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Animal 16 (2022) 100670

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

# Molecular programming of the hepatic lipid metabolism via a parental high carbohydrate and low protein diet in rainbow trout

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ARTICLE INFO

*Article history:*
Received 30 June 2022
Revised 11 October 2022
Accepted 13 October 2022
Available online 20 October 2022

*Keywords:*
Cholesterol Metabolism
Global DNA Methylation
Long-Chain Polyunsaturated Fatty Acids
Progeny

ABSTRACT

It is now recognised that parental diets could alter their offspring metabolism, concept known as nutritional programming. For agronomic purposes, it has been previously proposed that programming could be employed as a strategy to prepare individual for future nutritional challenges. Concerning cultured fish that belong to high trophic level, plant-derived carbohydrates are a possible substitute for the traditional protein-rich fishmeal in broodstock diet, lowering thus the dietary protein-to-carbohydrate ratio (**HC/LP nutrition**). However, in mammals, numerous studies have previously demonstrated that parental HC/LP nutrition negatively affects their offspring in the long term. Therefore, the question of possible adaptation to plant-based diets, *via* parental nutrition, should be explored. First, the maternal HC/LP nutrition induced a global DNA hypomethylation in the liver of their offspring. Interestingly at the gene expression level, the effects brought by the maternal and paternal HC/LP nutrition cumulated in the liver, as indicated by the altered transcriptome. The paternal HC/LP nutrition significantly enhanced cholesterol synthesis at the transcriptomic level. Furthermore, hepatic genes involved in long-chain polyunsaturated fatty acids were significantly increased by the parental HC/LP nutrition, affecting thus both hepatic and muscle fatty acid profiles. Overall, the present study demonstrated that lipid metabolism could be modulated *via* a parental nutrition in rainbow trout, and that such modulations have consequences on their progeny phenotypes.



## Implications

It is now recognised that parental nutrition could highly affect offspring metabolism in the long term. In farmed animals, modulation of parental diets has been proposed as a potential strategy to adapt the metabolism of their progeny and improve their growth. However, the underlying mechanisms are not yet fully understood and the effects of parental nutrition on progeny metabolism could also be unexpected. To question the possible adaptation of progeny metabolism through broodstock nutrition in aquaculture, the present study has investigated the modulation of progeny metabolism induced by a broodstock plant-derived carbohydrate nutrition in teleost fish rainbow trout.

## Introduction

Dietary stimuli exert at a critical developmental stage may lead to long-lasting consequences in the morphology, physiology and metabolism of an individual (Guo et al., 2020), in a gender-specific manner (Tarrade et al., 2015). This concept is known as nutritional programming. It has been previously hypothesised that such early nutritional stimulus could be adaptive, i.e. prepare an individual to face later nutritional challenges that would match the earlier one (Gluckman et al., 2016). Following this hypothesis, programming has been suggested as a potential strategy to modulate metabolic pathways of agronomical interests, and this is particularly true for cultured fish species (Hou and Fuiman, 2020).

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https://doi.org/10.1016/j.animal.2022.100670
1751-7311/

In an effort to promote a sustainable expansion of the aquaculture sector, formulations of aquafeed of cultured species have to evolve rapidly to reduce the use of fishmeal and fish oil (Hua et al., 2019). Aquafeed formulations have therefore been highly diversified over the years and new ingredients, less suitable than the traditional marine-derived ingredients, have to be incorporated (Jobling, 2012). Thus, several studies have tested to incorporate novel ingredients in broodstock diet in order to increase their offspring ability to use them, in sea bream (Izquierdo et al., 2015; Turkmen et al., 2017), and in zebrafish (Adam et al., 2018; Molinari et al., 2020). Nowadays, terrestrial plant ingredients are the most commonly used ingredients to replace fishmeal and fish oil for cultured species that belong to high trophic level such as salmonids (Tacon and Metian, 2008) but the use of complete plant-based diet is not yet feasible without negatively impairing growth performances (Callet et al., 2017).

Nevertheless, the use of programming (*via* broodstock nutrition) as a strategy to increase the proportion of terrestrial plant ingredients in salmonids aquafeed should however be carefully evaluated, and for two main reasons. First, it is not always possible to match the nutritional challenges that fish will face during their lifespan with the early nutritional stimulus received because broodstock and juveniles do not have the same nutritional requirements (Jobling, 2012). Second, mechanisms underlying the effects of nutritional programming are not yet fully understood and stimulus applied during phases with high plasticity could have unexpected effects. In mammals, numerous studies have previously demonstrated that parental nutrition reshapes their offspring epigenetic landscape and alters their metabolism (Guo et al., 2020).

The aim of this study was to investigate the effects of programming *via* broodstock nutrition on the ability of their offspring to grow with plant-based diets. To do so, offspring born from broodstock fed either a control diet formulated with the traditional protein-rich fishmeal (no carbohydrate diet, "**NC**") or a diet formulated with plant-derived carbohydrates, which is thus a high carbohydrate and low protein diet (namely "**HC/LP**" diet) (Callet et al., 2020), were challenged during 3 months with a complete plant-based diet, at the juvenile stage. The effects triggered by the parental nutrition are explored at both the phenotypical (zootechnical parameters) and at the molecular levels (global DNA methylation and transcriptomic approach) with a focus on liver as it is the key organ of intermediary metabolism.

## Material and methods

### *Experimental design*

To assess the effects of programming via parental nutrition in fish, juveniles rainbow trout (*Oncorhynchus mykiss*) produced from broodstock with different nutritional histories were challenged during a 3-month nutritional trial. Briefly, two-year-old female and male rainbow trout were fed either a control diet formulated with fishmeal (0% carbohydrate and 63.89% protein, namely "no carbohydrate diet", "**NC**") or a diet formulated with plant-derived carbohydrates (35.30% carbohydrate and 42.96% protein, namely "high carbohydrate – low protein" diet, "**HC/LP**"). Concerning female broodstock, the feeding trial lasted from the resumption of feeding after their first spawning (December) to the next spawning period in November. Concerning male broodstock, the feeding trial lasted from December to April, as a high mortality induced by a *Saprolegnia* infection in males fed the HC/LP diet occurred in April. From April to the next spawning period (November), all the males were fed the NC diet (Fig. 1 and see Callet et al., (2020)). At spawning, ova produced by female broodstock of similar body mass from the NC and HC/LP condition were fertilised with sperms from the NC and HC/LP males. Thus, four groups of offspring were obtained, including the NN (females NC × males NC), NH (females NC × males HC/LP), HN (females HC/LP × males NC), and HH (females HC/LP × males HC/LP) conditions. Since the first feeding (January), the offspring were fed with the commercial diet for seven months (Callet et al., 2021a). Then, 360 healthy offspring juveniles (initial BW: NN 82.89 ± 4.22 g, NH 92.22 ± 1.56 g, HN 86.22 ± 2.35 g, and HH 85.78 ± 1.60 g) were randomly selected and equally distributed into 12 tanks and fed with a complete plant-based diet for 12 weeks. Tanks were randomly distributed in the experimental facilities and named without any indication of the offspring's condition. This diet was formulated with terrestrial plant products only and was devoid of marine ingredients (Table 1). It was produced in the INRAE facility (Donzacq, France) as extruded pellets (BC45 BisVis Clextral®, France). The feeding trial was conducted from mid-July to mid-October, in a flow-through rearing system supplied with natural spring water at 17 ± 1 °C. Fish were fed three times per day to apparent satiation. The refused feed was removed from each tank, dried and weighed in order to carefully track the quantities of consumed feed for each tank.

### *Sampling*

Six hours after the last feeding, fish were anaesthetised with benzocaine (30 mg/l) and killed in a benzocaine bath at 60 mg/l. Before the beginning of the feeding trial (mid-July), six fish per condition were sampled, weighted, and stored at −20 °C for biochemical composition analysis. At the end of trial (mid-October), nine fish per condition (n = three fish per tank) were randomly sampled for biochemical composition analysis and nine additional fish (n = three fish per tank) were randomly sampled for further analyses. For the latter, fish were lengthwise measured and weighted. The digestive tract along with perivisceral tissues and the liver were removed and weighted in order to estimate the viscerosomatic index (**VSI**) and the hepatosomatic index (**HSI**) as follows: VSI (%) = 100 × viscera weight/Final BW and HSI (%) = 100 × liver weight/Final BW. The blood was removed from the caudal vein *via* heparinised syringes and centrifuged at 3 000*g* for 5 min (n = 9). Plasma was then recovered and stored at −20 °C until further analysis. The liver, the muscle and the digestive tract with the perivisceral tissues (for biochemical composition only) were removed on ice, immediately frozen in liquid nitrogen and stored at −80 °C. Zootechnical parameters were calculated as follows: Weight gain (**WG**,%) = 100 * (Final BW – Initial BW)/Initial BW; Feeding rate (**FR**, %BW/d) = 100 × dry feed intake/(days × (Initial BW + Final BW)/2); Feed efficiency (**FE**, %) = (100 × fresh BW gain) /dry feed intake; Protein retention efficiency (**PRE**, %) = (100 × body retained protein)/protein intake; Lipid retention efficiency (**LRE**, %) = (100 × body retained lipid)/lipid intake.

### *Chemical composition*

Proximate compositions of the feeds, whole body fish, liver, muscle, and digestive tract with perivisceral tissues were analysed as follows (n = 9 per condition): DM was determined by oven drying to constant weight at 105 °C; ash was determined via combustion in a muffle furnace at 600 °C to a constant weight; CP (N × 6.25) was determined by the Kjeldahl method after acid digestion. Crude lipid in the diet and whole body were determined by petroleum ether extraction (Soxthern method), while the total lipid content in the liver and the muscle were determined using dichloromethane/methanol (2:1, v/v), according to Folch et al. (1957). Determination of the hepatic glycogen and glucose content were performed from lyophilised liver. Glycogen content was

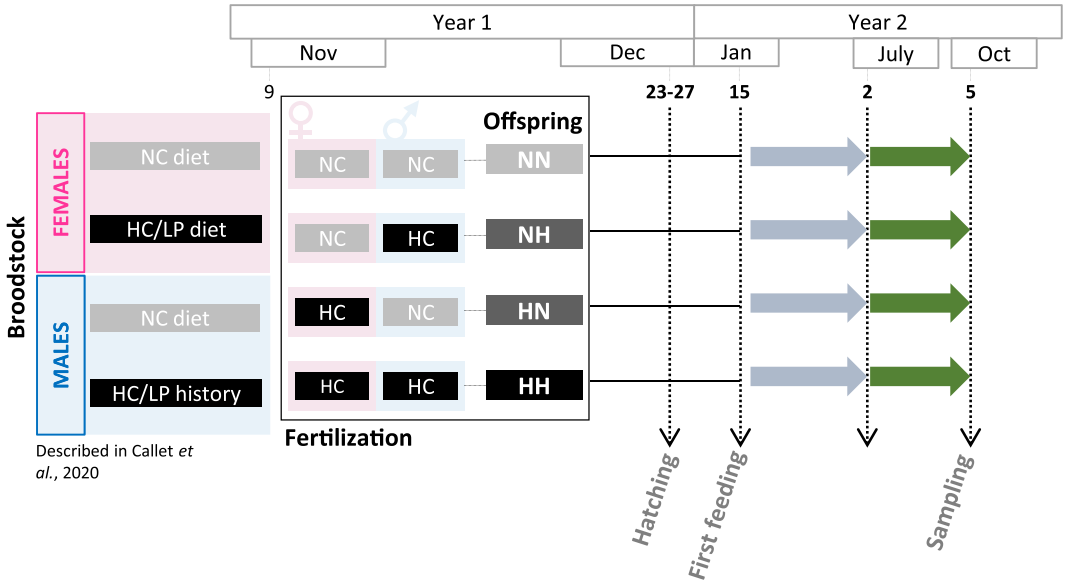


**Fig. 1.** Experimental design. Female and male rainbow trout broodstock were fed with either the NC diet (no carbohydrates) or the HC/LP (high carbohydrates/low protein) diet. Cross-fertilisations were carried out to obtain four groups of offspring: NN, NH, HN and HH. Offspring were then fed with a commercial diet (blue arrows) until July. To assess the effect of the parental HC/LP nutrition, offspring were then challenged during three months with a complete plant-based diet (green arrows). *Abbreviations: HC: high carbohydrate; LP: low protein, NN: offspring born from broodstock fed the control diet (No Carbohydrate); NH: offspring born from broodstock females fed the control diet and broodstock males fed the diet formulated with plant-derived carbohydrates (high carbohydrate and low protein nutrition); HN: offspring born from broodstock females fed the diet formulated with plant-derived carbohydrates ((high carbohydrate and low protein nutrition) and broodstock males fed the control diet; HH: offspring born from broodstock fed the diet formulated with plant-derived carbohydrates (high carbohydrate and low protein nutrition).*

**Table 1**
Formulation and chemical composition of the experimental diet (rainbow trout).

| Components | Values |
|---|---|
| Ingredients (%) | |
| Pregelatinised starch | 17.9 |
| Dicalcium Phosphate | 2.1 |
| Choline chloride 60% | 0.3 |
| Fava bean protein concentrate | 5 |
| Peas protein concentrate | |
| Wheat gluten | 11 |
| Corn gluten | 15 |
| Mix of vegetable oils (rapeseed, linseed, palm) | 14 |
| Soy-lecithine | 1 |
| L-lysine | 0.7 |
| L-methionine | 0.5 |
| Premix, INRAe[1] | 3.8 |
| Roasted guar meal | 4 |
| Soybean meal | 7 |
| Proximate composition | |
| DM (% diet) | 97.59 |
| CP (% DM) | 45.37 |
| Crude lipid (% DM) | 17.71 |
| Gross energy (kJ/g DM) | 24.38 |
| Ash (% DM) | 5.94 |
| Carbohydrates (% DM) | 17.79 |

[1] Mineral premix (g or mg/kg diet): calcium carbonate (40% Ca), 2.15 g; magnesium oxide (60% Mg), 1.24 g; ferric citrate, 0.2 g; potassium iodide (75% I), 0.4 mg; zinc sulphate (36% Zn), 0.4 g; copper sulphate (25% Cu), 0.3 g; manganese sulphate (33% Mn), 0.3 g; dibasic calcium phosphate (20% Ca, 18% P), 5 g; cobalt sulphate, 2 mg; sodium selenite (30% Se), 3 mg; KCl, 0.9 g; NaCl, 0.4 g (INRAe); Vitamin pre mix (IU or mg/kg diet): DL-α tocopherol acetate, 60 IU; sodium menadione bisulphate, 5 mg; retinyl acetate, 15 000 IU; DL-cholecalciferol, 3.000 IU; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; B12, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1 000 mg; biotin, 2.5 mg; calcium pantothenate, 50 mg; choline chloride, 2 000 mg (INRAe).

determined following the hydrolysis method described by Good et al. (1933). Samples were ground in HCl (1 mol/l), and aliquots were made for two separate parts (n = 9 per condition). As previously explained by Lazzarotto et al (2015), for the free glucose measurement, one of the aliquot samples was detected using the Amplite™ Fluorimetric Glucose Quantitation Kit (AAT Bioquest®, Inc., USA) after centrifuged at 10 000*g* for 10 min. For the glycogen measurement, the other part of the ground tissue was boiled at 100 °C for 2.5 h, adjusted to neutralisation by KOH (5 mol/l, VWR, USA), and determined using the same kits as above according to the manufacturer's instructions. Finally, glycogen content was evaluated by subtracting the free glucose content. In order to analyse fatty acid (**FA**) profile in the muscle and in the liver, fatty acid methyl esters (**FAMEs**) were prepared by acid catalysed transmethylation, using boron trifluoride according to Shantha and Ackman (1990). In order to have enough material for the analysis, FAMEs extracted from the liver were pooled (n = three per condition). FAMEs were then analysed in a Varian 3900 gas chromatograph equipped with a fused silica DB Wax capillary column (30 m × 0.25 mm internal diameter, film thickness 0.25 µm; JW Alltech, France). Injection volume was one $\mu$l, using helium as carrier gas (1 ml/min). The temperatures of the injector and the flame ionisation detector were 260 °C and 250 °C, respectively. The thermal gradient was as follows: 100–180 °C at 8 °C/min, 180–220 °C at 4 °C/min and a constant temperature of 220 °C for 20 min. Fatty acids were identified with reference to a known standard mixture (Sigma, St. Louis, MO, USA), and peaks were integrated using Varian Star Chromatography Software (Star Software, version 5). The results for individual FA were expressed as percentage of total identified FA methyl esters (Lazzarotto et al., 2015).

### *Plasma metabolites*

Plasma glucose, free fatty acids (**FFAs**), cholesterol and triglycerides concentrations were analysed using different kits (Glucose RTU, Fujifilm Wako, Sobioda, CHOD-PAP, Sobioda and PAP 150 bioMerieux, Marcy l'Etoile, France). The assays were carried out according to the manufacturer's instructions.

*RNA extraction, microarrays, cDNA labelling, hybridisation and quantitative PCR*

Total RNA was extracted from the frozen liver using Trizol® reagent (Invitrogen, Carlsbad, CA, United States) according to the manufacturer's instructions. Microarray analyses were performed on an Agilent-based microarray platform Rainbow Trout specific with 8 X 60 K probes per slide. For each condition, six RNA samples were selected thanks to their RNA integrity number. 150 ng of total RNA was first amplified, then labelled with Cy3-dye and finally hybridised on a sub-array, as described in Callet et al. (2021a). Slides were then washed and scanned (Agilent DNA Microarray Scanner, Agilent Technologies, Massy, France) using the standard parameters for a gene expression 8 × 60 K oligoarray (3 µm and 20 bits). Data were then obtained with the Agilent Feature Extraction software (10.7.1.1) and are available in the GEO database (ID: GSE184632). In order to further analyse gene mRNA level, 1 µg of the total RNA was reverse-transcribed into cDNA in duplicate using the SuperScript III RNase H-Reverse Transcriptase kit (Invitrogen, Carlsbad, CA, United States) with random primers (Promega, Charbonnieres-les-Bains, France). All of the quantitative real-time PCR (**qPCR**) assays were carried out in the Roche Lightcycler 480 system (Roche Diagnostics, Neuilly-sur-Seine, France). Two $\mu$l of diluted cDNA (2 µl cDNA diluted in 150 µl of water) was mixed with 3 µl of Light cycler 480 SYBR R Green I Master mix and diluted to obtain a final volume of 6 µl. Forward and reverse primers were used at a final concentration of 400 nM (Supplementary Table S1). Thermal cycling was initiated with an incubation at 95 °C for 10 min. Forty-five steps of PCR were performed, each one consisting of heating at 95 °C for 15 s for denaturing, and at 60 °C for 10 s for annealing and a third extension step at 72 °C for 15 s. Melting curves were systematically monitored (with a gradient of 0.5 °C/10 s from 55 °C to 94 °C) to confirm the specificity of the amplification reaction. Each PCR assay was run with replicate (duplicate of reverse transcription and PCR amplification), and negative controls were included. qPCR efficiency was measured by the slope of a standard curve using serial dilutions of cDNA (five dilutions of a pool of all conditions from D20 to D380, in triplicates). The relative expressions of genes assessed by qPCR were calculated by a mathematical method based on the real-time qPCR efficiencies, using a geometric mean from three reference genes (*actb*, *eef1a*, and *18s*) for the normalisation (Callet et al., 2021b).

*DNA extraction and global DNA methylation analysis*

DNA extraction was performed on frozen liver tissues. The samples were digested for 1 h at 57 °C in 800 µl of guanidine thiocyanate buffer (pH = 8; guanidine thiocyanate 1 M, EDTA 0.5 M, Tris Base 100 mM, 5% Tween 20, and 0.5% Triton X-100) supplemented with 10 µl of proteinase K (20 mg/ml; P6556, Sigma-Aldrich) and antioxidant mixture (8 µl of desferoxamine, 32 µl of histidine, and 8 µl of reduced glutathione). 800 µl of chloroform-alcohol isoamyl (24/1, v/v) and antioxidant mixture were added to each sample, and samples were placed on a rotating wheel (for 15 min at room temperature) and then centrifuged at 1 000*g* (for 15 min at room temperature). The upper phase was recovered, and 73.3 µl of NaCl (5 M) and 866 µl of 100% ice-cold ethanol were added. Samples were mixed by inversion and kept at −20 °C for at least 15 min and centrifuged at 10 000*g* (at 4 °C for 15 min). The pellets were washed in 1 ml of 70% ice-cold ethanol and centrifuged at 10 000*g* (at 4 °C for 15 min). Finally, the pellets were dried (45 °C for 10 min) and suspended in DNase-free water. The concentration and integrity of total DNA were measured by the NanoDrop (Thermo Fisher, USA) and agarose gel, respectively.

DNA samples were treated with RNase Cocktail (Invitrogen, Carlsbad, CA, United States) to remove RNA according to the manufacturer's procedure. Then, 1 µg of DNA was degraded into nucleosides with DNA Degradase Plus (Zymo Research, USA) according to the manufacturer's procedure. HPLC-UV analyses were conducted using a Waters® Alliance System (2695 separation module) coupled with a Waters 2487 dual $\lambda$ Absorbance Detector (Milford, MA, USA) based on the protocol of Kovatsi et al. (2012) with modifications described herein. Chromatographic separation was performed on a Luna C8 (5 µm, 150 * 4.6 mm) (Phenomenex, Torrance, CA, USA). The mobile phase was composed as follows: solvent A: 10 mM potassium phosphate buffer, pH = 3.7 ± 0.02, solvent B: 100% methanol. Linear gradient elution was employed as follows: 0–8.5 min, 98% A; 8.5–11.8 min, 97% A; 11.8–18.9 min, 73% A; 18.9–21.2 min, 65% A; 21.2–30 min, 98% A. The temperature of the column oven was 30 °C. The wavelength of UV detection was 277 nm. The standards of 2′-Deoxycytidine (**dC**), 5-Methyl-2′-deoxycytidine (**5-mdC**), 5-Hydroxymethyl-2′-deoxycytidine (**5-hmdC**), 5-Formyl- 2′-deoxycytidine (**5-fdC**) and 5-Carboethoxy-2′ deoxycytidine (**5-cadC**) were made using products from Berry and associates Inc (USA) (Liu et al., 2022). The *in vivo* global levels of 5-mC, 5-hmC, 5-fC, and 5-caC were calculated as the percentage of each individual's molar quantity divided by the total molar quantity of all the detected cytosine forms.

*Sex determination*

Extracted DNA was used to run PCR in order to amplify the master sex-determining gene, *sdY* gene (Yano et al., 2012). One $\mu$l of DNA was mixed with 1.25 µl of each primer at 10 µM (forward: CCCAGCACTGTTTTCTTGTCTCA; reverse: CTGTTGAAGAGCATCACAGGGTC), 1 µl dNTP mixture (Ozyme, 4 × 10 mM), and 5 l of 5× PCR Buffer (Promega) with 0.125 µl of Taq DNA Polymerase (Promega, 5 U/µl) in a total volume of 25 µl. Thermal cycling consisted of denaturation for 20 s at 94 °C followed by 35 cycles of 94 °C for 20 s, 59 °C for 20 s, and 72 °C for 20 s, with a final extension of 5 min at 72 °C. PCR products were electrophoresed on a 2% agarose gel in a 1.5× TAE buffer, stained with SYBRSafe, to reveal the presence or absence of *sdY*.

*Statistical analyses*

All of the statistical analyses were performed with the R software (v3.1.0). Results were expressed as the mean of all replicates ± the SE of the mean (**SEM**). Final body mass and length (n = 9) were analysed with linear model, using the "lme4" packages from the R software, with a significance threshold set at *P*-value = 0.05. Because sexual maturation had begun at the time of the sampling (gonado-somatic index and sex of the sampled fish are presented in Supplementary Table S2) and that the existence of potential effect of sexual dimorphism on metabolism has been previously described in fish (Robison et al., 2008), the effect of the sex was first tested. The maternal HC/LP nutrition, the paternal HC/LP nutrition and the interaction between those two last variables were then tested, with the tank treated as a random effect. The interaction between the sex of the fish and parental nutrition could not be tested as only females were sampled in the HH condition (see Supplementary Table S2). The best model was then chosen thanks to the Akaike Information Criterion (AIC). The distribution of residual errors were examined to check for the normality. In case of significant interaction between the maternal and the paternal nutrition, a Tukey posthoc comparison was done subsequently to decipher which condition significantly differ from the others. Tissue biochemical compositions (n = 9), muscle FA profiles (n = 9), hepatic global DNA methylation (n = 9) and hepatic gene mRNA levels (n = 6) were analysed with linear models, similarly as described for the final body mass and the length. An arcsine transformation was performed before statistical analyses on pro-

portions and relative mRNA levels were first transformed with a logarithmic transformation. Concerning feed intakes (n = 3 tanks/-condition), feed efficiencies (n = 3), nutrient retention (n = 3), whole body composition (n = 6) and hepatic FA profiles (n = 3), Wilcoxon tests were performed to estimate the effects of the paternal nutrition than the effects of the maternal nutrition. To detect the combined effect of paternal and maternal nutrition (namely parental effect), a Kruskal–Wallis non-parametric test was conducted on all these variables. A Tukey's posthoc analysis was followed in case of significance. Data from the microarray analysis were transformed with a logarithmic transformation, scale normalised and analysed using the package Limma (Ritchie et al., 2015). In order to find the differentially expressed genes resulting from the maternal, paternal, and both maternal and paternal nutritional history (namely parental effect), transcriptomes of HN, NH, and HH liver were successively compared with the transcriptomes of the control NN fish. For these three comparisons, Limma t-tests were performed, with a correction for multiple tests (cut-off *P*-value = 0.05 after a Benjamini-Hochberg correction), taking the sex of the fish into account.

## Results

### *Zootechnical performances*

At the end of the trial, fish weighted 342.6 ± 59.8 g and measured 274.3 ± 15.2 mm. Male offspring were significantly heavier and longer than female ones (+15.2% and + 4.2%, respectively), but no significant differences were observed among the four conditions (Table 2). In addition, no significant differences were observed in the weight gain (WG) whereas the feeding rate (FR) was significantly increased in the offspring of sires fed the HC/LP diet (+7.72%). In contrast, no significant differences in feed efficiency (FE) were detected in the offspring (Table 2). While no significant differences were detected on protein retention efficiency (PRE), the lipid retention efficiency (LRE) was slightly increased in the offspring of dams fed the HC/LP diet (*P*-value = 0.055).

### *Biochemical composition of tissues*

#### *Whole body*

No significant differences on whole body protein and ash contents were detected in the offspring after the challenge (Table 2). However, the whole lipid content was significantly higher in the offspring of sires fed the HC/LP diet (*P*-value = 0.006).

#### *Plasma*

Six hours after the last feeding, no significant differences in glucose, FFA and cholesterol plasmatic levels were detected among the offspring. Nevertheless, the triglycerides plasmatic levels tended to be increased in the offspring of dams fed the HC/LP diet (Table 3).

#### *Liver*

No significant differences were found in the biochemical composition of liver (protein, lipid, free glucose, glycogen) among the four conditions (*P*-value > 0.05). The hepatic lipid contents were only slightly decreased in male fish in comparison to the female ones, regardless of their parental nutritional history (Table 3). The hepatic fatty acid profiles of the offspring are presented in Supplementary Table S3. Interestingly, the levels of 16:0, 20:3n-6 and 22:5n-3 were significantly increased in the offspring of dams fed the HC/LP diet. More particularly, HH fish had a significantly higher level of hepatic 20:3n-6 than NN ones. While the levels of 16:1 and 18:1 were significantly decreased in the offspring of sires fed the HC/LP diet, the levels of 20:0, 20:1, 20:2n-6, 20:3n-3 and 20:4n-3 were significantly increased (Supplementary Table S3 and Fig. 2(a)).

**Table 2**
Effects of the parental HC/LP nutrition on zootechnical performances and biochemical composition, in rainbow trout.

| Item | NN | NH | HN | HH | *P*-values | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | sex | maternal | paternal | parental |
| Body mass (g) (n = 9) | 322.44 ± 60.41 | 357.67 ± 53.39 | 378.33 ± 58.99 | 316.11 ± 52.46 | 0.03 | – | – | – |
| Length (mm) (n = 9) | 270.22 ± 14.22 | 280.22 ± 19.22 | 279.78 ± 11.58 | 266.89 ± 12.14 | 0.04 | – | – | – |
| | NN | NH | HN | HH | *P*-values | | | |
| | | | | | maternal | paternal | parental | |
| Zootechnical performances (n = 3) | | | | | | | | |
| Feeding rate[1] (% BW/d) | 1.29 ± 0.05$^a$ | 1.42 ± 0.05$^b$ | 1.34 ± 0.01$^a$ | 1.43 ± 0.01$^b$ | 0.69 | 0.02 | 0.13 | |
| Feed efficiency[2] (%) | 84.08 ± 6.92 | 80.51 ± 6.16 | 95.54 ± 2.91 | 86.19 ± 1.07 | 0.15 | 0.15 | 0.22 | |
| Weight gain[3] (%) | 242.75 ± 9.88 | 251.57 ± 27.64 | 271.07 ± 14.00 | 254.20 ± 7.91 | 0.34 | 1.00 | 0.67 | |
| Nutrient retention (n = 3) | | | | | | | | |
| Protein retention efficiency[4] (%) | 32.13 ± 2.04 | 30.97 ± 2.41 | 36.42 ± 1.04 | 33.29 ± 0.85 | 0.20 | 0.20 | 0.32 | |
| Lipid retention efficiency[5] (%) | 61.91 ± 7.60 | 66.35 ± 3.31 | 72.84 ± 2.02 | 73.39 ± 1.68 | 0.06 | 1.00 | 0.29 | |
| Whole body composition (n = 6) | | | | | | | | |
| Protein (%DM) | 16.93 ± 0.18 | 16.99 ± 0.16 | 16.94 ± 0.16 | 17.11 ± 0.22 | 1.00 | 0.42 | 0.89 | |
| Lipid (%DM) | 13.33 ± 0.48$^a$ | 14.59 ± 0.54$^b$ | 13.20 ± 0.32$^a$ | 14.54 ± 0.20$^b$ | 1.00 | 0.01 | 0.06 | |
| Moisture (%) | 67.78 ± 0.42$^b$ | 66.32 ± 0.37$^a$ | 67.86 ± 0.49$^b$ | 66.30 ± 0.17$^a$ | 0.87 | 0.00 | 0.04 | |
| Ash (%DM) | 2.03 ± 0.08 | 2.01 ± 0.06 | 2.01 ± 0.11 | 2.01 ± 0.09 | 1.00 | 1.00 | 1.00 | |

Data are presented as means ± SEM. Final body mass and length were analysed by a linear model. The other parameters were analysed with Wilcoxon tests to test the paternal and the maternal nutrition effects. A Kruskal–Wallis non-parametric test, followed by a posthoc Tukey test in the case of a significant interaction, was performed to test the parental nutrition. Means with different lowercase letters in the same line are considered significantly different (*P*-values < 0.05).
Abbreviations: HC: high carbohydrate; LP: low protein, NN: offspring born from broodstock fed the control diet (No Carbohydrate); NH: offspring born from broodstock females fed the control diet and broodstock males fed the diet formulated with plant-derived carbohydrates (high carbohydrate and low protein nutrition); HN: offspring born from broodstock females fed the diet formulated with plant-derived carbohydrates ((high carbohydrate and low protein nutrition) and broodstock males fed the control diet; HH: offspring born from broodstock fed the diet formulated with plant-derived carbohydrates (high carbohydrate and low protein nutrition); IBW: initial BW; FBW: final BW.
[1] Feeding rate (FR, %BW/d) = 100 × dry feed intake/(days × (IBW + FBW)/2);
[2] Feed efficiency (FE, %) = (100 × fresh BW gain) /dry feed intake;
[3] Weight gain (WG, %) = 100 × (FBW - IBW)/IBW;
[4] Protein retention efficiency (PRE, %) = (100 × body retained protein)/protein intake;
[5] Lipid retention efficiency (LRE, %) = (100 × body retained lipid)/lipid intake.

**Table 3**
Effects of the parental HC/LP nutrition on tissue biochemical composition, in rainbow trout.

| Item | NN | NH | HN | HH | *P*-values sex | maternal | paternal | parental |
|---|---|---|---|---|---|---|---|---|
| Plasmatic parameters | | | | | | | | |
| Glucose (g/l) | 1.13 ± 0.3 | 1.11 ± 0.12 | 1.00 ± 0.19 | 1.00 ± 0.17 | - | - | - | - |
| FFA (mmol/l) | 0.25 ± 0.06 | 0.26 ± 0.04 | 0.26 ± 0.04 | 0.26 ± 0.05 | - | - | - | - |
| Cholesterol (g/l) | 2.27 ± 0.67 | 2.21 ± 0.76 | 2.58 ± 0.42 | 2.23 ± 0.3 | - | - | - | - |
| Triglycerides (g/l) | 3.78 ± 1.21 | 2.72 ± 1.29 | 4.99 ± 2.32 | 4.22 ± 2.14 | 0.37 | 0.10 | - | - |
| Hepatic tissue | | | | | | | | |
| HSI (%) | 1.00 ± 0.15 | 0.91 ± 0.11 | 0.94 ± 0.13 | 0.95 ± 0.1 | - | - | - | - |
| Protein (% DM) | 13.00 ± 1.2 | 13.12 ± 0.71 | 13.81 ± 2.85 | 13.6 ± 0.69 | - | - | - | - |
| Lipid (% DM) | 4.08 ± 0.3 | 4.48 ± 0.57 | 4.03 ± 0.41 | 4.29 ± 0.39 | 0.05 | | - | - |
| Free glucose (mg/g) | 33.09 ± 6.23 | 32.07 ± 6.5 | 30.12 ± 6.39 | 28.87 ± 3.84 | - | - | - | - |
| Glycogen (mg/g) | 282.76 ± 111.19 | 250.42 ± 107.76 | 312.6 ± 75.1 | 326.63 ± 80.24 | - | - | - | - |
| Muscle tissue | | | | | | | | |
| Protein (% DM) | 20.39 ± 0.54 | 20.62 ± 0.57 | 20.55 ± 0.48 | 20.25 ± 0.46 | - | - | - | - |
| Lipid (% DM) | 6.14 ± 1.03 | 5.32 ± 1.2 | 6.37 ± 1.17 | 5.03 ± 0.65 | 0.09 | 0.88 | 0.06 | - |
| Digestive Tract | | | | | | | | |
| VSI | 11.58 ± 0.52 | 10.63 ± 0.57 | 12.86 ± 0.68 | 10.37 ± 0.34 | 0.02 | - | - | - |
| Protein (% DM) | 6.93 ± 1.68 | 6.36 ± 1.09 | 7.47 ± 1.75 | 7.27 ± 1.02 | - | - | - | - |
| Lipid (% DM) | 48.28 ± 7.47$^{a}$ | 55.68 ± 4.19$^{b}$ | 52.23 ± 6.06$^{ab}$ | 51.74 ± 5.81$^{ab}$ | 0.06 | 0.90 | 0.27 | 0.02 |

Data are presented as means ± SEM and analysed by a linear model, followed by a posthoc Tukey test in the case of a significant interaction. Means with different lowercase letters in the same line are considered significantly different (*P*-values < 0.05).
Abbreviations: HC: high carbohydrate; LP: low protein, NN: offspring born from broodstock fed the control diet (No Carbohydrate); NH: offspring born from broodstock females fed the control diet and broodstock males fed the diet formulated with plant-derived carbohydrates (high carbohydrate and low protein nutrition); HN: offspring born from broodstock females fed the diet formulated with plant-derived carbohydrates ((high carbohydrate and low protein nutrition) and broodstock males fed the control diet; HH: offspring born from broodstock fed the diet formulated with plant-derived carbohydrates (high carbohydrate and low protein nutrition); FFA: free fatty acid; HSI: hepatosomatic index; VSI: viscerosomatic index.

*Muscle*

Whereas, no significant differences were found in the muscle protein content among the four conditions (*P*-value > 0.05), the muscle lipid content was slightly decreased in the offspring of sires fed the HC/LP diet (Table 3). The muscle fatty acid profiles of the offspring are presented in Supplementary Table S4. First, the fatty acid profiles of male fish significantly differ from the female ones. Of interest, they had a significantly lower 20:4n-6, 20:4n-3 and 22:6n-3 levels than female ones but they had a higher 18:2n-6, 22:2n-6 and 18:3n-3 levels. The levels of 20:0, 20:1, 20:2n-6, 22:2n-6 and 22:5n-6 were significantly lower in the offspring of dams fed the HC/LP diet. In contrary, the levels of 18:3n-6, 20:3n-6, 16:4n-3, 18:4n-3 and 20:4n-3 were significantly increased in those same fish. Finally, the proportions of 20:3n-3 and 20:4n-3 were significantly increased in the offspring of sires fed the HC/LP diet.

*Perivisceral tissues*

Male fish had a significantly lower VSI in comparison to female ones, regardless of their parental nutrition history (Table 3). No significant differences were detected in the protein content among the four conditions. NH fish had a significantly higher lipid content in the digestive tract than NN ones (Table 3).

*Hepatic global DNA methylation*

The hepatic abundance of cytosine was significantly higher in the offspring of dams fed the HC/LP diet. On the opposite, the hepatic abundance of 5-mdC was significantly decreased in the offspring of dams fed the HC/LP diet. The hepatic 5-hmdC levels were significantly higher in the NN fish in comparison to the other. Finally, neither 5-fdC nor 5-cadC was detected in the liver (Fig. 3).

*Results of transcriptomic analyses*

No probes were found differentially expressed between HN and NN. Only 3 probes were found differentially expressed between NH and NN. Finally, 294 probes were found differentially expressed between HH and NN fish in liver (Supplementary Table S5). Moreover, the differentially expressed genes were highly related to lipid and cholesterol metabolism. Thus, qPCR analysis was further conducted to confirm molecular events involved in lipid metabolism in the liver (Table 4).

*mRNA levels of genes involved in cholesterol metabolism*

The mRNA levels of *hmgcra*, *cyp51a*, *cyp51b*, *dhcr7b* and *abca1a* were on average increased in female offspring. The mRNA levels of *srebp2a*, *hmgcs1*, *mvdaa*, *mvdab*, *dhcr7* and *cyp51a1* were significantly increased in the offspring of sires fed the HC/LP diet (*P*-value > 0.05 and *P*-value = 0.08 for *cyp51a1*). The offspring of dams fed the HC/LP diet displayed a higher *mvdaa* mRNA levels and a lower *idi1* mRNA level than offspring of dams fed the NC diet. Finally, HN fish had a significantly lower *dhcr7* mRNA level than the others. Concerning the cholesterol elimination and transportation, HH fish had a significantly higher *lxrαb* mRNA level than HN fish. No significant differences were detected in the transcriptional expression of the other gene studied; expect for *abcg8* and *osbpl3ab*. The *abcg8* mRNA level was significantly increased in the offspring of the dams fed the HC/LP diet. The *osbpl3ab* mRNA level was significantly increased in the offspring of the sires fed the HC/LP diet (Table 4).

*mRNA levels of genes involved in lipid metabolism*

First, the mRNA levels of *srebp1c*, *acly* (global), *fasnb* and *g6pd* (global) were on average significantly increased in female offspring (Table 4). mRNA levels of both *acsf2a* and *acsf2d* were significantly decreased in the offspring of dams fed the HC/LP diet (Table 4). With respect to the hepatic lipogenesis, no significant differences were observed on the mRNA levels of genes involved in this pathway (*srebp*, *acly*, *aca* and *fasn*); except for *acaαb*. Offspring of sires fed the HC/LP diet displayed a lower *acaαb* mRNA level. Offspring of dams fed the HC/LP diet displayed a higher *g6pdb* mRNA level (Table 4). Regarding the fatty acid $\beta$-oxidation process (Table 4), *hadh*, *cpt1a*, *acox1* (global) mRNA levels were on average increased in female offspring. Moreover, mRNA level of *cpt1a* tended to decrease in the offspring of the sires fed the HC/LP diet. No signif-

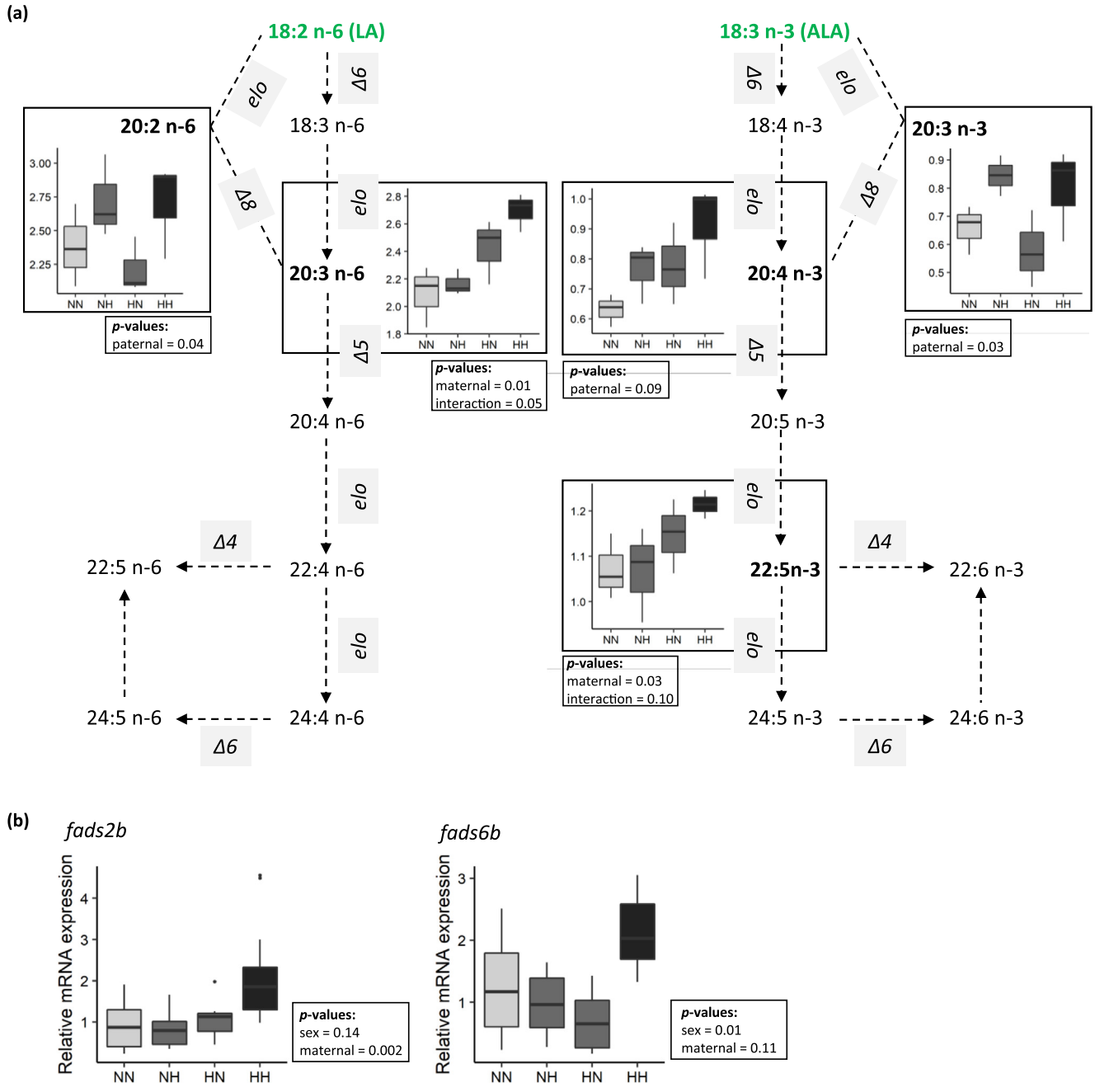


**Fig. 2.** Effect of the parental HC/LP nutrition on metabolism of polyunsaturated fatty acid biosynthesis, in rainbow trout. Biosynthetic pathways of long-chain polyunsaturated fatty acids (according to Monroig et al., 2011), along with (a) the hepatic proportion of fatty acids affected by the maternal HC/LP nutrition (20:3n-6 and 22:5n-3) or by the paternal HC/LP nutrition (20:2n-6, 20:3n-3 and 20:4n-3), (b) and the RNA levels of *fads2b* and *fads6b* of the offspring from the four conditions (NN, NH, HN and HH) after the 3-month complete plant-based diet challenge. The latter are coding for enzymes having a Delta5 and Delta6 desaturase activity.Abbreviations: HC: high carbohydrate; LP: low protein, NN: offspring born from broodstock fed the control diet (No Carbohydrate); NH: offspring born from broodstock females fed the control diet and broodstock males fed the diet formulated with plant-derived carbohydrates (high carbohydrate and low protein nutrition); HN: offspring born from broodstock females fed the diet formulated with plant-derived carbohydrates ((high carbohydrate and low protein nutrition) and broodstock males fed the control diet; HH: offspring born from broodstock fed the diet formulated with plant-derived carbohydrates (high carbohydrate and low protein nutrition).

icant differences were observed on the mRNA levels of the other genes involved in this fatty oxidation-related pathway (*hadh*, *acox*).

*mRNA levels of genes involved in long-chain polyunsaturated fatty acid biogenesis*

Regarding the polyunsaturated fatty acid biogenesis process in the liver, *scdbc*, *fads6b*, *fads2a*, *fads2c* and *elovl5a* mRNA levels were on average increased in female offspring (Fig. 2(B)). *fads2b* mRNA level sharply increased in the offspring of dams fed the HC/LP diet and *fads2c* mRNA level decreased in the offspring of sires fed the HC/LP diet. Interestingly, the HH fish showed markedly a higher level of *fads6b* than in the NN condition. No significant differences were found in the mRNA levels of genes coding for elongases (Table 4).

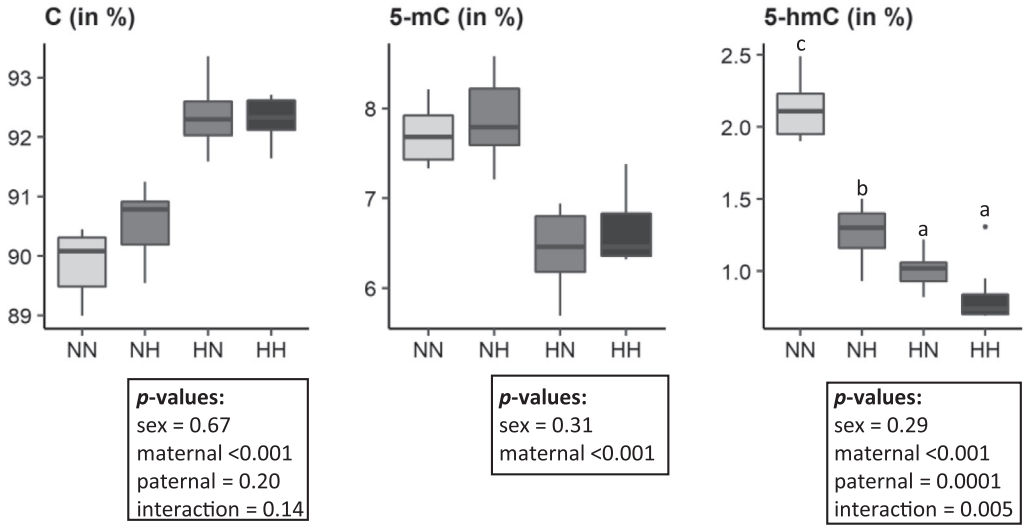


**Fig. 3.** Effect of the parental HC/LP nutrition on their offspring hepatic epigenetic landscape, in rainbow trout. Hepatic proportion of cytosine (C) and two intermediates of DNA methylation (5-mC and 5-hmC) in offspring from the four conditions (NN, NH, HN and HH) after the 3-month complete plant-based diet challenge. Abbreviations: HC: high carbohydrate; LP: low protein, NN: offspring born from broodstock fed the control diet (No Carbohydrate); NH: offspring born from broodstock females fed the control diet and broodstock males fed the diet formulated with plant-derived carbohydrates (high carbohydrate and low protein nutrition); HN: offspring born from broodstock females fed the diet formulated with plant-derived carbohydrates ((high carbohydrate and low protein nutrition) and broodstock males fed the control diet; HH: offspring born from broodstock fed the diet formulated with plant-derived carbohydrates (high carbohydrate and low protein nutrition).

## Discussion

In farmed animals, nutritional programming has been proposed as a potential strategy to modulate individual's metabolism with the aim of adapting them to face nutritional challenges (Hou et al., 2020). In aquafeed for higher trophic level teleost fish, fishmeal and fish oil need to be further replaced by plant terrestrial ingredients (Tacon and Metian, 2008). Concerning broodstock aquafeed, plant-derived carbohydrate is a possible substitute for the traditional protein-rich fishmeal (Washburn et al., 1990; Callet et al., 2020), triggering thus the diminution of the dietary protein-to-carbohydrate ratio (parental HC/LP diet). However, in mammals, numerous studies have previously demonstrated that HC/LP parental nutrition affect various metabolic pathways of their offspring in the long term, including the lipid metabolism (Lucas et al., 1993; Guo et al., 2020). The question of a possible adaptation, that is the possibility of improving the ability of the offspring to grow on plant-based diets, remains open and needs to be explored.

### *A global hepatic hypomethylation triggered by the maternal high carbohydrate and low protein nutrition*

The maternal HC/LP nutrition modulated the global DNA methylation landscape in the liver of their offspring challenged with a complete plant-based diet, as a diminution in both 5-methylcytosine (5-mC, *i.e.* hypomethylation) and 5-hydroxymethylcytosine (5-hmC) proportions was detected. Although the mechanisms underlying the transmission of DNA methylation modifications from parent to offspring are still to be discovered (Jiang et al., 2013; Wang and Bhandari, 2020), these results confirm the importance of addressing DNA methylation in studies focused on programming events. In addition, if affected genomic regions cannot be determined by global analysis of DNA methylation, changes identified in this parameter give an idea of the global genomic stability. Indeed, a decrease in global methylation levels is in favour of an opening of the chromatin which can in turn favour the expression of up-to-now repressed genes but also in certain cases lead to chromatin breakdown that can be deleterious to cells and more generally to the organism.

Interestingly, before the first feeding, the maternal HC-LP nutrition have also induced a decrease of 5-mC proportion in their progeny (whole fry) (Callet et al., 2021a). Therefore, the DNA methylation modifications persist over time (at least in the liver) and this, whatever the diet given, as the maternal HC/LP nutrition also triggered a decrease of both 5-mC and 5-hmC proportions in the liver of unchallenged offspring (personal and unpublished data). Such persistence of epigenetic modifications could be surprising as DNA methylation is a dynamic process, sensitive to environmental cues, but it has been previously demonstrated that some DNA methylation modifications that occurred during development have been retained over time in mammals (Murgatroyd et al., 2009; Cardenas et al., 2017). Finally, it is also recognised that those DNA methylation modifications, at both CpG and non-CpG sites, could alter gene expression (Fuso and Lucarelli, 2019). While 5-mC is indeed long recognised as a modification affecting chromatin stability and thereby gene expression, it is only more recently that studies in mammals have demonstrated that 5-hmC plays an active role in regulating gene expression in liver in mammals (Lin et al., 2017). In the present study, the modification of the global methylation landscape was indeed combined with the alteration of hepatic transcriptomes in the offspring. However, other epigenetic marks may have been affected by the parental nutrition and be at play, as offspring whose sires and dams were fed the HC/LP diet were the most affected (Supplementary Table S5), whereas DNA hypomethylation was induced by the maternal nutrition history regardless of the sire diet.

### *A parental high carbohydrate and low protein nutrition altered hepatic lipid metabolism in their offspring*

Among pathways affected in the offspring fed a complete plant-based diet *via* a parental HC/LP nutrition in trout, the lipid metabolism stands out, as indicated by both the molecular data (hepatic gene expression reported by microarray and qPCR data) and the phenotypes induced (whole body lipid content, muscle and hepatic FA profiles). Interestingly, the parental nutrition caused changes similar to those triggered by a direct complete plant-based feeding (Panserat et al., 2019), as detailed below.

**Table 4**
Effects of the parental HC/LP nutrition on mRNA levels of genes involved in lipid metabolism, in rainbow trout.

| | NN | | NH | | HN | | HH | | *P*-values | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | | | | | | | | | sex | maternal | paternal | parental |
| Cholesterol biosynthesis | | | | | | | | | | | | |
| *srebp2a* | 1.03 | ± 0.55 | 1.22 | ± 0.26 | 0.78 | ± 0.28 | 1.29 | ± 0.26 | 0.07 | 0.94 | 0.06 | – |
| *srebp2b* | 1.00 | ± 0.42 | 1.04 | ± 0.19 | 0.89 | ± 0.30 | 1.28 | ± 0.19 | 0.10 | – | – | – |
| *hmgcs1* | 0.82 | ± 0.70 | 1.79 | ± 0.88 | 0.72 | ± 0.31 | 1.91 | ± 0.93 | 0.12 | 0.54 | 0.02 | – |
| *Hmgcra* | 1.10 | ± 0.91 | 1.38 | ± 0.52 | 0.87 | ± 0.69 | 1.52 | ± 0.69 | 0.03 | – | – | – |
| *Hmgcrb* | 1.50 | ± 0.62 | 0.96 | ± 0.4 | 1.00 | ± 0.54 | 1.13 | ± 0.77 | – | – | – | – |
| *Mvdaa* | 0.68 | ± 0.59 | 1.25 | ± 0.4 | 0.87 | ± 0.49 | 2.47 | ± 1.27 | 0.25 | 0.03 | 0.00 | – |
| *Mvdab* | 0.70 | ± 0.36 | 2.25 | ± 2.06 | 0.58 | ± 0.31 | 1.62 | ± 0.80 | 0.20 | 0.82 | 0.01 | – |
| *idi1* | 1.50 | ± 0.91 | 1.94 | ± 1.07 | 0.94 | ± 0.81 | 0.81 | ± 0.53 | 0.09 | 0.05 | – | – |
| *cyp51a* | 0.83 | ± 0.77 | 1.78 | ± 0.87 | 0.74 | ± 0.55 | 2.28 | ± 1.35 | 0.00 | 0.43 | 0.08 | – |
| *cyp51b* | 1.13 | ± 0.67 | 1.17 | ± 0.54 | 0.97 | ± 0.68 | 1.19 | ± 0.28 | 0.02 | – | – | – |
| *dhcr7a* | 1.10 | ± 0.30b | 1.24 | ± 0.31b | 0.82 | ± 0.39a | 1.19 | ± 0.32b | 0.48 | 0.07 | 0.01 | 0.02 |
| *dhcr7b* | 1.20 | ± 0.43 | 1.17 | ± 0.51 | 0.92 | ± 0.73 | 1.21 | ± 0.40 | 0.00 | – | – | – |
| Cholesterol elimination | | | | | | | | | | | | |
| *Lxrαa* | 1.52 | ± 0.75 | 0.64 | ± 0.45 | 1.62 | ± 1.15 | 1.45 | ± 0.72 | 0.64 | 0.14 | 0.17 | – |
| *Lxrαb* | 1.16 | ± 0.31a | 0.97 | ± 0.25a | 0.86 | ± 0.20a | 1.15 | ± 0.17a | 0.31 | 0.98 | 0.78 | 0.03 |
| *abcg5* | 1.10 | ± 0.42 | 0.82 | ± 0.56 | 1.10 | ± 0.32 | 1.38 | ± 0.46 | – | – | – | – |
| *abcg8* | 1.00 | ± 0.41 | 1.00 | ± 0.48 | 1.16 | ± 0.48 | 1.21 | ± 0.31 | 0.07 | 0.05 | 0.15 | – |
| *abca1a* | 1.10 | ± 0.65 | 1.64 | ± 1.11 | 0.91 | ± 0.55 | 1.48 | ± 0.76 | 0.00 | – | – | – |
| *abca1b* | 1.09 | ± 0.61 | 1.39 | ± 0.71 | 0.98 | ± 0.29 | 1.02 | ± 0.51 | – | – | – | – |
| Cholesterol esterification | | | | | | | | | | | | |
| *acat1* | 1.09 | ± 0.44 | 1.00 | ± 0.29 | 1.02 | ± 0.18 | 1.05 | ± 0.28 | – | – | – | – |
| *acat2* | 0.94 | ± 0.29 | 1.04 | ± 0.26 | 1.06 | ± 0.27 | 1.10 | ± 0.13 | – | – | – | – |
| Lipid metabolism | | | | | | | | | | | | |
| *acsf2a* | 1.34 | ± 0.24 | 1.19 | ± 0.67 | 1.06 | ± 0.16 | 0.71 | ± 0.18 | 0.44 | 0.04 | 0.03 | – |
| *acsf2b* | 1.10 | ± 0.47 | 1.20 | ± 0.40 | 0.96 | ± 0.43 | 1.02 | ± 0.42 | – | – | – | – |
| *acsf2c* | 1.05 | ± 0.33 | 1.05 | ± 0.40 | 1.03 | ± 0.22 | 1.01 | ± 0.16 | – | – | – | – |
| *Acsfd* | 1.08 | ± 0.30 | 1.22 | ± 0.45 | 0.93 | ± 0.16 | 0.92 | ± 0.17 | 0.47 | 0.05 | – | – |
| Lipogenesis *de novo* | | | | | | | | | | | | |
| *srebf1* | 1.27 | ± 0.36 | 0.78 | ± 0.55 | 1.13 | ± 0.48 | 1.33 | ± 0.51 | – | – | – | – |
| *srebp1c* | 1.28 | ± 0.35 | 0.78 | ± 0.54 | 1.16 | ± 0.59 | 1.19 | ± 0.35 | 0.04 | 0.26 | 0.12 | – |
| *acly ± global* | 1.33 | ± 0.59 | 1.12 | ± 1.51 | 1.39 | ± 0.98 | 1.09 | ± 0.41 | 0.00 | – | – | – |
| *Aclyb* | 1.27 | ± 0.57 | 1.16 | ± 1.45 | 1.39 | ± 0.78 | 0.99 | ± 0.36 | – | – | – | – |
| *aca-αa* | 0.96 | ± 1.15 | 2.71 | ± 2.94 | 1.81 | ± 1.34 | 2.32 | ± 2.82 | – | – | – | – |
| *aca-αb* | 2.00 | ± 0.99 | 1.04 | ± 1.20 | 2.01 | ± 2.25 | 1.07 | ± 0.82 | 0.09 | 0.62 | 0.02 | – |
| *aca-βa* | 0.74 | ± 0.33 | 1.18 | ± 0.68 | 1.23 | ± 0.52 | 1.47 | ± 0.77 | – | – | – | – |
| *aca-βb* | 1.37 | ± 0.71 | 1.04 | ± 0.62 | 1.53 | ± 2.2 | 1.35 | ± 0.90 | – | – | – | – |
| *Fasna* | 1.71 | ± 0.93 | 1.03 | ± 1.81 | 2.06 | ± 1.99 | 1.59 | ± 0.83 | 0.16 | 0.20 | 0.14 | – |
| *Fasnb* | 1.92 | ± 1.37 | 1.26 | ± 2.08 | 1.64 | ± 1.34 | 1.41 | ± 0.60 | 0.07 | – | – | – |
| *fasn ± global* | 1.66 | ± 0.82 | 1.05 | ± 1.76 | 1.97 | ± 2.02 | 1.57 | ± 0.74 | 0.10 | 0.22 | 0.16 | – |
| *g6pd ± global* | 1.20 | ± 0.28 | 0.92 | ± 0.51 | 1.11 | ± 0.71 | 1.08 | ± 0.28 | 0.00 | 0.43 | 0.10 | |
| *g6pdb* | 0.85 | ± 0.32 | 0.93 | ± 0.40 | 1.13 | ± 0.45 | 1.47 | ± 0.54 | 0.89 | 0.06 | – | – |
| β-oxydation | | | | | | | | | | | | |
| *Hadh* | 0.98 | ± 0.41 | 0.90 | ± 0.32 | 1.27 | ± 0.23 | 1.06 | ± 0.26 | 0.00 | – | – | – |
| *cpt1a* | 1.28 | ± 0.49 | 0.91 | ± 0.36 | 0.93 | ± 0.42 | 1.18 | ± 0.24 | 0.00 | 0.27 | 0.06 | – |
| *acox1a* | 1.08 | ± 0.17 | 1.03 | ± 0.28 | 0.88 | ± 0.27 | 1.12 | ± 0.21 | 0.07 | 0.83 | 0.69 | 0.09 |
| *acox1 ± global* | 1.12 | ± 0.26 | 1.04 | ± 0.26 | 0.91 | ± 0.26 | 1.05 | ± 0.19 | 0.03 | – | – | – |
| *acox3* | 1.06 | ± 0.21 | 1.01 | ± 0.22 | 1.00 | ± 0.20 | 1.01 | ± 0.20 | – | – | – | – |
| Polyunsaturated FA biosynthesis | | | | | | | | | | | | |
| *Scdba* | 1.63 | ± 0.96 | 1.15 | ± 1.00 | 2.02 | ± 1.91 | 1.52 | ± 0.65 | – | – | – | – |
| *Scdbb* | 1.01 | ± 0.30 | 0.94 | ± 1.06 | 1.35 | ± 1.24 | 1.71 | ± 0.81 | 0.10 | 0.15 | – | – |
| *Scdbc* | 1.15 | ± 0.64 | 1.16 | ± 0.50 | 1.13 | ± 0.96 | 1.11 | ± 0.48 | 0.00 | 0.32 | 0.06 | 0.11 |
| *scdbd* | 1.42 | ± 0.84 | 1.04 | ± 1.42 | 1.29 | ± 1.36 | 2.11 | ± 1.84 | 0.14 | 0.16 | | |
| *fads6a* | 1.79 | ± 1.22 | 1.42 | ± 1.20 | 0.78 | ± 0.47 | 1.57 | ± 0.63 | – | – | – | – |
| *fads6b* | 1.24 | ± 0.75 | 0.97 | ± 0.47 | 0.71 | ± 0.44 | 2.13 | ± 0.59 | 0.01 | 0.11 | – | – |
| *fads2a* | 1.27 | ± 0.51 | 0.98 | ± 0.56 | 0.92 | ± 0.31 | 1.19 | ± 0.30 | <0.0001 | 0.30 | 0.11 | – |
| *fads2b* | 0.88 | ± 0.56 | 0.85 | ± 0.48 | 1.07 | ± 0.38 | 2.18 | ± 1.23 | 0.14 | 0.00 | – | – |
| *fads2c* | 1.32 | ± 0.36 | 0.90 | ± 0.22 | 1.12 | ± 0.42 | 0.90 | ± 0.32 | 0.01 | 0.67 | 0.01 | – |
| *elovl2b* | 1.23 | ± 0.79 | 1.08 | ± 0.27 | 1.15 | ± 0.74 | 0.98 | ± 0.34 | – | – | – | – |
| *elovl2 ± global* | 1.19 | ± 0.71 | 1.06 | ± 0.32 | 1.18 | ± 0.73 | 1.03 | ± 0.44 | – | – | – | – |
| *elovl5a* | 1.27 | ± 0.72 | 1.12 | ± 0.52 | 0.89 | ± 0.55 | 1.28 | ± 0.35 | 0.00 | – | – | – |
| *elovl5b* | 0.95 | ± 0.19 | 1.09 | ± 0.39 | 0.96 | ± 0.30 | 1.19 | ± 0.36 | 0.12 | – | – | – |

Data are presented as means ± SEM and analysed by a linear model, followed by a posthoc Tukey test in the case of a significant interaction. Means with different lowercase letters in the same line are considered significantly different (*P*-values < 0.05).
Abbreviations: HC: high carbohydrate; LP: low protein, NN: offspring born from broodstock fed the control diet (No Carbohydrate); NH: offspring born from broodstock females fed the control diet and broodstock males fed the diet formulated with plant-derived carbohydrates (high carbohydrate and low protein nutrition); HN: offspring born from broodstock females fed the diet formulated with plant-derived carbohydrates ((high carbohydrate and low protein nutrition) and broodstock males fed the control diet; HH: offspring born from broodstock fed the diet formulated with plant-derived carbohydrates (high carbohydrate and low protein nutrition); FA: fatty acid.

*Increase in whole body adiposity*

The use of diets formulated with terrestrial plant products and completely devoid of fish oil and fishmeal typically increase salmonids' overall adiposity (Torstensen et al., 2011). We demonstrated here that the decrease of fishmeal in the parental diet (*i.e.* HC/LP nutrition) has induced a similar phenotype in their offspring,

which was probably due to an increase in perivisceral fat rather than in muscle tissues. As the expression of genes related to de novo lipogenesis (except for aca$\alpha$b) and $\beta$-oxidation were not affected in the liver, such phenotype could emerge either from an augmentation of lipid retention and accumulation or an enhancement of de novo lipogenesis directly in perivisceral tissue, known to be an active site for lipogenesis (Bou et al., 2016). Interestingly, female offspring of sires fed a HC/LP (*i.e.* NH females) have accumulated 22.4% more fat in perivisceral tissues when they were fed with a commercial diet (Callet et al., 2021a). From these results, it appears that parental HC/LP nutrition induces this phenotype regardless of the given diet and constitutes therefore a constant outcome of the programming with parental HC/LP nutrition in trout. Nevertheless, in the present study, although the interaction between parental nutrition and offspring sex cannot be assessed (see statistical parts), males were more strongly affected than female offspring when fed a complete plant-based diet (+28.9% and 34.2% peri- visceral fat in NH and HN male offspring, respectively). The strong influence of the sex in programming in mammals has already been described (Gabory et al., 2009) and these results further demonstrate its importance in fish species, highlighting thus the importance of taking into account this parameter in programming studies.

*Enhancement of the cholesterol metabolism at the molecular level*

The paternal HC/LP nutrition in trout has also enhanced the typical increase of cholesterol biosynthesis observed when trout are directly fed a complete plant-based diet, which contains only plant sterols and lacks cholesterol (Zhu et al., 2018). The paternal HC/LP nutrition has indeed induced an increase in the hepatic cholesterol biosynthesis at the transcriptomic level, as seen by the augmentation of *srebp2* mRNA level, a master regulator of cholesterol biosynthesis (Horton et al., 2002), along with the mRNA levels of the downstream actors of this pathway. The parental HC/LP nutrition has also slightly affected the mRNA levels of *lxr$\alpha$b* in the HN and HH offspring. LXR$\alpha$ is one isoform of the LXRs family that plays a role in lipid sensing and avoids thus overload of cholesterol (Chawla et al., 2001). However, no specific patterns were identified in the expression of genes involved in cholesterol esterification and excretion (Zhu et al., 2018). These results suggest that the cholesterol excretion and esterification pathways were not affected by neither the maternal nor the paternal HC/LP nutrition, which is consistent with the unchanged plasmatic cholesterol levels observed.

Interestingly, similar inductions of cholesterol biosynthesis are typically observed in offspring of male rats fed a LP diet (Carone et al., 2010). It has been previously suggested that parental nutrition could induce constant effects across species (Langley-Evans, 2009). However, in the present study, the male broodstock have been affected by a *Saprolegnia* infection as previously described (Callet et al., 2020). Therefore, it could not be excluded that sires have been selected during the trial, and it is not possible to decipher if the effect observed is triggered by the selection or by the HC/LP nutrition.

*Enhancement of the long-chain poly unsaturated fatty acid biosynthesis*

Vegetable oils are abundant in linoleic acid (LA, 18:2n-6) and $\alpha$-linolenic acid (ALA,18:3n-3), but lack long-chain (LC) PUFA. Freshwater fish, such as rainbow trout, have the ability to convert ALA and LA; and the partial or total replacement of dietary fish oil and fishmeal by plant ingredients stimulates the process of desaturation of polyunsaturated fatty acids (Panserat et al., 2019; Geay et al., 2011). As for the other pathways described in above paragraphs, the parental HC/LP nutrition has increased the hepatic PUFA biogenesis of their offspring, as seen by the augmentation of *fads6b* and *fads2b* hepatic mRNA levels, genes catalysing the rate-limiting step of fatty acid desaturation process in many fish species (Perera et al., 2020). The hepatic unsaturated FA profiles were subsequently affected and the proportion of the intermediates and products of the desaturation pathway (20:2n-6, 20:3n-6, 20:3n-3 and 22:5n-3) were significantly increased in the liver.

In gilthead sea bream, manipulation of broodstock nutrition, but with a different stimulus (vegetable oils), also shaped the expression of genes involved in PUFA biosynthesis and more particularly enhanced the gene expression of hepatic *fads6* in their progeny (Izquierdo et al., 2015; Perera et al., 2020; Xu et al., 2020). Together, these results confirm that the desaturation pathway could be modulated by broodstock nutrition in agronomical high-value species. Such modulation could be of great relevance for the aquaculture sector. Indeed, due to the reduction of marine ingredients in aquafeed, EPA (20:5n-3) and DHA (22:6n-3) quantities decreased over the years in salmonids, while they have important nutritional values for human consumers (Sprague et al., 2016).

Unfortunately, in the present study, the effect of parental HC/LP nutrition was restricted to the liver and the parental nutrition did not lead to an increase of EPA, or DHA in fish flesh. Nevertheless, n-3 LC-PUFA plays also important physiological roles for fish and in particular, dietary n-3 LC-PUFA has been shown to enhance the immune system in fish (Montero and Izquierdo, 2010). Their higher synthesis *via* a parental HC/LP nutrition might therefore present some advantage to improve the health of their progeny. At the same time, transcriptomic analyses revealed that the parental HC/LP nutrition have altered the expression of some genes involved in immunity in their offspring but only challenges to test their resistance to different pathogens could confirm the advantage of such results.

## Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100670.

## Ethics approval

The study was conducted according to the guidelines of the French and European regulations on animal welfare (Decree 2001 464, 29 May 2001 and Directive 2010/63/EU, respectively). This protocol and the project as a whole were approved by the French National Consultative Ethics Committee (reference numbers 2015112018112159 and 201511201756973).

## Data and model availability statement

The microarray data are available at NCBI GEO: GSE:184632. The models were not deposited in an official repository. The models that support the study findings are available upon reasonable request to the corresponding author.

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by H.L. (molecular analyses, biochemical analyses), T.C. (molecular analyses), C.H. (global DNA methylation), A.S. (biochemical analyses), L.L. (biochemical analyses). Formal analyses, visualisation, and writing by H.L and T.C. All authors have read and agreed to the published version of the manuscript.

## Declaration of interest

None.

## Acknowledgements

The author H. Li thanks the financial assistance provided by China Scholarship Council (File No. 201904910576).

## Financial support statement

The present study was supported by the French National Research Agency (ANR-18-CE20-0012-01 "SweetSex").

## References

Adam, A.-C., Skjrven, K.H., Whatmore, P., Moren, M., Lie, K.K., 2018. Parental high dietary arachidonic acid levels modulated the hepatic transcriptome of adult zebrafish (*Danio rerio*) progeny. PloS One 13, 0201278.

Bou, M., Todorcevic, M., Torgersen, J., Skugor, S., Navarro, I., Ruyter, B., 2016. De novo lipogenesis in atlantic salmon adipocytes. Biochimica et Biophysica Acta (BBA)-General Subjects 1860, 86–96.

Callet, T., Medale, F., Larroquet, L., Surget, A., Aguirre, P., Kerneis, T., Labbe, L., Quillet, E., Geurden, I., Skiba-Cassy, S., 2017. Successful selection of rainbow trout (*Oncorhynchus mykiss*) on their ability to grow with a diet completely devoid of fishmeal and fish oil, and correlated changes in nutritional traits. PLoS ONE 12, 0186705.

Callet, T., Hu, H., Larroquet, L., Surget, A., Liu, J., Plagnes-Juan, E., Maunas, P., Turonnet, N., Mennigen, J.A., Bobe, J., 2020. Exploring the impact of a low-protein high-carbohydrate diet in mature broodstock of a glucose-intolerant teleost, the rainbow trout. Frontiers in physiology 11, 303.

Callet, T., Li, H., Coste, P., Glise, S., Heraud, C., Maunas, P., Mercier, Y., Turonnet, N., Zunzunegui, C., Panserat, S., 2021a. Modulation of energy metabolism and epigenetic landscape in rainbow trout fry by a parental low protein/high carbohydrate diet. Biology 10, 585.

Callet, T., Li, H., Surget, A., Terrier, F., Sandres, F., Lanuque, A., Panserat, S., Marandel, L., 2021b. No adverse effect of a maternal high carbohydrate diet on their offspring, in rainbow trout (*Oncorhynchus mykiss*). PeerJ 9, 12102.

Cardenas, A., Rifas-Shiman, S.L., Agha, G., Hivert, M.F., Litonjua, A.A., DeMeo, D.L., Lin, X., Amarasiriwardena, C.J., Oken, E., Gillman, M.W., 2017. Persistent DNA methylation changes associated with prenatal mercury exposure and cognitive performance during childhood. Scientific reports 7, 1–13.

Carone, B.R., Fauquier, L., Habib, N., Shea, J.M., Hart, C.E., Li, R., Bock, C., Li, C., Gu, H., Zamore, P.D., 2010. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell 143, 1084–1096.

Chawla, A., Repa, J.J., Evans, R.M., Mangelsdorf, D.J., 2001. Nuclear receptors and lipid physiology: opening the x-files. Science 294, 1866–1870.

Folch, J., Lees, M., Stanley, G.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of biological chemistry 226, 497–509.

Fuso, A., Lucarelli, M., 2019. Cpg and non-cpg methylation in the diet-epigenetics eurodegeneration connection. Current nutrition reports 8, 74–82.

Gabory, A., Attig, L., Junien, C., 2009. Sexual dimorphism in environmental epigenetic programming. Molecular and cellular endocrinology 304, 8–18.

Geay, F., Ferraresso, S., Zambonino-Infante, J.L., Bargelloni, L., Quentel, C., Andeputte, M., Kaushik, S., Cahu, C.L., Mazurais, D., 2011. Effects of the total replacement of fish-based diet with plant-based diet on the hepatic transcriptome of two european sea bass (Dicentrarchus labrax) half-sibfamilies showing different growth rates with the plant-based diet. BMC Genomics 12, 1–18.

Gluckman, P.D., Buklijas, T., Hanson, M.A., 2016. The developmental origins of health and disease (DOHaD) concept: past, present, and future. Academic Press, Washington, DC, USA, pp. 1–15.

Good, C.A., Kramer, H., Somogyi, M., 1933. The determination of glycogen. Journal of Biological Chemistry 100, 485–491.

Guo, T., Luo, F., Lin, Q., 2020. You are affected by what your parents eat: Diet, epigenetics, transgeneration and intergeneration. Trends in Food Science & Technology 100, 248–261.

Horton, J.D., Goldstein, J.L., Brown, M.S., 2002. Srebps: activators of the complete program of cholesterol and fatty acid synthesis in the liver. The Journal of Clinical Investigation 109, 1125–1131.

Hou, Z., Fuiman, L.A., 2020. Nutritional programming in fishes: insights from mammalian studies. Reviews in Fish Biology and Fisheries 30, 67–92.

Hua, K., Cobcroft, J.M., Cole, A., Condon, K., Jerry, D.R., Mangott, A., Praeger, C., Vucko, M.J., Zeng, C., Zenger, K., 2019. The future of aquatic protein: implications for protein sources in aquaculture diets. One Earth 1, 316–329.

Izquierdo, M., Turkmen, S., Montero, D., Zamorano, M., Afonso, J., Karalazos, V., Fernandez-Palacios, H., 2015. Nutritional programming through broodstock diets to improve utilization of very low fishmeal and fish oil diets in gilthead sea bream. Aquaculture 449, 18–26.

Jiang, L., Zhang, J., Wang, J.J., Wang, L., Zhang, L., Li, G., Yang, X., Ma, X., Sun, X., Cai, J., et al., 2013. Sperm, but not oocyte, dna methylome is inherited by zebrafish early embryos. Cell 153, 773–784.

Jobling, M., 2012. National Research Council (NRC): Nutrient Requirements of Fish and Shrimp. Springer, Berlin, Germany.

Kovatsi, L., Fragou, D., Samanidou, V., Njau, S., Kouidou, S., Bailey, A., 2012. Evaluation of 5-methyl-2-deoxycytidine stability in hydrolyzed and nonhydrolyzed dna by hplc-uv. Bioanalysis 4, 367–372.

Langley-Evans, S.C., 2009. Nutritional programming of disease: unravelling the mechanism. Journal of Anatomy 215, 36–51.

Lazzarotto, V., Corraze, G., Leprevost, A., Quillet, E., Dupont-Nivet, M., Medale, F., 2015. Three-year breeding cycle of rainbow trout (Oncorhynchus mykiss) fed a plant-based diet, totally free of marine resources: consequences for reproduction, fatty acid composition and progeny survival. PLoS ONE 10, e0117609.

Lin, I.-H., Chen, Y.-F., Hsu, M.-T., 2017. Correlated 5-hydroxymethylcytosine (5hmc) and gene expression profiles underpin gene and organ-specific epigenetic regulation in adult mouse brain and liver. PloS One 12, 0170779.

Liu, J., Heraud, C., Véron, V., Laithier, J., Burel, C., Prézelin, A., Panserat, S., Marandel, L., 2022. Hepatic Global DNA Hypomethylation Phenotype in Rainbow Trout Fed Diets Varying in Carbohydrate to Protein Ratio. The Journal of Nutrition 152, 29–39.

Lucas, A., Baker, B., Desai, M., Hales, C., 1993. Nutrition in pregnant or lactating rats programs lipid metabolism in the offspring. British Journal of Nutrition 76, 605–612.

Molinari, G.S., McCracken, V.J., Wojno, M., Rimoldi, S., Terova, G., Kwasek, K., 2020. Can intestinal absorption of dietary protein be improved through early exposure to plant-based diet? PLoS ONE 15, 0228758.

Monroig, Ó., Navarro, J.C., Tocher, D.R., 2011. Long-chain polyunsaturated fatty acids in fish: recent advances on desaturases and elongases involved in their biosynthesis. Universidad Autónoma de Nuevo León, Monterre, México, pp. 257–283.

Montero, D., Izquierdo, M., 2010. Welfare and health of fish fed vegetable oils as alternative lipid sources to fish oil. In: Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds, CRC Press (Taylor and Francis Group). FL, USA, Boca Raton, pp. 439–485.

Murgatroyd, C., Patchev, A.V., Wu, Y., Micale, V., Bockmuhl, Y., Fischer, D., Holsboer, F., Wotjak, C.T., Almeida, O.F., Spengler, D., 2009. Dynamic DNA methylation programs persistent adverse effects of early-life stress. Nature neuroscience 12, 1559–1566.

Panserat, S., Hortopan, G., Plagnes-Juan, E., Kolditz, C., Lansard, M., Skiba-Cassy, S., Esquerre, D., Geurden, I., Medale, F., Kaushik, S., 2019. Differential gene expression after total replacement of dietary fish meal an fish oil by plant products in rainbow trout (*Oncorhynchus mykiss*) liver. Aquaculture 294, 123–131.

Perera, E., Turkmen, S., Simo-Mirabet, P., Zamorano, M.J., Xu, H., Naya-Catala, F., Izquierdo, M., Perez-Sanchez, J., 2020. Stearoyl-coa desaturase (*scd1a*) is epigenetically regulated by broodstock nutrition in gilthead sea bream (*Sparus aurata*). Epigenetics 15, 536–553.

Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., Smyth, G.K., 2015. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic acids research 43), 47.

Robison, B.D., Drew, R.E., Murdoch, G.K., Powell, M., Rodnick, K.J., Settles, M., Stone, D., Churchill, E., Hill, R.A., Papasani, M.R., 2008. Sexual dimorphism in hepatic gene expression and the response to dietary carbohydrate manipulation in the zebrafish (*Danio rerio*). Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 3, 141–154.

Shantha, N., Ackman, R., 1990. Nervonic acid versus tricosanoic acid as internal standards in quantitative gas chromatographic analyses of fish oil longer-chain n|3 polyunsaturated fatty acid methyl esters. Journal of Chromatography B: Biomedical Sciences and Applications 533, 1–10.

Sprague, M., Dick, J.R., Tocher, D.R., 2016. Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006–2015. Scientific reports 6, 1–9.

Tacon, A.G., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285, 146–158.

Tarrade, A., Panchenko, P., Junien, C., Gabory, A., 2015. Placental contribution to nutritional programming of health and diseases: epigenetics and sexual dimorphism. Journal of Experimental Biology 218, 50–58.

Torstensen, B.E., Espe, M., Stubhaug, I., Lie, 2011. Dietary plant proteins and vegetable oil blends increase adiposity and plasma lipids in atlantic salmon (Salmo salar L.). British Journal of Nutrition 106, 633–647.

Turkmen, S., Zamorano, M.J., Fernandez-Palacios, H., Hernandez-Cruz, C.M., Montero, D., Robaina, L., Izquierdo, M., 2017. Parental nutritional programming and a reminder during juvenile stage affect growth, lipid metabolism and utilisation in later developmental stages of a marine teleost,

the gilthead sea bream (*Sparus aurata*). British Journal of Nutrition 118, 500–512.
Wang, X., Bhandari, R.K., 2020. DNA methylation reprogramming in medaka fish, a promising animal model for environmental epigenetics research. Environmental Epigenetics 6, 008.
Washburn, B.S., Frye, D.J., Hung, S.S., Doroshov, S.I., Conte, F.S., 1990. Dietary effects on tissue composition, oogenesis and the reproductive performance of female rainbow trout (*Oncorhynchus mykiss*). Aquaculture 90, 179–195.
Xu, H., Ferosekhan, S., Turkmen, S., Afonso, J.M., Zamorano, M.J., Izquierdo, M., 2020. Influence of parental fatty acid desaturase 2 (*fads2*) expression and diet on gilthead seabream (*Sparus aurata*) offspring fads2 expression during ontogenesis. Animals 10, 2191.
Yano, A., Guyomard, R., Nicol, B., Jouanno, E., Quillet, E., Klopp, C., Cabau, C., Bouchez, O., Fostier, A., Guiguen, Y., 2012. An immune-related gene evolved into the master sex-determining gene in rainbow trout, *Oncorhynchus mykiss*. Current Biology 22, 1423–1428.
Zhu, T., Corraze, G., Plagnes-Juan, E., Quillet, E., Dupont-Nivet, M., Skiba-Cassy, S., 2018. Regulation of genes related to cholesterol metabolism in rainbow trout (*Oncorhynchus mykiss*) fed a plant-based diet. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 314, 58–70.