the diets, because none of the plant-based products contain n-3 LC-PUFAs, such as eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA), known to play a key role in fish reproduction and development [8; 9]. A few studies have also been conducted on the concomitant replacement of FM and FO. These studies showed lower growth performance in fish fed the plant-based diet, an effect mainly linked to FM replacement [10; 11].

The metabolic consequences of FM and FO replacement by alternative protein or fatty acid sources are numerous and mediated by several interacting pathways. Nutrigenomic tools (*i.e.* transcriptomics) are increasingly used to investigate molecular events taking place in a genome receiving nutritional signals and responding to them through characteristic metabolic processes in the organism [12]. Nutrigenomics studies in farmed fish have addressed the replacement of different percentages of FM and/or FO with plant ingredients in diets [13; 14; 15], and the effects of such replacement are well characterized in the hepatic transcriptome of salmonids [10; 16; 17; 18] and marine species such as sea bass [14]. For example, the replacement of fish oil by vegetable oils was found to be mainly associated with modification of genes involved in cholesterol and fatty acid biosynthesis [16; 19], while the substitution of fish meal by plant proteins was found to be associated with a decreased capacity for protein biosynthesis and variation in nitrogen metabolism in rainbow trout [17]. The replacement of both fish meal and fish oil by plant-based ingredients in the diet of rainbow

trout was associated with changes in nucleic acid and glucose metabolism, in addition to the aforementioned changes in lipid and protein metabolism[10]. Other studies have investigated the intestinal gene expression profile in response to different levels of dietary replacement of marine ingredients by plant products in several fish species, such as Atlantic salmon (*Salmo salar*)[20; 21; 22] , gilthead sea bream (*Sparus aurata L.*)[23] and Atlantic cod (*Gadus morhua*)[24]. However, most of these studies were carried out on growing fish and there is still a gap in the understanding of the effects of plant-based diets on the rest of the life cycle (broodstock and early stages). In addition to the already recognised importance of broodstock nutrition on progeny survival and development, nutrients contained in the yolk sac, transmitted by broodstock to developing progeny, are also known to influence the characteristic gene expression of offspring by modifying or interacting with transcription factor or DNA structure [25]. The effects of the maternal dietary history on reproduction and metabolic capacities of the progeny are still poorly documented, especially when broodstock are fed a totally plant-based diet without any FM and FO, and thus devoid of n-3 LC-PUFA, over the whole life cycle. In an earlier trial, we showed that broodstock produced viable offspring even when reared exclusively with a plant-based diet [26]. We also showed that trout are capable of synthesizing n-3 LC-PUFAs from the dietary precursor (α -linolenic acid, 18:3 n-3) and of incorporating them into ova, which in fish represent the main sources of nutrients utilized by the embryo [27] and later by the developing alevin.

The early life stages of fish represent a transitional ontogenetic period of simultaneous growth and organ/tissue differentiation, during which fish undergo the transition from endogenous to exogenous feeding, *i.e.* from yolk consumption to ingestion of external food [28]. Moreover, previous studies carried out on developing larvae [29; 30] showed that gene expression, and the subsequent activation of the related metabolic pathways, is differentially regulated with advancing ontogenesis. Thus, regulation of gene expression during this phase is considered to be a key mechanism underlying the management of the biological process required for harmonious development over this phase of life, during which nutritional input is of great importance.

In order to characterise the effects of broodstock nutritional history as well as those of first feeding diets with different proportions of FM and FO and plant ingredients, the whole body transcriptome of rainbow trout alevins was characterised at two different developmental stages: (i) before first feeding (end of endogenous feeding period) to assess the effects of maternal nutritional history and (ii) after 3 weeks of feeding (exogenous feeding alevins) to assess both the effects of maternal nutritional background and those of first feeding diets.

Experimental methods

Diets

Broodstock

The broodstock diets were the same as those previously described in Lazzarotto et al.[26]. Briefly, broodstock were fed either a commercial (COM) diet composed of FM, FO and plant-based ingredients (45% FM and 50% FO replaced by plant ingredients), or an experimental plant-based (VEG) diet, completely free of marine FM and FO, which were replaced by plant protein sources (22% corn gluten, 26% soybean meal, 33% wheat gluten, 7% durum wheat, 8% white lupin and 4% dehulled peas) and vegetable oils (50% rapeseed oil, 30% linseed oil, and 20% palm oil), respectively.

Alevins

Three different first-feeding experimental diets with different dietary levels of FM and FO replacement were formulated and manufactured (INRA-NuMéa) : a marine (M) diet, based on marine resources (no replacement), a commercial-like (C) diet, containing both marine and plant-based ingredients (replacement of 46% FM and 69% of FO), and a completely plant-based diet (V), with total replacement of marine FM and FO by plant-based proteins and vegetable oils. The ingredients and composition of the three diets are provided in Table 1. In order to obtain total replacement of fish products, only plant-based proteins and vegetable oils (7% rapeseed oil, 7% linseed oil and 4% palm oil) were used in the V-diet, whereas the M and C-diets contained FO (12% and 8%, respectively). Consequently, the V-diet contained no n-3 LC-PUFAs, whereas it contained a high level of 18:3 n-3, mainly derived from linseed oil, compared to the other two experimental diets (Table 2).

Animals and experimental plan

The experiment was carried out in strict accordance with EU legal frameworks relating to the protection of animals used for scientific purposes (Directive 2010/63/EU) and according to the *National Guidelines for Animal Care of the French Ministry of Research* (Decree N°. 2001-464, May 29, 2001). It was approved by the Ethics Committee of INRA (INRA 2002-36, April 14, 2002) and the scientist in charge of the experimentation received training and personal authorisation (N°B64 10 003). Female rainbow trout were produced at the INRA facilities (PEIMA, Sizun, France - permit number B29-277-02). During the trial, they were reared under natural photoperiod

and temperature conditions. At the beginning of the trial, female fish were randomly divided into two groups, fed from first feeding and through a 3-year life cycle, with either the broodstock commercial (COM) diet or the broodstock plant-based (VEG) diet [26]. At spawning ova produced by 10 female trout/group (3-year-old females) of similar body weight from each dietary treatment were fertilized with a pool of sperm from males fed a commercial diet. Eggs were transferred to our experimental hatchery (INRA, Lees Athas, France – permit number A64-104-1) where the water temperature is around 7°C all year long. Just before first feeding (62 days post-fecundation) body weights and survival rates of alevins were recorded and whole body samples of fry were collected.

The remaining alevins from both cohorts were subsequently split into three groups of fish. Each group (four replicates) received one of the three experimental diets from first feeding, *i.e.* diet-M, diet-C or diet-V. After 3 weeks of feeding, survival rates and body weights of alevins were recorded and whole body alevins samples were collected for subsequent analysis. All the samples were frozen in liquid nitrogen and stored at -80°C until analysis.

Lipid and fatty acid analysis

Total lipids of whole body alevins collected before first feeding (pool=15 alevins/maternal group) and after 3 weeks of feeding (pool=15 alevins/dietary group), were extracted and quantified gravimetrically according to Folch et al. [31]. Neutral (NL) and polar (PL) lipid fractions were separated on silica cartridges (Sep-Pak, Waters, Ireland) [32] and fatty acid methyl esters (FAME) were prepared according to Shantha & Ackman [33]. FAMEs were then analysed by gas chromatography as previously described in detail [26].

RNA extraction

Total RNA was extracted from individual whole body swim-up fry (n=8/maternal group) and alevins (n=8/dietary group) using the TRIzol® reagent method (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's recommendations. The quantity and quality of extracted RNA were analysed using a spectrophotometer (ND-1000, NanoDrop) and a Bioanalyzer (Agilent Technologies, Kista, Sweden), respectively.

cRNA synthesis, labelling and purification

Cy3-labelled experimental cRNA samples (n=8/treatment) were generated using the Agilent "One-Color Microarray-based Gene Expression Analysis" (Low Input Quick Amp Labeling-LIQA) kit, according to manufacturer's instructions. The method uses T7 RNA Polymerase Blend, which simultaneously amplifies target material and incorporates Cyanine 3-CTP (Cy3). For each sample 150ng of total RNA were used to generate fluorescent cRNA. Agilent Spike-In was included in

each reaction. After the denaturation step (10 min in circulating bath at 65°C) and cRNA synthesis step (2hr at 40°C), the reactions were incubated at 70°C for 15 min to inactivate the AffinityScript enzyme. To perform the labelling reaction, cRNA samples were each mixed with 6µL of Transcription Master Mix cocktail, containing Cy3-dye, and then incubated at 40°C for two hours. Purification was performed using Quiagen RNeasy mini spin columns, eluting in 30uL of RNase-free water.

Microarray hybridization and scanning

Cy3-labelled cRNA sample yields (> 0.825µg cRNA) and specific activity (> 6pmol of Cy3/µg of cRNA) were verified using a NanoDrop ND-1000: 600ng of Cy3-cRNA were fragmented and hybridized on a sub-array, following the LIQA kit instructions. The transcriptomic analysis was conducted using a custom-commercial 8x60K oilgoarray (Agilent Technologies, Massy, France; Gene Expression Omnibus (GEO) accession no. GPL15840). The hybridization reactions were allowed to continue for 17 hours in a rotating hybridization oven (65°C), prior to washing according to the manufacturer's instructions. Slides were scanned with an Agilent Scanner (Agilent DNA Microarray Scanner, Agilent technologies, Massy, France) using the standard parameters for a gene expression 8x60K oligoarray (3µm – 20 bits). Data were then obtained with the Agilent Feature Extraction software (10.7.1.1), according to the appropriate GE protocol (GE1_107_Sep09). The data is deposited in NCBI's GEO (GSE74271).

Quantitative RT-PCR

Six individual samples (single whole body swim-up fry or alevin) per experimental condition were used as biological replicates. Total RNA (1µg) was reverse-transcribed to cDNA with SuperScript III RNase H reverse transcriptase (Invitrogen, Carlsbad, CA, USA) using oligo dT Primers. Real-time PCR was performed in the iCycler iQ TM (BIO-RAD, Hercules, CA, USA). Quantitative PCR (q-PCR) analyses for gene expression were performed using the Roche Lightcycler 480 system (Roche Diagnostics, Neuilly-sur-Seine, France). The assays were performed using 2 µl of diluted cDNA mixed with 3 µl of Light cycler 480 SYBR® Green I Master mix in a total volume of 6 µl, using forward and reverse primers at a final concentration of 400 nM. Primer design was performed using the Primer 3 software. Specific primer pairs were designed with an overlapping intron, when possible, using known trout sequences in nucleotide databases (GeneBank and INRA-Sigenae). Database accession numbers and the sequences of forward and reverse primers used to test each gene are provided as supplementary files (Supplementary Table 1 a-b).

Thermal cycling was initiated with the incubation at 95°C (10 min) for hot-start iTaq TM DNA polymerase activation. Forty-five cycles of PCR were performed, each consisting of a heating step

at 95°C (15 sec) for denaturing, a second step at 60°C (10 sec) for annealing and a third extension step at 72°C (15 sec). Following the final cycle of the PCR, melting curves were systematically monitored (with a gradient of 0.5°C/10 s from 55°C to 94°C) to ensure that only one target fragment was amplified. Samples without reverse transcriptase and samples without RNA were run for each reaction as negative controls. mRNA levels of all target genes were normalized with the housekeeping gene α-elongation factor 1 (*EF1-α*), previously used as a reference gene in salmonids [(34)]. The expression levels were calculated according to the threshold cycle ($\Delta\Delta C_T$) method [(35)].

Statistical analysis and data mining

Data of weight, survival, lipid content and fatty acids of whole body alevins (collected before 1st feeding and after 3 weeks of feeding) are presented as means ± standard deviation (SD). Data were analysed statistically using the R-software (version 2.14.0) and the Rcmdr package. The normality and homogeneity of variance of the variables were tested with Shapiro-Wilk's test and Levene's test, respectively. Data for alevins collected before first feeding were analysed by an independent sample t-test to assess the effects of the different broodstock nutritional histories, when both conditions were satisfied. The variables with non-parametric distribution were either normalized with an arcsin transformation or, if the criteria were still not met (some fatty acids), compared using a non-parametric paired Wilcoxon test. Data for alevins collected after 3 weeks of feeding were analysed using a two-way ANOVA (*p-value <0.05*) to assess the effects of the *nutritional broodstock history* and the *first feeding diets*. The variables with non-parametric distribution were normalized with an arcsin transformation. Data from microarray analysis were normalized and analysed statistically using GeneSpring software (12.6, Agilent). Data were scale-normalized using the median value of each array to identify differentially expressed genes between conditions. An unpaired t-test was performed to determine the effects of the *nutritional broodstock history* on the transcriptome of alevins collected before first feeding, (Benjamini Hochberg FDR correction, *p-value* cut- off 0.05). For analysis of whole body alevins collected after 3 weeks of feeding, differentially expressed genes were obtained by two-way ANOVA, with the different *broodstock nutritional histories* and *first feeding diets* as independent variables (Benjamini-Hochberg correction, *p-value* cut-off *0.05*). For all genes found to be differentially expressed, Gene Ontology (GO) annotations (biological process, cellular component, molecular functions) were obtained using the Expression Analysis Systematic Explorer (EASE) software version 2.0 [(36)]. Significant enrichment of GO was tested by using EASE software and the Benjamini correction (score < 0.05). Gene expression data obtained by RT-qPCR were tested for normality and homogeneity of variances with Shapiro-Wilk's test and Levene's test, respectively. When variances were not normally distributed a logarithmic transformation was performed. To assess the effects of the

nutritional broodstock history and the *first feeding diets*, gene expression was analysed by two-way ANOVA (*p-value <0.05*). *Post hoc* comparisons were made using Tukey's range test and differences were considered statistically significant at $p<0.05$. Correlation of the mRNA measurement by microarray with that by reverse transcription PCR (RT-PCR) for two of the tested genes, chosen as examples, is provided as supplementary material (Supplementary Figure 1).

Results

Growth performance

Survival rates and weight of alevins before first feeding and after three weeks of feeding are given in Table 3. No statistically significant differences in survival were found in alevins from the different experimental groups, either before first feeding or after the 3-week feeding challenge.

Before first feeding, alevins developing from VEG-fed females had significantly lower body weights (-13%, *p-value <0.001*) compared to those from COM-fed females. The initial slight difference in weight resulting from the maternal nutritional history (VEG *vs* COM) was maintained after 3 weeks of feeding, irrespective of the diets fed to the alevins. After the 3-week feeding trial, alevins responded to the three dietary treatments (M, C or V) irrespective of maternal nutritional history, with lower growth when fed the V-diet (V *vs* M: -27%; V *vs* C: -15%).

Alevin whole body lipid composition

Data on alevins collected before first feeding were presented in detail in Lazzarotto et al.[26] and are summarized in the Supplementary file 1. Briefly, before first feeding there were no significant differences in lipid content between alevins originating from COM-fed (5.9% of fresh weight) and VEG-fed (5.6% of fresh weight) females. The whole body lipid content of fry mainly comprised neutral lipids (NL: 70%; polar lipids, PL: 30%) in progeny from both broodstock groups (COM and VEG).

In alevins collected after the 3-week feeding challenge (Table 4), we observed an effect of both maternal nutritional history and first feeding diets on the whole body lipid content, whereas no interaction between the two factors was found. Lipid content was significantly higher in progeny from the VEG-fed females that received the M-diet for three weeks (5% of fresh weight), whereas the progeny from the COM-fed females that received the V-diet had the lowest whole body lipid content (4% of fresh weight). No significant differences were found between the other treatment groups.

The respective proportions of NL and PL were similar in all experimental groups (70% NL and 30% PL), and were therefore not affected by dietary treatments.

FA composition

Alevins before first feeding

Data on FA profiles of whole body alevins collected before first feeding were presented in detail in Lazzarotto et al. [26] and were used in the present study (Supplementary File 1) as a starting point for comparison with data from 3-week fed alevins. We found that alevins of females fed the VEG-diet had higher n-6 PUFA, 18:2 n-6, ARA and 18:3 n-3 levels before the first feeding than those from COM-fed females. In contrast, higher percentages of n-3 PUFA, EPA and DHA were found in progeny from COM-fed females, with the exception of the PL fraction, where no significant differences were found in DHA content between groups. Lower amounts of EPA + DHA were found in alevins from VEG-fed females (1.4 mg alevin^{-1}) than in alevins from COM-fed females (2.6 mg alevin^{-1}) (Table 5).

Alevins after 3 weeks of feeding

After 3 weeks of feeding, the fatty acid composition of whole body alevin samples reflected those of the respective experimental first-feeding diets M, C or V (Table 4).

Polar lipid fraction

All FA classes (except SAT) were significantly affected by both broodstock nutritional history and the dietary treatment.

Lower percentages of SAT were found in fish fed the C and V- diets, compared to M-fed fish. Levels of MUFA were higher in fish fed the C and V-diets, with higher values in fish from VEG-fed females. The percentage of total n-6 PUFA (reflecting mainly 18:2 n-6) was higher when FM and FO were replaced by plant-ingredients (C and V-diet), with higher levels in progeny from females fed the VEG-diet. On the other hand, levels of n-3 PUFA were significantly higher in progeny from COM-fed females, EPA and DHA levels being the lowest with the V-diet.

Neutral lipid fraction

Lower levels of SAT were found in alevins fed the C and V diets, the lowest levels being found in progeny from broodstock fed the VEG-diet. Higher percentages of MUFA were found in alevins fed the C-diet in both broodstock groups (mainly due to the higher 18:1 content).

Alevins originating from females fed the VEG-diet exhibited higher (or equal) levels of n-6 PUFA than those from the COM-fed broodstock, with higher levels with the V-diet compared to the other groups. Alevins receiving the C-diet had values intermediate between the M and V-fed alevins (V>C>M). These differences were related to the greater quantities of linoleic acid in alevins fed the

V-diet. Higher proportions of ARA were found in alevins fed the V-diet and values in progeny of VEG-fed females were higher.

Lower n-3 PUFA levels were found in progeny from VEG-fed females compared to progeny from COM-fed females. Alevins fed the V-diet had lower percentages of n-3 PUFA compared to alevins fed the C or M-diet. Higher (or equal) proportions of 18:3 n-3 were found in alevins originating from females fed the VEG-diet compared to those from COM-fed females. Percentages of 18:3 n-3 were higher in alevins fed the V-diet, compared to those fed the C or M-diet, irrespective of broodstock nutritional history. On the other hand, lower percentages of EPA and DHA were found in alevins originating from females fed the VEG-diet. Alevins fed the V-diet had lower EPA and DHA values than alevins fed the other experimental diets (C or M).

Amounts of EPA + DHA

The difference in quantity of EPA+DHA (mg alevin^{-1}) originating from the maternal nutritional history (COM *vs* VEG) still remained after three weeks of feeding, with lower levels recovered in progeny from VEG-fed females, irrespective of the first feeding diets (Table 5). After 3 weeks of feeding, lower levels of EPA+DHA were found in progeny fed the V-Diet, irrespective of the broodstock nutritional history (COM, V *vs* M: -62% and V *vs* C: -40%; VEG, V *vs* M: -65% and V *vs* C: -44%).

Transcriptomics

Microarray results

Transcriptome of alevins collected before first feeding

Although 3185 genes exhibited fold changes (FC) >1.5, 624 (FC >2), and 114 (FC >3) between progeny originating for COM and VEG-fed females (Table 6a), none of the changes was statistically significant (*p-value >0.05, false discovery rate >5%*).

Transcriptome of alevins collected after 3 weeks of feeding

Two-way ANOVA analysis of the transcriptome profile of whole body alevins collected 3 weeks after first feeding revealed that 71 genes were significantly differentially expressed in response to the broodstock nutritional background, and 249 gene features in response to the first-feeding diets. No significant interaction between the nutritional background of female broodstock and first-feeding diets was detected at the level of gene expression (Table 6b). The GO enrichment analysis highlighted changes in expression of genes involved in different GO categories (Figure 1a-b). In the following discussion we will focus on the main overrepresented processes, which are principally involved in metabolism-related biological processes.

Effects of broodstock nutritional history

As regards analysis of the effects of broodstock nutritional history (VEG *vs* COM) on gene expression in alevins, 54 of the 71 differentially expressed probes had an assigned gene annotation. The GO enrichment analysis highlighted changes in metabolism-related biological processes (EASE score <0.05). In particular, 11 genes involved in carbohydrate metabolism and energy pathways (20% of annotated genes) were found to be down-regulated in the transcriptome of whole-body alevins originating from females fed the VEG-diet, compared to alevins from COM-fed broodstock. The GO enrichment also indicated differential expression of 12 genes related to muscle growth and contraction (22% of annotated genes). For these genes, microarray analysis also revealed overall down-regulation in the transcriptome of whole bodies of progeny of females fed the VEG-diet, compared to those of COM-fed females (Table 7).

Effects of first feeding diets

Of the 249 probes corresponding to genes differentially expressed in response to the first feeding diets, 133 had an assigned gene annotation. GO enrichment for the biological process was performed to interpret this list of genes further. The GO enrichment analysis revealed overrepresentation of biological processes related to amino acid/protein metabolism (16 genes, 17% of annotated genes), lipid/cholesterol metabolism (13 genes, 14% of annotated genes), carbohydrate and energy metabolism (11genes, 12% of annotated genes), transport and catabolism (9 genes, 10% of annotated genes) and muscle contraction (7 genes, 8% of annotated genes). The other GO processes affected by the first feeding diets (oxidation-reduction process, transcription/translation and trans-sulfuration pathways) and their respective percentages are shown in Figure 1b. The microarray analysis showed up-regulation of the genes involved in both amino acid/protein metabolism and lipid and cholesterol metabolism with the introduction of plant-based ingredients in the diets (Table 8). By studying the expression of genes involved into carbohydrate and energy metabolism, we observed down-regulation of glucokinase (GCK) with the C-diet, and this effect became more evident when fish were fed the V-diet. In contrast, up-regulation of hexokinase (HK2) was found with the C-diet, which became more pronounced with the V-diet. Down-regulation of genes involved in muscle contraction was also observed in the transcriptome of fish fed the C and V-diets, compared to those fed the M-Diet. Genes involved in transport and catabolism were up-regulated in fish fed the plant-based C and V diets. A complete list of the pathways which have been found to be affected by first feeding diet is provided as Supplementary Table 2.

Real time quantitative PCR

Effects of broodstock nutritional history on gene expression

Of the genes found to be differentially expressed by microarray approach, four of the genes involved in muscle growth and contraction were analysed by RT-qPCR (ACTA1, CKM, MYBPC1, MYBPC2), and are presented in Supplementary Figure 2a. The analysis revealed down-regulation of these genes in progeny originating from females fed the VEG-diet, confirming the microarray results. In addition, RT-qPCR showed an effect of the first feeding diets ($p<0.01$) for ACTA1 and an interaction between the broodstock nutritional history and the first feeding diets for CKM ($p<0.05$) that were not evident on microarray analysis.

Of the genes involved in carbohydrate metabolism and energy pathways (Supplementary Figure 2b), PGK1 was up-regulated in progeny from VEG-fed females, not confirming microarray analysis. Expression levels of five other genes (PYGM, PYGL, 6PFKM, SDHA and GPD1) were not significantly changed when measured by RT-qPCR.

Effects of first feeding diets on gene expression

A number of genes involved in amino acid and protein metabolism (IARS, LARS, EPRS and DARS) were assayed by RT-qPCR, confirming the up-regulation with the V-diet observed with the microarray analysis (Supplementary Figure 3a). With regard to cholesterol metabolism, two genes involved in cholesterol synthesis were analysed by RT-qPCR (HMGCR and HMGCS1), and the results are presented in Supplementary Figure 3b. Up-regulation of these genes was observed with the introduction of plant-based ingredients in the diet (C-diet and V-diet). Among the genes involved in carbohydrate metabolism that showed changed expression in the array analysis, three were also analysed by RT-qPCR (GCK, HK2 and LDHA). GCK and LDHA were down-regulated with the V-Diet, whereas up-regulation of HK2 expression was observed, confirming the microarray results (Supplementary Figure 3c).

Discussion

This study is to our knowledge the first investigation into the effects of a totally plant-based diet (no FM or FO) on the whole body transcriptome of rainbow trout alevins. It is also one of the first studies investigating the consequences of long-term feeding broodstock (3 years) a totally plant-based diet on the ability of progeny to respond to different first feeding diets with a replacement of marine ingredients rate of up to 100%. The relatively low values of FC found in this study (although statistically significant) suggest that the modifications induced by the diets, and therefore the metabolic consequences of the dietary replacement, are not so drastic. It is also important to bear in mind that one of the limitations of transcriptomic analysis in early stages might be linked to the use of RNA extracted from whole body fish, because such sample types include a mixture of

different organs. The use of this kind of sample thus does not provide information about the regulation of expression in a specific organ and/or tissue.

Plant based diets do not have detrimental effects on survival but affect growth of alevins

We recently demonstrated that feeding broodstock the VEG-diet throughout a 3-year life cycle had no detrimental effects on survival but resulted in lower body weight of fry before first feeding compared to those originating from COM-fed females [26]. Survival levels after the 3-week feeding challenge did not differ between alevins fed any of the three experimental diets (M, C or V), irrespective of the broodstock nutritional history (COM or VEG). We found that a 50% replacement rate (C-diet) resulted in lower body weights and the effect was more pronounced with total replacement (M > C > V), irrespective of the maternal nutritional history. The concomitant replacement of marine ingredients by plant-protein sources and vegetable oils is known to be responsible for a reduction in feed intake and feed efficiency [37; 38; 39; 40], resulting in reduced growth performance [41]. This effect is believed to be mainly related to the replacement of FM but not to FO substitution in rainbow trout [17; 42], European seabass [43] or gilthead sea bream [44].

Maternal nutritional history has no visible effect on whole-body transcriptome of alevins before first feeding

Early embryonic development in teleosts is governed until the start of zygotic transcription by maternally supplied mRNA that is incorporated into the oocyte during oogenesis [45]. Maternal mRNA is critical to embryonic development since it implements basic biosynthetic processes, directs first mitotic divisions, and defines initial cell fate and embryonic patterning [46]. Given the previous findings on the effects of a plant-based diet on the transcriptome of adult fish and the importance of broodstock nutrition for the development of progeny, our hypothesis was that broodstock nutritional history can affect the progeny transcriptome. However, our results did not demonstrate any significant regulation in the whole body transcriptomic profile of alevins before the first feeding, despite differences in body weight and FA profile. These results suggest that no trans-generational effects linked to maternal nutritional background are present or visible at a molecular level at this specific developmental stage. One possible explanation could be that the transcriptional differences are governed by specific tissues such as the liver that are present in smaller proportions in whole individuals at this specific stage of development. The liver represents only a small proportion (around 1%) of alevin whole body components. Such a small proportion might have prevented detection of the transcriptional differences at the level of the whole individual. Nevertheless, we used the same type of sample to analyse the transcriptome of alevins after 3 weeks of feeding and we found a number of genes linked to intermediary metabolism that

were differentially expressed i.e. according to the broodstock origin. Thus, the hypothesis of a "whole body diluted effect" related to the sample type probably cannot fully explain the absence of significant maternal effect.

Maternal nutritional history and first-feeding diets affect the whole-body transcriptome of alevins after three weeks of feeding

Muscle growth/contraction, as well as metabolism-related biological processes, constitute the largest group among the GO terms associated with the genes found differentially expressed in response to both broodstock nutritional history and first feeding diets of exogenous feeding alevins. In the following discussion we therefore focus on specific actors involved in metabolism from a nutrigenomic point of view in relation to different levels of FM/FO dietary replacement. However, since no interaction was found between the two factors, the effects of broodstock nutritional history and first feeding diets are discussed separately.

Effects of broodstock nutritional history

In contrast to what was observed in alevins collected before first feeding, we found a significant effect of the maternal dietary background on the transcriptomic profile of alevins after 3 weeks of exogenous feeding. One of the possible reasons to explain these results can be found in the switch of alevins from endogenous (vitellus) to exogenous feeding (external feeding). Indeed, the initiation of exogenous feeding is known to alter gene expression, through the activation of different metabolic pathways [(29)].

A set of genes related to different aspects of muscle development and contraction were found to be down-regulated in progeny from females fed the VEG-diet, compared to those from COM-fed broodstock. In particular, we observed down-regulation of creatine kinases (CKM-CKB) and myomesins (MYOM1 and MYOM2), which are involved in the structure of the contractile muscles, as well as down-regulation of alpha actin (ACTA1), which is the major constituent of the contractile apparatus. Down-regulation of myosin (*myosin heavy chain*, MYH2) and slow and fast type myosin-binding proteins-C (MYBPC1 and MYBPC2, respectively) was also observed in alevins from VEG-fed females. In fish, as in other vertebrates, skeletal muscle formation (myogenesis) involves the specific control of several myogenic regulatory factors which control processes such as specification, activation and differentiation of myogenic cells[(47)]. Once myogenic cells are activated, they proliferate and differentiate; finally, in the later stage of differentiation the expression of different genes that encode structural muscle protein, such as myosin light chain, actin and myosin heavy chain is up-regulated, marking sarcomeric assembly [(48)]. The down-regulation of the major

muscular actors observed in the present study in progeny originating from VEG-fed females could therefore be mainly related to the delayed growth, and specifically muscle mass growth and development, rather than to the metabolism. Moreover, after three weeks of feeding the differences in body weight observed between groups in response to broodstock nutritional background became more evident. This increased difference could thus have helped in making the transcriptional changes detectable.

Furthermore, an overall decrease in expression of genes related to carbohydrate and energy metabolism was found. For example, a specific form of muscular phosphofructokinase (PFKM), an actor of glycolysis, the main pathway providing energy for swimming activity in fish white muscle, was less strongly expressed in fish fed diets containing plant ingredients. Another gene encoding creatine kinase (CK) was also associated with the glycolysis-related gene expression pattern. Previous studies on larva development of European sea bass [30] showed that these genes were increasingly expressed throughout larva growth, linked to development of skeletal muscle [49]. These findings suggest that the delayed growth recorded in fish from VEG-fed females in our study may be linked to delayed muscle differentiation.

Considering the expression of genes involved in carbohydrate metabolism and energy pathways, the results obtained by microarray analysis were not confirmed by RT-qPCR. This might be due to the fact that the primers designed for RT-qPCR do not necessarily match exactly the probes on the array, as it has been previously observed in a study on Atlantic salmon liver [18]. Indeed, due to the whole genome duplication that occurred in salmonids [50], transcriptomic and gene expression studies are often more challenging due to the presence of duplicated and highly similar genes whose transcripts might be differentially regulated.

Effects of first feeding diets

The dietary replacement of both marine proteins and oil sources by plant ingredients has been shown to result in changes in protein metabolism [10; 21]. Interestingly, we found up-regulation of 11 aminoacyl-tRNA synthetases, which catalyse the ligation of specific amino acids to their cognate tRNA and thereby assemble the building blocks of RNA translation and protein synthesis [51], with the plant-based diets. The results thus showed concomitantly higher expression of three initiation factors and a translation elongation factor in fish fed the V and C-diet. Taken together, these results seem to suggest that the replacement of FM and FO dietary sources by plant-based ingredients led to higher levels of protein synthesis. Previous studies in fish have shown that protein synthesis rates differ between tissues [52; 53]. In our study, we focused on the early stages, a period of major changes in development, during which fish go through differential rates of relative growth of organs, called

allometry [54], in order to meet the specific needs of this critical developing stage and to ensure that the most essential organs for primary functions are developed first, followed by the development of organs with lower priority for survival [55]. According to these assumptions, and considering the delay in (muscle) growth found in fish fed the plant-based diets, we can hypothesize that the differences in gene expression between groups were mostly linked to the delay in development of the plant-fed groups. However, since a number of processes have key roles in protein and AA metabolism, the biological significance of the changes in gene expression observed is limited and we prefer to treat this hypothesis with caution.

As for the broodstock nutritional history-related effects, down-regulation of genes involved in muscle contraction was also found in response to the first feeding diets in progeny receiving diets containing increasing levels of plant ingredients. These findings seem to confirm our previous hypothesis, reflecting the delay in growth and muscle development induced by plant-based diets.

Another metabolic pathway significantly affected by dietary FM and FO replacement was that of sterol metabolism. Our results suggest a general up-regulation of expression levels of genes involved in cholesterol metabolism in fish fed the diets containing increased levels of plant ingredients, namely the C-diet and the V-diet. Among the genes we found differentially expressed, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), a transmembrane glycoprotein involved in the rate-limiting step of sterol biosynthesis, was increased, as reported in European sea bass fed a diet where fish oil was replaced by vegetable oils [14]. In previous studies with Atlantic salmon [19] and rainbow trout [10], the authors found up-regulation of genes involved in cholesterol biosynthesis. Plant ingredients are in fact rich in phytosterols that can interfere with cholesterol metabolism, while diets based on marine FM and FO contain greater amounts of cholesterol [56]. The positive effects on genes of cholesterol biosynthesis pathways found in our study confirmed that trout fed the plant-based diets were capable of responding to the reduced dietary cholesterol levels in the diets as early as 3 weeks from first feeding. Indeed, the cholesterol content in our experimental diets was lower in the V-diet (0.34%) and the C-diet (0.52%) than in the M-diet (0.66%).

Our findings also suggest differential regulation of genes involved in different steps of glucose metabolism with the introduction of plant ingredients in the diet. In alevins fed the V-diet we observed up-regulation of HK2, a gene involved in the first step of the glycolysis pathway [57], and down regulation of GCK, which is involved in maintaining the hepatic glucose balance. Focusing on the latter, a previous study with rainbow trout, gilthead seabream and common carp [58] showed that nutritional induction of GCK gene expression and activity was associated with a high dietary carbohydrate (starch) intake. In our study, the down-regulation of GCK may have been linked to the lower level of dietary starch in the C- and V-diets (around 10% *vs* 13.5% in the M-diet). The low

level of expression could also explain the absence of induction of genes involved in lipogenesis, this process being induced when glucose is in excess. Alpha-enolase (ENO1), which participates in the conversion of glucose to pyruvate, a key intermediate at the intersection of multiple metabolic pathways including lipogenesis, was slightly down-regulated in fish fed the C- and V-diets, as previously observed in salmon fed rapeseed oil compared to those fed FO [16; 18].

Effects of broodstock nutritional history and first feeding diets on fatty acid profile of alevins

In a previous study we showed that feeding broodstock a totally plant-based diet (VEG) throughout the life cycle affects the fatty profile of progeny (before first feeding) in both PL and NL fractions [26]. In the present study, the analysis of whole body FA composition of alevins showed higher percentages of 18:2 n-6 and 18:3 n-3 in those originating from broodstock fed the VEG-diet and in response to the V-diet. These results reflected the higher dietary content of these FA and were consistent with findings in many studies on feeding fish vegetable oils [24; 59; 60; 61]. Moreover, non-negligible amounts of n-3 LC-PUFAs (EPA and DHA) were found in both PL and NL fractions, although the dietary intake was nil with the plant-based diet. These results suggest active bioconversion from dietary precursor 18:3 n-3, and subsequent activation of the LC-PUFA biosynthesis pathway. Previous studies analysing fish transcriptome responses after dietary substitution of FO with vegetable oils have shown that lipid metabolism is highly affected [18; 19; 23; 62; 63], regardless of the vegetable oil used. For instance in these studies, genes involved in LC-PUFA biosynthesis were overrepresented among the differentially expressed genes in Atlantic salmon post-smolts [19] and in juvenile rainbow trout [10]. The biosynthesis of n-3 LC-PUFAs in vertebrates involves consecutive desaturation and elongation reactions which convert the 18:3n-3 (α-linolenic acid) to longer-chain more unsaturated FA of the same series, including EPA and DHA [2]. Two types of enzyme are responsible for this conversion, namely fatty acid desaturases and elongases. The former introduce a double bond in the fatty acyl chain from the carboxyl group, and elongases account for the condensation of activated fatty acids with malonyl-CoA in the FA elongation pathway. The analysis of our transcriptomic data on alevins, did not show any significant changes in the expression of genes involved in this pathway. A possible explanation of this result may be that we used RNA extracted from whole body alevins, including a mixture of different organs. Indeed, the use of this kind of sample does not allow unambiguous interpretation of the diet-induced regulation of gene expression, because regulation of genes in the liver and intestine, the main tissues in which the bioconversion of LC-PUFA occurs, can be masked by the mean expression pattern throughout the other organs/tissues of whole fish, especially the muscle. Moreover, when comparing the amounts of EPA + DHA (mg alevin^{-1}) in whole body alevins at our starting point (before first feeding) and at the end of the trial (after 3 weeks of feeding), we

observed a decrease in their relative quantities in alevins fed the V-Diet, irrespective of the broodstock nutritional history. Indeed, during the 3-week feeding trial V-fed alevins from both COM and VEG-fed females used around 54% and 36% of the amounts of EPA+DHA they had at the beginning of the trial, respectively. These results suggest that the reserves in terms of n-3 LC-PUFA provided by the mother through the egg (vitellus) are enough to satisfy the needs of alevins during early development, and therefore they do not need to activate the bioconversion pathway at this stage.

The present study confirmed that increasing replacement of fishmeal and fish oil by plant ingredients (up to total replacement) in the rainbow trout diet allowed fish to survive and grow, but with slight differences in terms of weight. The replacement of marine sources by plant-based ingredients in both broodstock and first feeding diets resulted in significant effects on the transcriptome of whole body alevins after 3 weeks of feeding. However, the relatively low values of FC found in this study (although statistically significant) suggest that the modifications induced by the diets, and therefore the metabolic consequences of the dietary replacement, are not too drastic. An organ-dedicated approach would be more informative and precise to improve understanding of the effects of external input, and specifically the replacement of FM and FO by plant ingredients.

Overall, these results improve the understanding of mechanisms and pathways activated by concomitant FM and FO replacement in diets for rainbow trout. These results also provide a framework for additional research on the consequences of maternal nutrition with reduced levels of fish meal and fish oil on the physiological and metabolic responses of progeny to different replacement rates in the first feeding diets. The results open up avenues to further reduction of the reliance of aquaculture on marine fishery resources by using plant-based diets over the full life cycle of fish, including broodstock and the early stages. Indeed, the limited negative consequences, despite the suppression of FM and FO, suggest that larger proportions of FM and FO can be replaced by plant ingredients in diets for trout broodstock and alevin, compared with what is currently practiced.

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Conflict of interest

None.

Authorship

Formulated research questions and designed the study: FM GC. Performed the experiments: VL LL FM GC. Analysed the data: VL GC DM FM. Wrote the paper: VL GC FM.

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Tables

Table 1. Ingredients and composition of first-feeding diets

Diets	**Diet-M**	**Diet- C**	**Diet-V**
Ingredients (%)			
Fish meal *	**65.0**	**30.0**	**0.0**
Corn gluten	0.0	13.0	24.0
Soybean meal 48	0.0	6.0	2.0
Wheat gluten	0.0	10.0	22.0
Soy protein concentrate	0.0	10.0	20.0
White lupin	0.0	0.4	2.5
Peas	0.0	4.0	0.0
Rapeseed meal 00	0.0	6.2	2.3
Extruded whole wheat	21.1	1.3	0.0
Fish oil †	**11.7**	**8.1**	**0.0**
Rapeseed oil	0.0	8.1	6.7
Linseed oil	0.0	0.0	6.7
Palm oil	0.0	0.0	3.6
Min.-Vit. premix	2.0	2.0	2.0
Soy lecithin	0.0	0.0	2.0
L-lysine	0.0	0.3	1.5
L-Methionine	0.0	0.01	0.3
CaHPO4.2H20 (18%P)	0.0	0.0	2.9
Attractant Mix	0.0	0.0	1.5
Composition (% DM)			
Dry Matter (DM, %)	94.3	95.3	95.5
Crude protein	48.9	53.3	52.9
Crude fat	21.5	22.1	21.8
Energy (kJ/g DM)	23.0	24.2	24.1
Cholesterol	0.70	0.55	0.36

* Origin co-fishery products - all species

† Origin co-fishery products – sardines
Diet-M, marine FM-FO-based diet;
Diet-C, commercial-like FM-FO & plant-based diet;
Diet-V, experimental 100% plant-based diet.

Table 2. Fatty acid composition (% total fatty acids) of experimental diets

	Diet-M	**Diet-C**	**Diet-V**
Fatty acid			
Saturated	**30.8**	**20.9**	**18.5**
MUFA	**33.2**	**41.9**	**38.3**
18:2 n-6 (LA)	3.2	12.5	21.5
20:2 n-6	0.2	0.1	0.04
20:3 n-6	0.1	0.02	0.0
20:4 n-6 (ARA)	0.7	0.4	0.0
22:2 n-6	0.0	0.0	0.02
Σ n-6	**4.3**	**13.1**	**21.5**
18:3 n-3 (ALA)	1.1	4.8	21.3
18:4 n-3	2.1	1.2	0.0
20:3 n-3	0.1	0.0	0.0
20:4 n-3	0.6	0.3	0.0
20:5 n-3 (EPA)	11.1	6.7	0.0
22:5 n-3	1.1	0.7	0.0
22:6 n-3 (DHA)	6.7	4.2	0.0
Σ n-3	**23.3**	**18.1**	**21.3**

MUFA, Monounsaturated fatty acid; LA, linoleic acid; ARA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid;
Diet-M, marine FM-FO-based diet;
Diet-C, commercial-like FM-FO & plant-based diet;
Diet-V, experimental 100% plant-based diet.

Table 3. Survival rates and weights of alevins collected before first feeding and after 3 weeks of feeding

	Progeny-COM						**Progeny-VEG**						***Significance***		
Before first feeding[1]			Mean	SD					Mean	SD			B		
Survival (% hatched)			84.0	7.0					78.0	13.0			*ns*		
Weight (mg)			135.0	1.0					118.0	1.0			*		
	M		**C**		**V**		**M**		**C**		**V**		***Significance***		
After 3wks feeding[2]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	B	D	BxD
Survival (% fed alevins)	99.8	0.2	99.7	0.3	100.0	0.0	99.4	0.5	99.0	0.3	99.5	0.6	*ns*	*ns*	*ns*
Weight (mg)	268.0[a]	7.0	229.0[b]	4.0	190.0[c]	4.0	246.0[a]	4.0	210.0[b]	4.0	179.0[c]	1.0	*	**	*ns*

B, broodstock nutritional history effect; D, 1[st] feeding diet effect; BxD, interaction. Statistical differences were determined by independent sample t-test[1] or by two-way ANOVA[2] followed by Tukey's HSD comparison test, when appropriate.*, *p<0.05;* **, *p<0.01*; ***, *p<0.001*; *ns*: not significant.

Table 4.Total lipid content (percentage of fresh weight) and fatty acid composition (percentage of total fatty acid) of polar and neutral lipid fractions of whole body alevins collected after 3 weeks of feeding.

	Progeny-VEG						*Progeny-COM*						*Significance*		
	Diet-M		Diet-C		Diet-V		Diet-M		Diet-C		Diet-V				
Whole body lipids	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*B*	*D*	*BxD*
Total lipids	5.0	0.3	4.7	0.4	4.5	0.4	4.6	0.2	4.5	0.3	4.0	0.4	*	*	*ns*
PL	70.5	1.6	70.5	1.4	71.3	2.2	70.4	1.7	71.2	0.7	68.2	1.4	*ns*	*ns*	*ns*
NL	29.5	1.6	29.5	1.4	28.7	1.4	29.6	1.7	28.8	0.7	31.8	1.4	*ns*	*ns*	*ns*
PL fraction															
Saturated	32.5	1.2^{a}	30.0	1.0^{bc}	29.3	0.6^{bc}	31.5	1.4^{ab}	28.4	0.8^{c}	30.6	1.1^{ac}	*ns*	***	*
MUFA	18.5	0.6	20.9	0.5	19.0	0.5	17.5	0.4	20.3	0.2	18.4	0.4	***	***	*ns*
18:2 n-6	2.5	0.2	4.9	0.2	7.4	0.4	2.2	0.0	4.7	0.2	7.1	0.1	**	***	*ns*
ARA	3.3	0.2	3.8	0.1	4.6	0.2	2.4	0.1	2.5	0.2	2.6	0.1	**	***	*ns*
∑ n-6 PUFAs	7.3	0.3^{e}	10.6	0.2^{c}	14.6^{a}	0.4	5.3	0.2^{f}	8.3	0.2^{d}	11.3	0.1^{b}	***	***	***
18:3 n-3	0.8	0.1	1.5	0.1	3.3	0.2	0.4	0.0	1.2	0.0	3.1	0.1	***	***	*ns*
EPA	9.0	0.4	7.2	0.2	5.5	0.1	9.6	0.5	8.1	0.4	6.3	0.2	***	***	*ns*
DHA	23.9	2.5	22.6	1.7	21.3	0.3	28.6	2.3	27.8	1.9	23.3	1.4	***	**	*ns*
∑ n-3 PUFAs	36.5	2.7	33.9	1.8	33.6	0.4	41.2	2.3	39.5	2.1	35.8	1.6	***	**	*ns*
NL fraction															
Saturated	23.3	0.1	17.6	0.2	17.6	2.6	24.9	1.5	19.1	0.8	18.2	0.5	*	***	*ns*
MUFA	37.8	0.3^{c}	44.0	0.3^{a}	38.3	0.9^{c}	34.3	0.7^{d}	42.3	0.3^{b}	34.1	0.4^{d}	***	***	***
18:2 n-6	7.6	0.4^{c}	14.5	0.1^{b}	18.2	0.4^{a}	6.9	0.4^{c}	14.8	0.2^{b}	18.8	0.2^{a}	***	***	***
ARA	1.2	0.1	1.0	0.1	1.0	0.2	1.0	0.1	0.7	0.0	0.7	0.0	***	***	*ns*
∑ n-6 PUFAs	10.7	0.5	17.6	0.1	22.0	0.8	9.1	0.4	16.7	0.2	21.4	0.3	***	***	*ns*
18:3 n-3	3.0^{d}	0.2	5.4	0.1^{b}	10.8	0.4^{a}	1.7	0.1^{e}	4.3	0.1^{c}	10.4	0.2^{a}	***	***	**
EPA	8.6	0.6	4.4	0.2	1.6	0.3	10.0	0.5	5.5	0.5	3.3	0.1	***	***	*ns*
DHA	8.3	0.6	6.1	0.4	3.4	1.0	10.9	2.0	7.0	1.2	6.4	0.4	***	***	*ns*
∑ n-3 PUFAs	25.7	0.8	20.1	0.6	21.3	1.9	28.5	2.8	20.6	1.8	25.7	0.5	**	***	*ns*

B, broodstock nutritional history effect; D, 1st feeding diet effect; BxD, interaction. PL, Polar Lipids; NL, Neutral lipids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Statistical differences were determined by two-way ANOVA followed by Tukey's HSD comparison test, when appropriate. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; *ns*, not significant.

Table 5. EPA and DHA content (mg alevin^{-1}) in whole body alevins collected before first feeding and after 3 weeks of feeding.

	EPA+DHA		
	before 1st feeding		3 weeks' feeding
		diets	
Progeny-COM	2.6	Diet-M	3.2
		Diet-C	2.0
		Diet-V	1.2
Progeny-VEG	1.4	Diet-M	2.6
		Diet-C	1.6
		Diet-V	0.9

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Table 6a. Whole body transcriptome of alevins collected before first feeding: Fold Changes and number of differentially expressed genes between groups (VEG-fed *vs* COM-fed).

Fold Change	Number of genes	*Significance*
FC > 1.5	3185	*Ns*
FC > 2.0	624	*Ns*
FC > 3.0	114	*Ns*

FC, Fold changes; *ns*, not significant

Table 6b. Whole body transcriptome of alevins collected after 3 weeks of feeding.

Factor	Number of genes	*P-value*
Broodstock nutritional history	71	*<0.05*
First feeding diet	249	*<0.05*
B x D	0	-

B, Broodstock nutritional history; D, First feeding diet; B x D, interaction.
Data were obtained by two-way ANOVA ($p<0.05$, Benjamini-Hochberg correction, *p-value* cut-off 0.05)

Table 7. Impact of broodstock nutritional history on whole body transcriptome of alevins collected after three weeks of feeding.

			Fold change (FC)			
Probe Name	**Gene Symbol**	**Description**	***VEG-M**/COM-M*	***VEG-C**/COM-C*	***VEG-V**/COM-V*	*p-value*
Muscle contraction/cell motility						
CUST_8882_PI425536763	**ACTA1**	**actin, alpha 1, skeletal muscle**	**-1.3**	**-1.2**	**-1.2**	***0.012***
CUST_7196_PI425536763	ACTN2	actinin, alpha 2	-1.3	-1.1	-1.4	*0.022*
CUST_8889_PI425536763	ACTB	actin, beta	-1.2	-1.2	-1.2	*0.029*
CUST_21547_PI425536763	CKB	creatine kinase, brain	-1.2	-1.2	-1.2	*0.039*
TC126460	**CKM**	**creatine kinase, muscle**	**-1.3**	**-1.2**	**-1.2**	***0.003***
CUST_21418_PI425536763	**MYBPC1**	**myosin binding protein C, slow type**	**-1.3**	**-1.3**	**-1.3**	***0.019***
CUST_20928_PI425536763	**MYBPC2**	**myosin binding protein C, fast type**	**-1.4**	**-1.3**	**-1.3**	***0.010***
CUST_21270_PI425536763	MYH2	myosin heavy chain	-1.1	-1.3	-1.5	*0.041*
TC98395	MYOM1	myomesin 1	-1.3	-1.2	-1.6	*0.037*
TC128672	MYOM2	myomesin 2	-1.3	-1.2	-1.3	*0.049*
CUST_21086_PI425536763	NEB	Nebulin	-1.5	-1.4	-1.4	*0.025*
CUST_2237_PI425536763	TXN	Thioredoxin	-1.3	-1.2	-1.2	*0.029*
Carbohydrate metabolism/Energy pathways						
CUST_8962_PI425536763	FH	fumarate hydratase	-1.3	-1.2	-1.2	*0.029*
CUST_21445_PI425536763	GPI	glucose phosphate isomerase	-1.4	-1.5	-1.5	*0.019*
TC96901	**GPD1**	**glycerol-3-phosphate dehydrogenase 1 (soluble)**	**-1.3**	**-1.5**	**-1.5**	***0.008***
CUST_3410_PI425536763	MDH1	Malate dehydrogenase 1	-1.2	-1.3	-1.2	*0.041*
TC100795	**PFKM**	**phosphofructokinase, muscle**	**-1.5**	**-1.6**	**-1.7**	***0.014***
CUST_8938_PI425536763	**PGK1**	**phosphoglycerate kinase 1**	**-1.3**	**-1.3**	**-1.2**	***0.034***
CUST_8841_PI425536763	PGM1	phosphoglucomutase 1	-1.4	-1.3	-1.4	*0.049*
TC109193	PHKA1	phosphorylase kinase alpha 1	-1.6	-1.7	-1.7	*0.026*
CUST_9021_PI425536763	**PYGL**	**phosphorylase, glycogen, liver**	**-1.2**	**-1.3**	**-1.4**	***0.032***
CUST_8835_PI425536763	**PYGM**	**phosphorylase, glycogen, muscle**	**-1.4**	**-1.4**	**-1.6**	***0.025***
CUST_22399_PI425536763	**SDHA**	**succinate dehydrogenase complex, subunit A, flavoprotein (Fp)**	**-1.3**	**-1.4**	**-1.5**	***0.006***

Genes tested by RT-qPCR are in bold. Fold changes refer to progeny developing from VEG-fed females compared to progeny from COM-fed females.

Table 8. Impact of experimental first feeding diets on whole body transcriptome of alevins after three weeks of feeding (main Biological Processes impacted).

			Fold change (FC)				
Probe Name	**Gene Symbol**	**Description**	***COM-C/COM-M***	***COM-V/COM-M***	***VEG-C/VEG-M***	***VEG-V/VEG-M***	*p-value*
Biological Process							
Amino acids/protein metabolism							
TC105786	AARS	alanyl-tRNA synthetase	+ 1.1	+ 1.4	+ 1.0	+ 1.3	*0.046*
CUST_8078_PI425536763	**EPRS**	**glutamyl-prolyl-tRNA synthetase**	**+ 1.0**	**+ 1.4**	**+ 1.1**	**+ 1.4**	***0.002***
CUST_5873_PI425536763	**DARS**	**aspartyl-tRNA synthetase**	**+ 1.1**	**+ 1.5**	**+ 1.0**	**+ 1.2**	***0.001***
CUST_11065_PI425536763	HARS	histidyl-tRNA synthetase	+ 1.1	+ 1.6	+ 1.0	+ 1.9	*0.001*
CUST_9823_PI425536763	**IARS**	**isoleucyl-tRNA synthetase**	**+ 1.6**	**+ 1.6**	**+ 1.3**	**+ 3.1**	***0.037***
TC99236	**LARS**	**leucyl-tRNA synthetase**	**+ 1.1**	**+ 1.3**	**+ 1.1**	**+ 1.2**	***0.017***
CUST_2969_PI425536763	NARS	asparaginyl-tRNA synthetase	+ 1.2	+ 1.7	+ 1.1	+ 1.3	*0.001*
TC113600	QARS	glutaminyl-tRNA synthetase	+ 1.2	+ 1.5	+ 1.1	+ 1.3	*0.007*
CUST_1945_PI425536763	SARS	seryl-tRNA synthetase	+ 1.1	+ 1.6	+ 1.0	+ 1.5	*0.001*
CUST_27479_PI425536763	TARS	threonyl-tRNA synthetase	+ 1.2	+ 1.6	+ 1.1	+ 1.3	*0.025*
CUST_6009_PI425536763	WARS	tryptophanyl-tRNA synthetase	+ 1.2	+ 1.6	+ 1.1	+ 1.2	*0.016*
TC108527	PHGDH	phosphoglycerate dehydrogenase	+ 1.3	+ 4.3	- 1.1	+ 2.2	*<0.001*
CUST_5305_PI425536763	EEF1E1	eukaryotic translation elongation factor 1 epsilon 1	+ 1.1	+ 1.5	+ 1.1	+ 1.3	*0.007*
TC97482	EIF2B1	eukaryotic translation initiation factor 2B, subunit 1 alpha	+ 1.1	+ 1.6	+ 1.2	+ 1.2	*0.005*
CUST_14068_PI425536763	EIF2B3	eukaryotic translation initiation factor 2B, subunit 3 gamma	+ 1.0	+ 1.6	+ 1.1	+ 1.4	*0.015*
TC95289	EIF2S2	eukaryotic translation initiation factor 2, subunit 2 beta	- 1.1	+ 2.8	+ 1.0	+ 1.9	*<0.001*

Table 8 (Continued)

Probe Name	Gene Symbol	Description	*Fold change (FC)*				
			COM-C/COM-M	***COM-V/COM-M***	***VEG-C/VEG-M***	***VEG-V/VEG-M***	*p-value*
Cholesterol/ Lipid metabolism							
CUST_16218_PI425536763	**HMGCR**	**3-hydroxy-3-methylglutaryl-CoA reductase**	**+ 1.5**	**+ 1.7**	**+ 1.4**	**+ 1.4**	***0.035***
TC114256	**HMGCS1**	**3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)**	**+ 1.7**	**+ 2.1**	**+ 1.2**	**+ 1.3**	***0.004***
CUST_8711_PI425536763	SQLE	squalene epoxidase	+ 1.6	+ 1.7	+ 1.5	+ 1.6	*0.009*
TC121390	CYB5R2	cytochrome b5 reductase 2	+ 1.4	+ 1.5	+ 1.3	+ 1.4	*0.011*
CUST_2668_PI425536763	CYP2F1	cytochrome P450, family 2, subfamily F, polypeptide 1	- 1.0	- 2.3	- 1.5	- 2.3	*0.003*
TC121294	CYP51A1	cytochrome P450, family 51, subfamily A, polypeptide 1	+ 1.5	+ 1.9	+ 1.5	+ 1.6	*0.001*
TC112425	CYP46A1	cytochrome P450, family 46, subfamily A, polypeptide 1	+ 1.0	- 1.3	+ 1.3	- 1.5	*0.005*
TC107840	IDI1	isopentenyl-diphosphate delta isomerase 1	+ 1.6	+ 1.7	+ 1.2	+ 1.4	*0.006*
TC130899	INSIG1	insulin induced gene 1	+ 1.3	+ 1.7	+ 1.0	+ 1.3	*0.003*
CUST_12877_PI425536763	INSIG2	insulin induced gene 2	+ 1.5	+ 1.7	+ 1.2	+ 1.2	*0.029*
CUST_5335_PI425536763	MVD	mevalonate (diphospho) decarboxylase	+ 1.6	+ 1.9	+ 1.1	+ 1.3	*0.026*
CUST_28240_PI425536763	LSS	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	+ 1.6	+ 1.9	+ 1.3	+ 1.0	*0.007*
CUST_16670_PI425536763	HSD17B7	hydroxysteroid (17-beta) dehydrogenase 7	+ 1.3	+ 1.7	+ 1.2	+ 1.4	*0.011*

Table 8 (Continued)

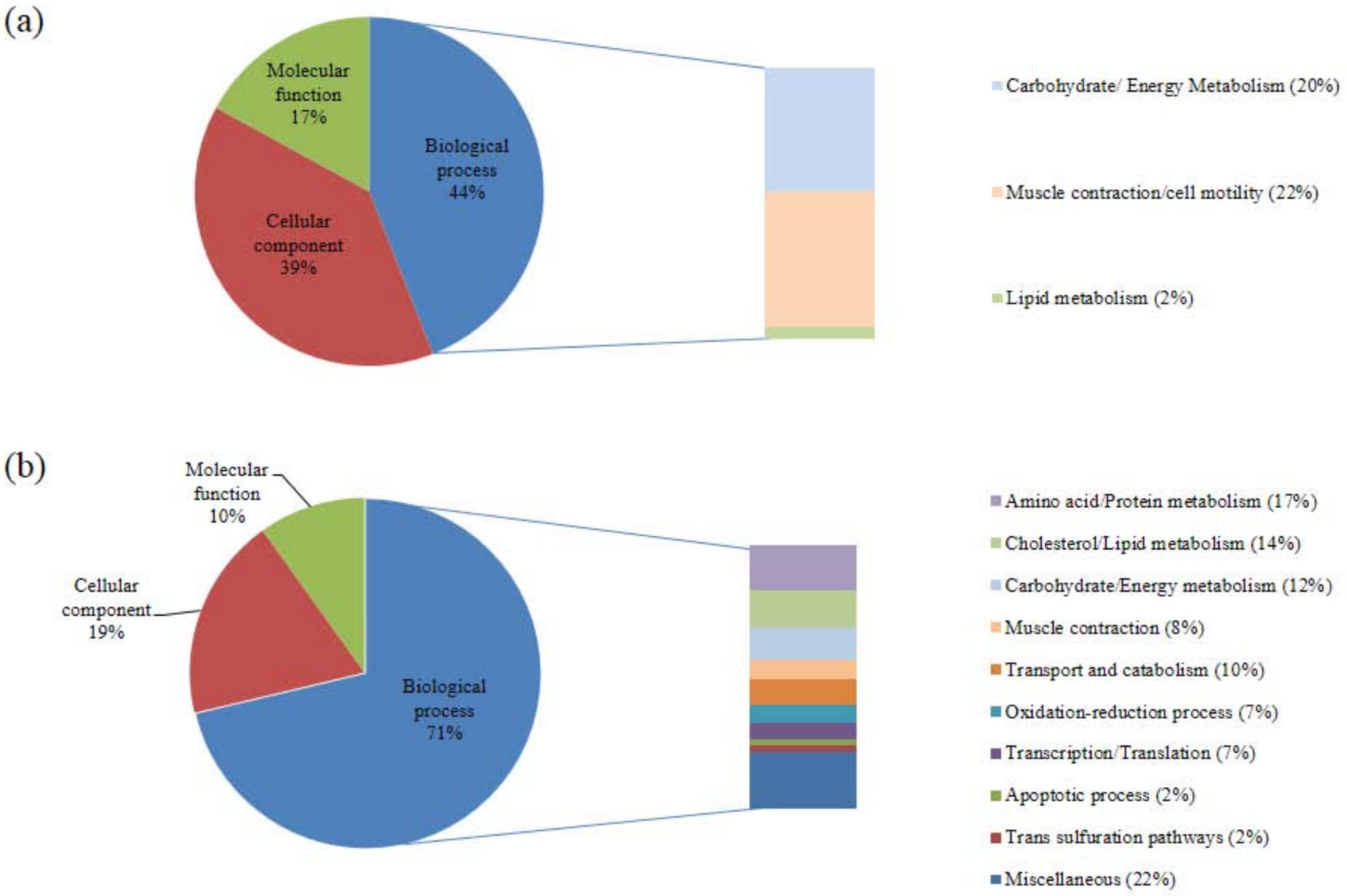
Probe Name	Gene Symbol	Description	*Fold change (FC)*				
			COM-C/COM-M	***COM-V/COM-M***	***VEG-C/VEG-M***	***VEG-V/VEG-M***	*p-value*
Carbohydrate/ Energy metabolism							
CUST_8779_PI425536763	ENO1	enolase 1, (alpha)	- 1.0	- 1.5	- 1.3	- 1.7	*0.004*
CUST_21534_PI425536763	ENO2	enolase 2 (gamma, neuronal)	+ 1.0	+ 1.5	+ 1.5	+ 1.4	*0.012*
CUST_21688_PI425536763	ENO3	enolase 3, beta muscle	- 1.0	- 1.5	- 1.3	- 1.7	*0.046*
CUST_6475_PI425536763	**GCK**	**glucokinase (hexokinase 4)**	**- 1.5**	**- 2.1**	**- 1.4**	**- 1.6**	***0.031***
CUST_2361_PI425536763	**HK2**	**hexokinase 2**	**+ 1.1**	**+ 1.3**	**+ 1.2**	**+ 1.2**	***0.017***
CUST_21617_PI425536763	**LDHA**	**lactate dehydrogenase A**	**- 1.1**	**- 1.7**	**- 1.3**	**- 1.9**	***0.043***
CUST_21434_PI425536763	LDHB	lactate dehydrogenase B	- 1.0	- 1.9	- 1.3	- 1.7	*0.030*
TC95453	G6PC	glucose-6-phosphatase, catalytic subunit	- 1.4	- 2.7	- 1.2	- 1.7	*0.012*
CUST_11963_PI425536763	GSK3A	glycogen synthase kinase 3 alpha	+ 1.1	+ 1.3	+ 1.3	+ 1.4	*0.012*
CUST_8266_PI425536763	ATP5C1	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	+ 1.1	- 1.2	+ 1.1	- 1.3	*0.011*
CUST_5461_PI425536763	ATP5J	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit F6	- 1.1	- 1.2	- 1.2	- 1.3	*0.047*
Muscle contraction							
CUST_8882_PI425536763	ACTA1	actin, alpha 1, skeletal muscle	- 1.1	- 1.4	-1.0	- 1.3	*0.017*
CUST_7817_PI425536763	ACTN2	actinin, alpha 2	- 1.1	- 1.3	- 1.2	- 1.3	*0.025*
CUST_18923_PI425536763	GAMT	guanidinoacetate N-methyltransferase	+ 1.1	- 1.7	+ 1.0	- 2.0	*0.027*
TC102031	TCAP	titin-cap	- 1.2	+ 1.9	+ 1.0	+ 1.7	*0.017*
TC96295	TNNI2	troponin I type 2 (skeletal, fast)	- 1.2	- 1.6	- 1.1	- 1.5	*0.027*
CUST_20992_PI425536763	TNNT2	troponin T type 2 (cardiac)	- 1.2	- 1.5	- 1.3	- 1.7	*0.002*

Table 8 (Continued)

			Fold change (FC)				
Probe Name	**Gene Symbol**	**Description**	***COM-C/COM-M***	***COM-V/COM-M***	***VEG-C/VEG-M***	***VEG-V/VEG-M***	*p-value*
CUST_8764_PI425536763	TNNT3	troponin T type 3 (skeletal, fast)	- 1.1	- 1.5	- 1.3	- 1.6	*0.001*
Transport and Catabolism							
CUST_23987_PI425536763	SLC3A2	solute carrier family 3 (amino acid transporter heavy chain), member 2	+ 1.0	+ 1.9	+ 1.3	+ 2.6	*<0.001*
TC120357	SLC15A1	solute carrier family 15 (oligopeptide transporter), member 1		- 2.4	- 1.6	- 2.5	*< 0.001*
CUST_27328_PI425536763	SLC1A4		+ 1.0	+ 4.0	+ 1.2	+ 3.2	*< 0.001*
TC119001	NXT2	nuclear transport factor 2-like export factor 2	+ 1.3	+ 1.9	+ 1.1	+ 1.2	*0.005*
TC98320	NDUFA10	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10,	+ 1.0	+ 1.4	+ 1.0	+ 1.8	*0.045*
CUST_8732_PI425536763	CAPN3	calpain 3	+ 1.1	- 1.2	+ 1.1	- 1.3	*0.048*
TC128968	CASP8	caspase 8, apoptosis-related cysteine peptidase	+ 1.0	+ 1.5	+ 1.5	+ 1.8	*0.036*
TC103006	CHIA	chitinase, acidic	- 1.3	- 1.9	- 1.3	- 1.7	*0.035*
TC118835	ACR	acrosin	- 1.1	+ 1.9	+ 1.1	+ 1.5	*<0.001*

Genes tested by RT-qPCR are in bold. Fold changes refer to progeny fed C or V-diet compared to fish fed the M diet.

Figure 1 a-b. Whole-body alevins transcriptome: proportions of different GO-categories represented by differentially expressed genes obtained by a 2-way ANOVA (FDR 0.05). **A**: *broodstock nutritional history effect*; **B**: *first feeding diet effect*.



Supplementary material

Supplementary Table 1a-b. RT-qPCR-Primers

a. Primer sequences for real-time q-PCR assays for transcripts expressed differentially in response to broodstock nutritional history (COM - VEG)

Gene	*Primer 5'-3' (FW)*	*Primer 5'-3' (RV)*	*Annealing temperature, C°*
Muscle growth/contraction			
ACTA1	AAAACAGGCCAGGGACAACA	CCTGGTATTGCTGCCCGTAT	60
CKM	TGCGTTGGTCTGAAAAGGATTGA	TCTCCTCAAACTTGGGGTGTGT	60
MYBPC1	CCAGCATCCAGAACCATCCT	TACACTGGGGAAGGTCGACA	60
MYBPC2	GTGAGTGTCCGTTTGTTGCC	CTGCCAAGTGAGACTGACGT	60
Carbohydrate/energy metabolism			
PYGM	TGCAATGTGTGTCGGTGTTG	AAGTTCCCTGGAGACCACGA	60
PYGL	AACCGACACCTCCACTTCACC	CCTGCATCTTCCTCCATCTC	60
6PFKM	GAGGGCGAAGATGAAGCTTG	GGGACCTCGAGATGAACGTA	60
SDHA	TGGTGTTTGGACGTGCCTGC	AACACAGCGGCGTGGTTCTG	60
GPD1	CTTCGCCCGGATATTCTGCA	GACCCTGGAGCTTCTGCCCA	60
PGK1	TTCGGCACAGCACACAGAGC	AAAGGGCCTGGCTGGTTTCTCC	60
Reference gene			
EF1-α	TCCTCTTGGTCGTTTCGCTG	ACCCGAGGGACATCCTGTG	59

ACTA1, actin-alpha 1; CKM, creatin kinase muscle; MYBPC1, myosin binding protein-C slow type; MYBPC2, myosin binding protein-C fast type; PYGM, phosphorylase glycogen muscle; PYGL, phosphorylase glycogen liver; 6PFKM, 6-phosphofructokinase muscle; SDHA, succinate dehydrogenase complex, subunit A, flavoprotein; GPD1, glycerol-3-phosphate dehydrogenase 1; PGK1, phosphoglycerate kinase 1; EF1-α, α-elongation factor-1

b. Primer sequences for real-time q-PCR assays for transcripts expressed differentially in response to first feeding diets (M-C-V)

Gene	*Primer 5'-3' (FW)*	*Primer 5'-3' (RV)*	*Annealing temperature, C°*
Carbohydrate metabolism			
GCK	GCACGGCTGAGATGCTCTTTG	GCCTTGAACCCTTTGGTCCAG	60
HK2	CGCCGTGGTCGATAAGAT	TGATGAGAGCCGCCCCCTTT	60
LDHA	ATGCGTGCTGGGCAACAGTG	GCTGATAAATTAACCCTCCGC	60
Lipid/cholesterol metabolism			
HMGCR	GAACGGTGAATGTGCTGTGT	GACCATTTGGGAGCTTGTGT	60
HMGCS1	AGTGGCAAAGAGAGGGTGTG	TTCTGGTTGGAGACGAGGAG	60
Amino acids/protein metabolism			
DARS	GACCTGGCGGACATTGTGAA	GAGAGGGCCATTCACCACAA	60
EPRS	GTCGTCTGATGCCCTCTTGA	TGAAGCAGGGTCAGTGTGTG	60
IARS	ACATCGTGACTCGCTTCGCC	CTACAACCGTCAGATACGCGG	60
LARS	CGGCAGTGACATGAATGCAG	CCACTGGCCACAATGCTTTC	60
Reference gene			
EF1-α	TCCTCTTGGTCGTTTCGCTG	ACCCGAGGGACATCCTGTG	59

GCK, glucokinase; HK2, hexokinase-2; LDHA, lactate dehydrogenase-A; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; HMGCS1, 3-hydroxy-3-methylglutaryl-CoA synthetase ; DARS, aspartyl-

tRNA synthetase ; EPRS, glutamyl-tRNA synthetase ; IARS, isoleucyl-tRNA synthetase ; LARS, leucyl-tRNA synthetase; EF1-α, α-elongation factor-1

Supplementary Table 2. Global **e**ffects of different first feeding diets on whole body transcriptome of alevins after three weeks of feeding.

			Fold change (FC)				
Probe Name	**Gene Symbol**	**Description**	***COM-C/COM-M***	***COM-V/COM-M***	***VEG-C/VEG-M***	***VEG-V/VEG-M***	*p-value*
Biological Process							
Amino acids/protein metabolism							
TC105786	AARS	alanyl-tRNA synthetase	+ 1.1	+ 1.4	+ 1.0	+ 1.3	*0.046*
CUST_8078_PI425536763	**EPRS**	**glutamyl-prolyl-tRNA synthetase**	**+ 1.0**	**+ 1.4**	**+ 1.1**	**+ 1.4**	***0.002***
CUST_5873_PI425536763	**DARS**	**aspartyl-tRNA synthetase**	**+ 1.1**	**+ 1.5**	**+ 1.0**	**+ 1.2**	***0.001***
CUST_11065_PI425536763	HARS	histidyl-tRNA synthetase	+ 1.1	+ 1.6	+ 1.0	+ 1.9	*0.001*
CUST_9823_PI425536763	**IARS**	**isoleucyl-tRNA synthetase**	**+ 1.6**	**+ 1.6**	**+ 1.3**	**+ 3.1**	***0.037***
TC99236	**LARS**	**leucyl-tRNA synthetase**	**+ 1.1**	**+ 1.3**	**+ 1.1**	**+ 1.2**	***0.017***
CUST_2969_PI425536763	NARS	asparaginyl-tRNA synthetase	+ 1.2	+ 1.7	+ 1.1	+ 1.3	*0.001*
TC113600	QARS	glutaminyl-tRNA synthetase	+ 1.2	+ 1.5	+ 1.1	+ 1.3	*0.007*
CUST_1945_PI425536763	SARS	seryl-tRNA synthetase	+ 1.1	+ 1.6	+ 1.0	+ 1.5	*0.001*
CUST_27479_PI425536763	TARS	threonyl-tRNA synthetase	+ 1.2	+ 1.6	+ 1.1	+ 1.3	*0.025*
CUST_6009_PI425536763	WARS	tryptophanyl-tRNA synthetase	+ 1.2	+ 1.6	+ 1.1	+ 1.2	*0.016*
TC108527	PHGDH	phosphoglycerate dehydrogenase	+ 1.3	+ 4.3	- 1.1	+ 2.2	*<0.001*
CUST_5305_PI425536763	EEF1E1	eukaryotic translation elongation factor 1 epsilon 1	+ 1.1	+ 1.5	+ 1.1	+ 1.3	*0.007*
TC97482	EIF2B1	eukaryotic translation initiation factor 2B, subunit 1 alpha	+ 1.1	+ 1.6	+ 1.2	+ 1.2	*0.005*
CUST_14068_PI425536763	EIF2B3	eukaryotic translation initiation factor 2B, subunit 3 gamma	+ 1.0	+ 1.6	+ 1.1	+ 1.4	*0.015*
TC95289	EIF2S2	eukaryotic translation initiation factor 2, subunit 2 beta	- 1.1	+ 2.8	+ 1.0	+ 1.9	*<0.001*

Probe Name	Gene Symbol	Description	*Fold change (FC)*				
			COM-C/COM-M	***COM-V/COM-M***	***VEG-C/VEG-M***	***VEG-V/VEG-M***	*p-value*
Cholesterol/ Lipid metabolism							
CUST_16218_PI425536763	**HMGCR**	**3-hydroxy-3-methylglutaryl-CoA reductase**	**+ 1.5**	**+ 1.7**	**+ 1.4**	**+ 1.4**	***0.035***
TC114256	**HMGCS1**	**3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)**	**+ 1.7**	**+ 2.1**	**+ 1.2**	**+ 1.3**	***0.004***
CUST_8711_PI425536763	SQLE	squalene epoxidase	+ 1.6	+ 1.7	+ 1.5	+ 1.6	*0.009*
TC121390	CYB5R2	cytochrome b5 reductase 2	+ 1.4	+ 1.5	+ 1.3	+ 1.4	*0.011*
CUST_2668_PI425536763	CYP2F1	cytochrome P450, family 2, subfamily F, polypeptide 1	- 1.0	- 2.3	- 1.5	- 2.3	*0.003*
TC121294	CYP51A1	cytochrome P450, family 51, subfamily A, polypeptide 1	+ 1.5	+ 1.9	+ 1.5	+ 1.6	*0.001*
TC112425	CYP46A1	cytochrome P450, family 46, subfamily A, polypeptide 1	+ 1.0	- 1.3	+ 1.3	- 1.5	*0.005*
TC107840	IDI1	isopentenyl-diphosphate delta isomerase 1	+ 1.6	+ 1.7	+ 1.2	+ 1.4	*0.006*
TC130899	INSIG1	insulin induced gene 1	+ 1.3	+ 1.7	+ 1.0	+ 1.3	*0.003*
CUST_12877_PI425536763	INSIG2	insulin induced gene 2	+ 1.5	+ 1.7	+ 1.2	+ 1.2	*0.029*
CUST_5335_PI425536763	MVD	mevalonate (diphospho) decarboxylase	+ 1.6	+ 1.9	+ 1.1	+ 1.3	*0.026*
CUST_28240_PI425536763	LSS	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	+ 1.6	+ 1.9	+ 1.3	+ 1.0	*0.007*
CUST_16670_PI425536763	HSD17B7	hydroxysteroid (17-beta) dehydrogenase 7	+ 1.3	+ 1.7	+ 1.2	+ 1.4	*0.011*

Probe Name	Gene Symbol	Description	Fold change (FC)				
			COM-C/COM-M	***COM-V/COM-M***	***VEG-C/VEG-M***	***VEG-V/VEG-M***	*p-value*
Carbohydrate/ Energy metabolism							
CUST_8779_PI425536763	ENO1	enolase 1, (alpha)	- 1.0	- 1.5	- 1.3	- 1.7	*0.004*
CUST_21534_PI425536763	ENO2	enolase 2 (gamma, neuronal)	+ 1.0	+ 1.5	+ 1.5	+ 1.4	*0.012*
CUST_21688_PI425536763	ENO3	enolase 3, beta muscle	- 1.0	- 1.5	- 1.3	- 1.7	*0.046*
CUST_6475_PI425536763	**GCK**	**glucokinase (hexokinase 4)**	**- 1.5**	**- 2.1**	**- 1.4**	**- 1.6**	***0.031***
CUST_2361_PI425536763	**HK2**	**hexokinase 2**	**+ 1.1**	**+ 1.3**	**+ 1.2**	**+ 1.2**	***0.017***
CUST_21617_PI425536763	**LDHA**	**lactate dehydrogenase A**	**- 1.1**	**- 1.7**	**- 1.3**	**- 1.9**	***0.043***
CUST_21434_PI425536763	LDHB	lactate dehydrogenase B	- 1.0	- 1.9	- 1.3	- 1.7	*0.030*
TC95453	G6PC	glucose-6-phosphatase, catalytic subunit	- 1.4	- 2.7	- 1.2	- 1.7	*0.012*
CUST_11963_PI425536763	GSK3A	glycogen synthase kinase 3 alpha	+ 1.1	+ 1.3	+ 1.3	+ 1.4	*0.012*
CUST_8266_PI425536763	ATP5C1	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	+ 1.1	- 1.2	+ 1.1	- 1.3	*0.011*
CUST_5461_PI425536763	ATP5J	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit F6	- 1.1	- 1.2	- 1.2	- 1.3	*0.047*
Muscle contraction							
CUST_8882_PI425536763	ACTA1	actin, alpha 1, skeletal muscle	- 1.1	- 1.4	-1.0	- 1.3	*0.017*
CUST_7817_PI425536763	ACTN2	actinin, alpha 2	- 1.1	- 1.3	- 1.2	- 1.3	*0.025*
CUST_18923_PI425536763	GAMT	guanidinoacetate N-methyltransferase	+ 1.1	- 1.7	+ 1.0	- 2.0	*0.027*
TC102031	TCAP	titin-cap	- 1.2	+ 1.9	+ 1.0	+ 1.7	*0.017*
TC96295	TNNI2	troponin I type 2 (skeletal, fast)	- 1.2	- 1.6	- 1.1	- 1.5	*0.027*
CUST_20992_PI425536763	TNNT2	troponin T type 2 (cardiac)	- 1.2	- 1.5	- 1.3	- 1.7	*0.002*

			Fold change (FC)				
Probe Name	**Gene Symbol**	**Description**	***COM-C/COM-M***	***COM-V/COM-M***	***VEG-C/VEG-M***	***VEG-V/VEG-M***	*p-value*
CUST_8764_PI425536763	TNNT3	troponin T type 3 (skeletal, fast)	- 1.1	- 1.5	- 1.3	- 1.6	*0.001*
Transport and Catabolism							
CUST_23987_PI425536763	SLC3A2	solute carrier family 3 (amino acid transporter heavy chain), member 2	+ 1.0	+ 1.9	+ 1.3	+ 2.6	*<0.001*
TC120357	SLC15A1	solute carrier family 15 (oligopeptide transporter), member 1		- 2.4	- 1.6	- 2.5	*< 0.001*
CUST_27328_PI425536763	SLC1A4		+ 1.0	+ 4.0	+ 1.2	+ 3.2	*< 0.001*
TC119001	NXT2	nuclear transport factor 2-like export factor 2	+ 1.3	+ 1.9	+ 1.1	+ 1.2	*0.005*
TC98320	NDUFA10	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10,	+ 1.0	+ 1.4	+ 1.0	+ 1.8	*0.045*
CUST_8732_PI425536763	CAPN3	calpain 3	+ 1.1	- 1.2	+ 1.1	- 1.3	*0.048*
TC128968	CASP8	caspase 8, apoptosis-related cysteine peptidase	+ 1.0	+ 1.5	+ 1.5	+ 1.8	*0.036*
TC103006	CHIA	chitinase, acidic	- 1.3	- 1.9	- 1.3	- 1.7	*0.035*
TC118835	ACR	Acrosin	- 1.1	+ 1.9	+ 1.1	+ 1.5	*<0.001*
Oxidation-reduction process							
CUST_8851_PI425536763	MTHFD2	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	- 1.2	+ 2.2	+ 1.4	+ 2.1	*<0.001*

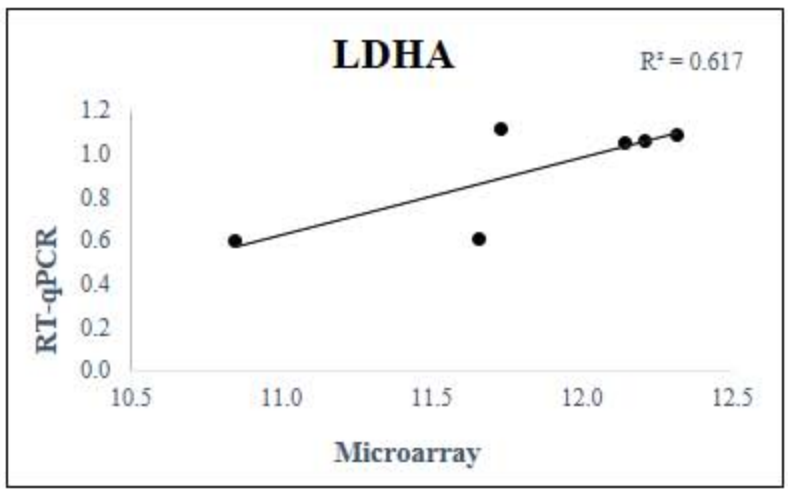
Probe Name	Gene Symbol	Description	*Fold change (FC)*				
			COM-C/COM-M	***COM-V/COM-M***	***VEG-C/VEG-M***	***VEG-V/VEG-M***	*p-value*
CUST_9084_PI425536763	DHODH	dihydroorotate dehydrogenase (quinone)	+ 1.4	+ 1.5	+ 1.1	+ 1.4	*0.002*
CUST_7365_PI425536763	COX15	cytochrome c oxidase assembly homologue 15 (yeast)	+ 1.0	+ 1.8	- 1.1	+ 1.1	*0.026*
CUST_4068_PI425536763	NDUFS7	NADH dehydrogenase (ubiquinone) Fe-S protein 7, 20kDa (NADH-coenzyme Q reductase)	- 1.1	- 1.2	- 1.1	- 1.2	*0.047*
CUST_9914_PI425536763	TM7SF2	transmembrane 7 superfamily member 2	+ 2.0	+ 2.2	+ 1.5	+ 1.6	*0.001*
CUST_3410_PI425536763	MDH1	malate dehydrogenase 1, NAD (soluble)	- 1.1	- 1.3	- 1.3	- 1.4	*0.023*
Transcription/Translation							
CUST_7617_PI425536763	ATF3	activating transcription factor 3	- 1.1	+ 2.4	+ 1.2	+ 2.8	*<0.001*
TC117725	ATF4	activating transcription factor 4	+ 1.0	+ 2.2	+ 1.3	+ 2.8	*<0.001*
CUST_28448_PI425536763	ATF5	activating transcription factor 5	+ 1.2	+ 2.4	+ 1.3	+ 2.5	*0.011*
CUST_13247_PI425536763	CEBPB	CCAAT/enhancer binding protein (C/EBP), beta	+ 1.4	+ 2.1	- 1.0	+ 1.6	*<0.001*
CUST_20799_PI425536763	MEF2C	myocyte enhancer factor 2C	+ 1.2	+ 1.4	+ 1.0	+ 1.4	*0.035*
TC127379	MAP2K6	mitogen-activated protein kinase kinase 6	- 1.2	- 1.3	- 1.1	-1.4	*0.025*
Apoptotic process							
TC119464	GADD45G	growth arrest and DNA-damage-inducible, gamma	+ 1.0	+ 1.7	+ 1.1	+ 1.4	*0.035*
TC113845	NMT1	N-myristoyltransferase 1	+ 1.1	+ 3.4	+ 1.2	+ 2.7	*<0.001*

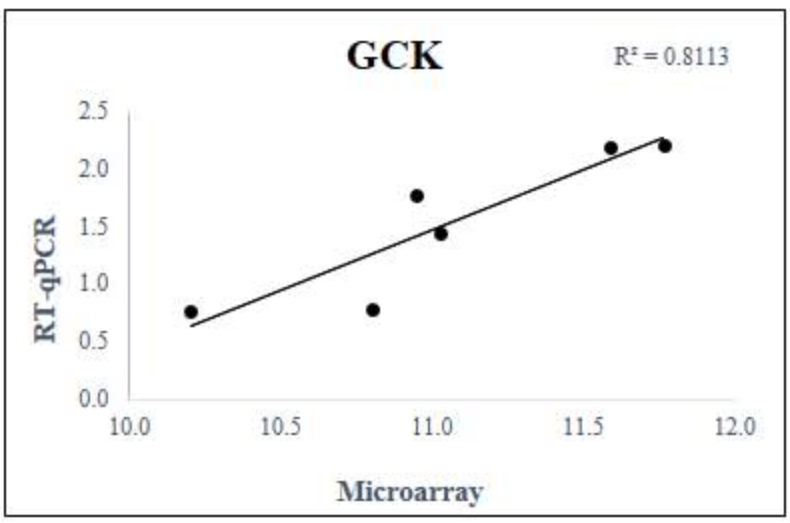
Probe Name	Gene Symbol	Description	*Fold change (FC)*				
			COM-C/COM-M	***COM-V/COM-M***	***VEG-C/VEG-M***	***VEG-V/VEG-M***	*p-value*
Trans-sulfuration pathways							
TC129723	CBS	cystathionine-beta-synthase	+ 1.7	+ 2.9	+ 1.2	+ 1.7	*0.020*
CUST_5923_PI425536763	CTH	cystathionase (cystathionine gamma-lyase)	+ 1.2	+ 1.7	+ 1.2	+ 1.2	*0.035*
Others							
CUST_5617_PI425536763	FBLN2	fibulin 2	+ 1.1	+ 1.4	+ 1.0	+ 1.2	*0.021*
TC111317	LCT	Lactase	- 1.5	- 4.7	- 2.6	- 4.8	*<0.001*
CUST_3595_PI425536763	ADK	adenylate kinase	+ 1.1	- 1.2	+ 1.0	- 1.3	*0.030*
CUST_28134_PI425536763	FBXO25	F-box protein 25	- 2.3	- 2.3	- 1.7	- 1.6	*0.041*
TC104210	MEP1A	meprin 1 alpha	- 1.5	- 6.1	+ 1.0	- 2.5	*<0.001*
CUST_2492_PI425536763	CCRN4L	CCR4 carbon catabolite repression 4-like (*S. cerevisiae*)	+ 1.0	+ 1.3	+ 1.0	+ 1.3	*0.011*
TC95024	HAGH	hydroxyacylglutathione hydrolase	+ 1.0	+ 1.4	+ 1.0	+ 1.5	*0.043*
TC95784	HMGB3	high mobility group box 3	+ 1.1	+ 1.9	+ 1.0	+ 1.4	*<0.001*
CUST_5459_PI425536763	RAB15	RAB15, member RAS oncogene family	- 1.2	+ 1.9	+ 1.2	+ 2.4	*0.036*
TC112850	RGN	regucalcin (senescence marker protein-30)	- 1.2	+ 1.3	+ 1.3	+ 4.3	*0.002*
CUST_577_PI425536763	SMYD1	SET and MYND domain containing 1	- 1.1	- 1.3	- 1.2	- 1.5	*0.021*
TC108261	TP53BP1	tumor protein p53 binding protein 1	+ 1.0	+ 1.4	- 1.0	+ 1.2	*0.047*
TC119917	XBP1	X-box binding protein 1	+ 1.0	+ 1.7	+ 1.0	+ 1.4	*0.005*
TC110767	PISD	phosphatidylserine decarboxylase	+ 1.4	+ 1.8	+ 1.4	+ 1.7	*0.009*

Probe Name	Gene Symbol	Description	*Fold change (FC)*				
			COM-C/COM-M	***COM-V/COM-M***	***VEG-C/VEG-M***	***VEG-V/VEG-M***	*p-value*
TC95665	EBP	emopamil binding protein	+ 1.5	+ 1.6	+ 1.4	+ 1.3	*0.018*
CUST_8110_PI425536763	TPI	triose phosphate isomerase	- 1.1	- 1.6	- 1.1	+ 1.3	*0.046*
TC118127	APOBEC2	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 2	+ 1.3	+ 1.3	+ 1.2	+ 1.1	*0.027*
TC108960	GLA	galactosidase, alpha	+ 1.0	+ 1.7	+ 1.1	+ 1.9	*0.009*
TC109455	FDPS	farnesyl diphosphate synthase	+ 1.6	+ 2.5	+ 1.3	+ 1.5	*0.010*
CUST_27735_PI425536763	FN1	fibronectin 1	- 1.0	+ 1.7	+ 1.3	+ 2.7	*0.015*

Genes tested by RT-qPCR are in bold. Fold changes refer to progeny fed C or V-diet compared to fish fed the M diet.

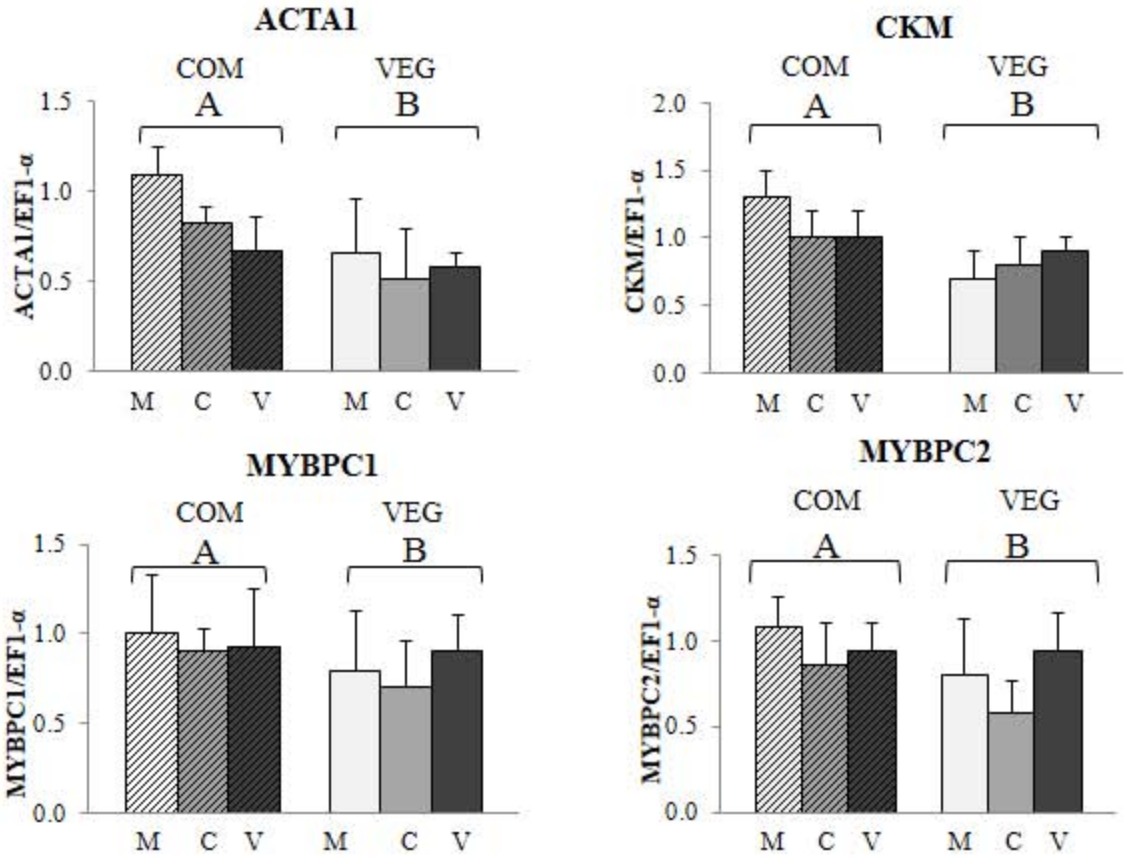
Supplementary Figure 1. Examples of correlation of the measurement of mRNA by microarray to that by RT-qPCR.



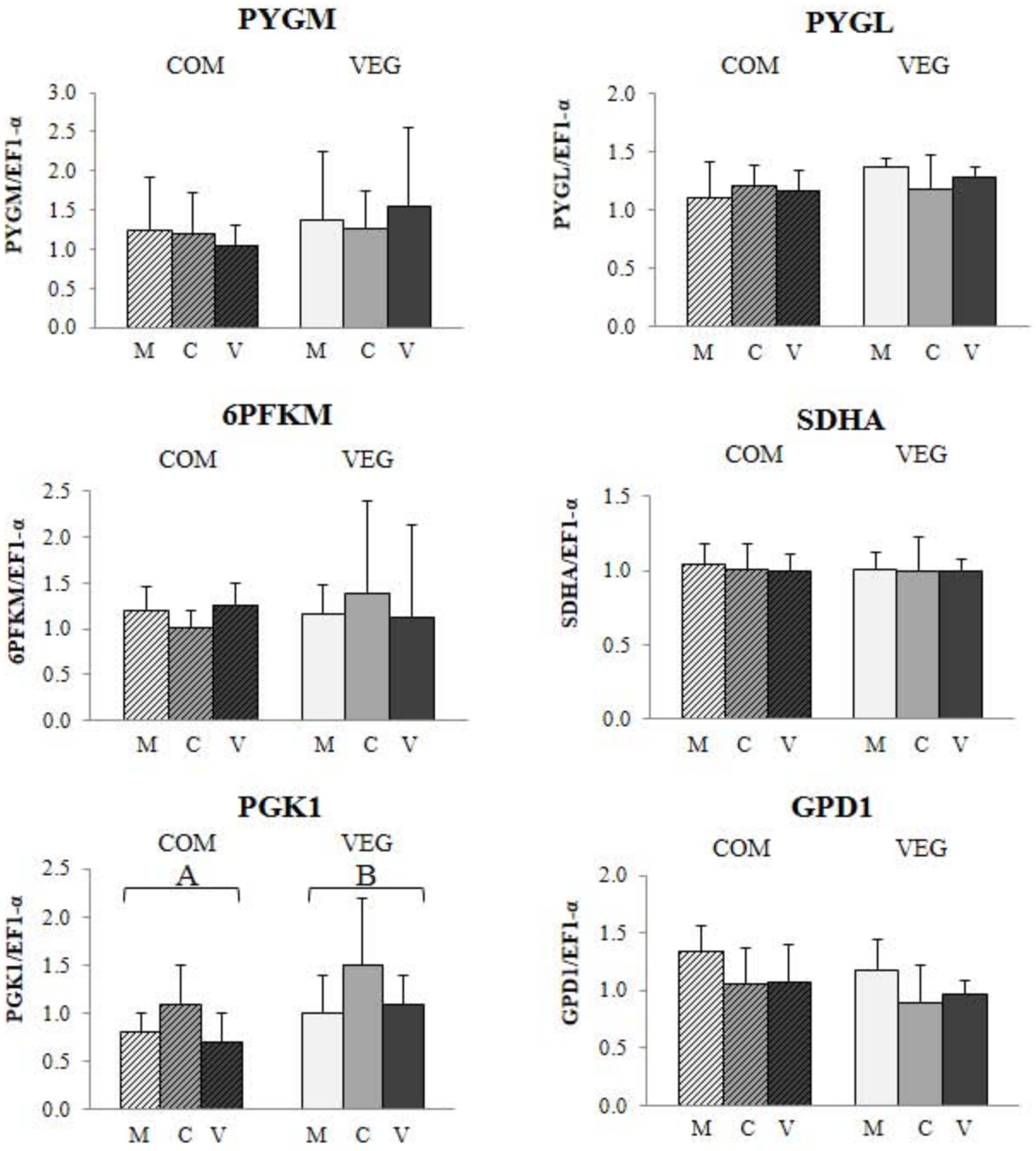


Supplementary Figure 2. Gene expression of selected genes differentially expressed in response to broodstock nutritional history (COM *vs* VEG) and involved in (a) muscle growth/contraction, (b) carbohydrate/energy metabolism. Expression values are normalized by elongation factor-1 alpha (EF1α)-expressed transcripts. Data are presented as mean ± S.D. (n=6 individuals/treatment) and were analyzed using two-way ANOVA ($p<0.05$). Values not sharing a common lowercase letter are significantly different from each other ($p<0.05$).

Supplementary Figure 2a. Muscle growth/contraction. Actin-alpha skeletal muscle (ACTA1), muscle creatine kinase (CKM), myosin binding protein-C slow type (MYBPC1) and fast type (MYBPC2) mRNA levels were measured using RT-qPCR.



Supplementary Figure 2b. Carbohydrate/energy metabolism. Glycogen phosphorylase- muscle (PYGM) and liver (PYGL), phosphofructokinase-muscle (6PFKM), succinate dehydrogenase complex-A (SDHA), glycerol-3-phosphate dehydrogenase2 (GPD1) and phosphoglycerate-kinase 1 (PGK1) mRNA levels were measured using RT-qPCR.



Supplementary Figure 3. Gene expression of selected genes differentially expressed in response to alevin diets (M-C-V) and involved in (a) amino acid/protein metabolism, (b) lipid/cholesterol metabolism and (c) carbohydrate/energy metabolism. Expression values are normalized by elongation factor-1 (EF1-α) expressed transcripts. Data are presented as mean ± S.D. (n=6 individuals/treatment) and were analyzed using two-way ANOVA ($p<0.05$). Values not sharing a common lowercase letter are significantly different from each other ($p<0.05$).

Supplemenatry Figure 3a. AA/protein metabolism. Isoleucyl-tRNA synthetase (IARS), aspartyl-tRNA synthetase (DARS), glutamyl-prolyl-tRNA synthetase (EPRS) and leucyl-tRNA synthetase (LARS) mRNA levels were measured using real-time quantitative PCR.

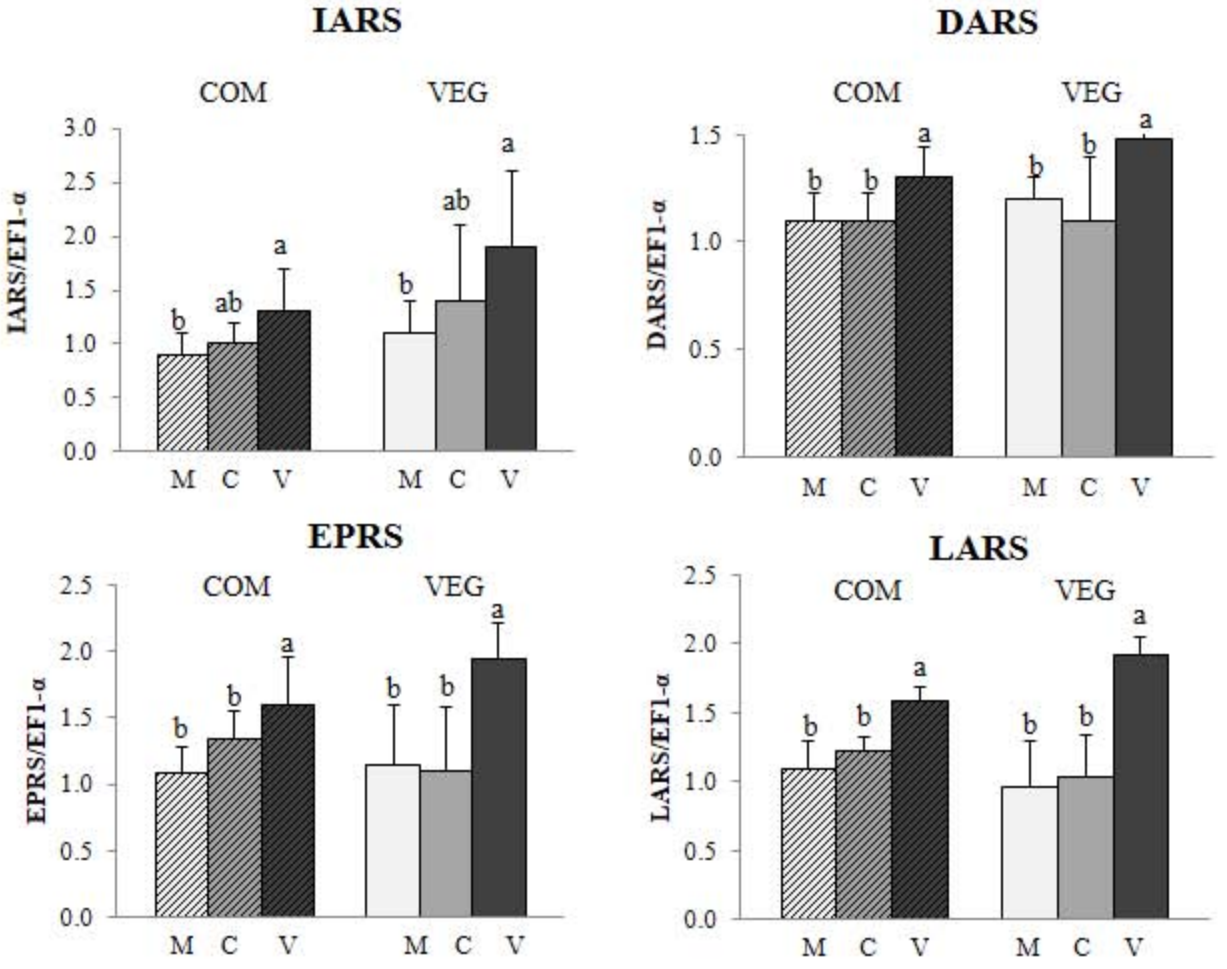
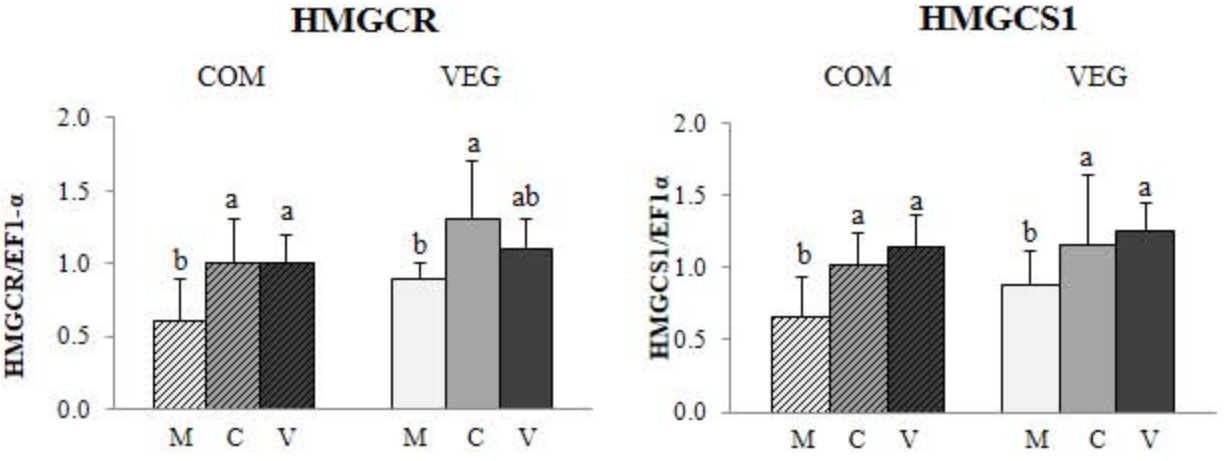


Figure3b. Lipid/cholesterol metabolism. 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS1) mRNA levels were measured using RT-qPCR.



Supplementary Figure 3c. Carbohydrate/energy metabolism. Glucokinase (GCK), hexokinase-2 (HK2) and lactate dehydrogenase-A (LDHA) mRNA levels were measured using RT-qPCR.