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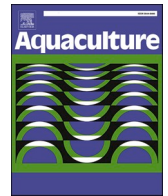
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# A two-year plant-based diet alters the fatty acid profile and enzymatic and non-enzymatic lipid metabolites, in the eggs and fry of female rainbow trout

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## ABSTRACT

To promote sustainable aquaculture, plant-based ingredients are increasingly being used to replace fish meal (FM) and fish oil (FO) in diets, affecting critical fatty acid (FA) profiles in broodstock diets. These profiles are essential for reproduction and offspring survival. The absence of traditional fish-derived nutrients results in an altered FA composition, particularly a reduction in omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs). This change is detrimental to trout reproduction over the long term. Current research gaps, especially regarding FA profiles and related metabolites, limit our understanding of the reproductive consequences of omega-3 deficient plant-based diets. To fill this knowledge gap, a two-year trial was conducted to compare the reproductive effects (spawning performances, egg quality, embryo development) of two diets on rainbow trout females: a plant-based diet containing linseed (10.8 %), palm (5 %), and sunflower oils (2.7 %), and a commercial diet with high FM (27 %) and FO (12.1 %) content. Both diets were similar in terms of protein (44 %), lipids (23.4 %), and energy (25 kJ/g). The study spanned from initial feeding to first reproduction, and fatty acid profiles and metabolites (selected enzymatic and non enzymatic metabolites from FA) were examined in the broodstock diet, female muscle tissue, eggs, and fry. Trout fed a plant-based diet showed reduced reproductive traits such as lower weight and fecundity, but egg and embryo quality remained high. Survival and hatching rates were similar to those on commercial diets. The study revealed for the first time the critical role of maternal diet in the generation of lipid metabolites in the offspring, particularly DHA (F<sub>4</sub>-neuroprostane, maresin, 14-HDoHE) and AA (F<sub>2</sub>-isoprostane, prostaglandin E and F<sub>2</sub>, 8-12-15-HETEs) oxylipins, highlighting the complex dietary influences on fish reproduction. This work underlines that a plant-based diet significantly affects the reproductive performance of broodstock trout by altering fatty acid profiles and metabolite levels and highlights the delicate balance between dietary components, oxylipins, and reproductive functions in fish, suggesting the need for more comprehensive studies of their interrelated effects.

## 1. Introduction

For decades, global consumption of aquatic food products has grown exponentially, quintupling overall. Now, since 2020, aquaculture has increased by 6.6 %, now contributing over 57 % of aquatic animal products for direct human consumption (FAO, 2024). To meet this growing demand, the fishing industry has expanded rapidly, effectively feeding an ever-increasing global population. However, this relentless

pursuit of profit and the large-scale industrialization of fishing led to overexploitation of resources by the end of the 20th century. Overfishing, pollution, and mismanagement led to a drastic decline in these aquatic resources. In 2021, more than 37.7 % of the world's fishstocks were exploited at biologically unsustainable levels (FAO, 2024). In response to this situation, the introduction of fishing quotas and the rising global demand for fish has disrupted the marine fishing industry, prompting the expansion of the aquaculture sector. By 2022,

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aquaculture will provide 55 % of aquatic food products for human consumption, compared to 45 % from capture fisheries (FAO, 2024). This trend has made aquaculture the primary production sector capable of meeting the growing demand for aquatic products, making it a key element in global food security. However, to ensure a sustainable future, it is imperative that this development takes place in a responsible manner, limiting its impact on the environment. In this context, the development of aquaculture feed formulations is a major challenge. Despite the potential of aquaculture to feed a growing world population, it is essential to reduce dependence on fish meal (FM) and fish oil (FO), considered the gold standard of aquaculture ingredients, is essential. Efforts are underway to replace these ingredients with readily available plant-based alternatives in the diets of aquatic species. Among salmonids, rainbow trout (*Oncorhynchus mykiss*) farming is the most important freshwater production in Europe. Over the past two decades of research (Aragão et al., 2022), plants have emerged as an important alternative to FM/FO, serving as a major source of proteins and lipids. This transition is particularly notable in the diet of rainbow trout, where the proportion of plant-based ingredients has increased from 25 % in 2000 to 82 % in 2018. Despite the rapid expansion of aquaculture, the reliance on FM and FO remains substantial, estimated at over 16 million tonnes in 2020. Thus, although a significant reduction in the level of FM/FO in the diet can be achieved, there are still several difficulties to be overcome in order to fully replace FM/FO with plant ingredients, even in salmonids. Among the various effects observed in trout is a reduction in growth rate (de Francesco et al., 2004; Panserat et al., 2008; Vilhelmsson et al., 2004), which is associated with a combination of reduced food intake (Aas et al., 2022; Baranek et al., 2023; Gomes et al., 1995; Heraud et al., 2022; Lazzarotto et al., 2018; Roy et al., 2022) and low feed efficiency (Panserat et al., 2009; Richard et al., 2006). In addition to the effects on fish physiology, the complete replacement of FM/FO in aquaculture diets leads to change in the composition of micro and macronutrients. FM/FO are known for their high nutritional value and richness in polyunsaturated omega-3 fatty acids ( $\omega$ 3 LC-PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are completely absent in plant raw materials (Turchini et al., 2009). These fatty acids (FA) play critical roles throughout the life cycle of rainbow trout, particularly in reproduction, egg quality, and fry development, in addition to their importance in ensuring fillet quality for human consumption (Rosenlund et al., 2011). In both salmonids and many farmed fish species, sexual maturation and gonadal development occur at the expense of stored energy, including lipids (Manor et al., 2015). Consequently,  $\omega$ 3 LC-PUFAs are preferentially incorporated into the eggs (Bromage and Roberts, 1995), providing energy reserves for oocytes and fry development. The absence of these  $\omega$ 3 LC-PUFAs in trout diets leads to immature gonads, resulting in lower hatching rates, increased fry malformations, and reduced progeny survival (Izquierdo et al., 2001; Lazzarotto et al., 2015; Salze et al., 2005; Sargent et al., 1997). Although salmonids have the ability to biosynthesize DHA through the desaturation and elongation of alpha-linolenic acid (ALA; found in plant components such as linseed oil) (Lazzarotto et al., 2015; Tocher, 2003), studies have shown that this natural conversion process is insufficient to compensate for the lack of dietary  $\omega$ 3 LC-PUFAs, resulting in a significant reduction of these FA in fish tissues (Turchini and Francis, 2009). Moreover, FA are highly susceptible to oxidation, especially  $\omega$ -3 and  $\omega$ -6 LC-PUFAs, which can lead to the formation of NEO-PUFAs (Galano et al., 2018). These oxylipins synthesized by enzymatic or non-enzymatic oxidation of LC-PUFAs are bioactive downstream lipid mediators. While these oxylipins are increasingly recognized for their biological roles in mammals (Dyall et al., 2022; Galano et al., 2018; Roy and Le Guennec, 2017), few studies investigated their effects in fish. The first study in rainbow trout dates back 28 years (Knight et al., 1995), and showed their presence in various tissues, notably in muscle and ovaries. A more recent study demonstrated that different FA dietary compositions significantly impact the levels of enzymatic and non-enzymatic metabolites in trout brain (Roy et al., 2020b), which may explain

changes in brain activity, especially in the absence of EPA and DHA in whole plant-based diets (Roy et al., 2020a). However, to our knowledge, no study has quantified and investigated the levels of these FA metabolites in trout diets and their effect during the trout reproductive period. While some investigated the effects of a DHA/EPA-deficient plant diet on fish growth or flesh quality, the effects of a plant diet throughout the life cycle, particularly on initial reproductive performance and fry survival, focusing on FA profiles and their enzymatic and non-enzymatic metabolites, have not been investigated to date. Giving the critical role of FA, particularly LC-PUFAs, in successful reproduction, egg quality, and fry development (Fernández-Palacios et al., 1995; Lazzarotto et al., 2015; Støttrup et al., 2013), this represents a notable gap in the understanding of how fish reproduction is impacted when fed a plant-based diet. In our study, we investigated the reproductive performance of rainbow trout fed a plant-based diet (V-diet) vs commercial diet (C-diet) from their initial feeding to their first reproduction (spanning 2 years of feeding). In addition, we investigated the incorporation of LC-PUFAs and their enzymatic and non-enzymatic metabolites into female muscle, eggs and in the transgenerational effects on fry survival. The aim of this comprehensive analysis was to assess the effect of diet on the overall FA profile in reproductive female trout. By identifying specific molecules, our aim was to establish potential FA markers indicative of poor reproductive performance in farmed trout.

## 2. Materials and methods

### 2.1. Ethics statement

The trial was conducted in accordance with the Guidelines of the National Legislation on Animal Care of the French Ministry of Research (decree no. 2013-118, 1 February 2013) and following European Union legal frameworks related to the protection of animals used for scientific purposes (i.e. Directive 2010/63/EU). The scientists in charge of the trial received training and personal authorization. The trial was conducted at INRAE's NuMeA facilities (doi:10.15454/GPYD-AM38) and approved by the ethical committee (C2EA-73) of INRAE "Comité d'éthique Aquitaine poissons oiseaux" (INRAE agreement no. 21699, 19 December 2019). All efforts were made to minimize the number of fish used and their suffering. This experiment, which lasted a total of two years, made it possible to produce this article, but others analyses (Heraud et al., 2022) and samples were performed using other animals during this trial.

### 2.2. Fish and rearing

Mono sex female rainbow trout were held until their first reproduction (2 years). The first year of rearing was carried out in the INRAE experimental facilities at Donzacq (permit no. A40-228.1, Landes, France Landes) under natural photoperiod at a temperature of 17 °C. This first year of the breeding was previously described (Baranek et al., 2023). The fish were then transferred to the INRAE's experimental facilities Fish Farm of Léas-Athas (permit no. A64.104.1, Pyrenees Atlantiques, France). The rearing was carried out in 2m<sup>3</sup> tanks at a biomass of 22 kg m<sup>-3</sup> under natural photoperiod at 8 °C, optimal water temperature for RT reproduction and egg incubation ( $n = 22$  and 19 females for C diet and V diet respectively). All fish were fed manually twice a day until apparent satiety. Water flow rate adjusted to provide a dissolved oxygen concentration greater than 90 % of saturation.

### 2.3. Experimental diets

For this experiment, two isoproteic, isoenergetic and isolipidic diets were formulated using Allix® software (A-Systems, Versailles, France) and manufactured at INRAE's experimental facility at Donzacq using a twin-screw extruder (45 BCE, Cletral, Firminy, France): a commercial-like diet (C-diet) containing a mixture of FM (27 %), FO (12.1 %) and

plant ingredients compared to a plant-based diet, without of FM and FO (V-diet). The dietary treatment was applied from first feeding until the end of the trial (as described in detail (Baranek et al., 2023)). The composition and granulometry of the diets were adjusted during rearing to meet the physiological and energetic requirements of the different life stages of the fish (54 % protein in the diet up to 20 g, then 43 % until the end of the trial) (National Research Council, 2011). The ingredients and composition of the diets used during the first stage of rearing phase (before the transfer of the fish to the Léés-Athas site) was previously described (Baranek et al., 2023). The ingredients and composition of the diets used for female broodstock are presented in Table 1.

The nutrient composition of the diets was analyzed by drying the samples to constant weight at 105 °C for 24 h. Crude protein content was determined by the Kjeldahl method after acid digestion and estimated by multiplying nitrogen by 6.25. Gross energy was determined in an adiabatic bomb calorimeter (IKA, Heitersheim Gribheimer, Germany). Starch content was determined by an enzymatic method (Megazyme). Ash content was determined by combustion in a muffle furnace (550 °C for 8 h). Total lipid for diet but also for females muscle, eggs and fry were extracted (with an Ultra-Turrax (IKA-Werke, Germany) tissue (females muscle, eggs and alevins) disrupter) and measured gravimetrically according to the Folch method (Floch et al., 1957) using dichloromethane instead of chloroform. FA methyl esters were prepared by acid-catalyzed transmethylation of total lipids using boron trifluoride (BF<sub>3</sub>) in methanol (14 %) with 0.01 % butylated hydroxytoluene (BHT) as an antioxidant according to Shantha and Ackman (Shantha and Ackman,

**Table 1**  
Ingredients and composition of experimental diets.

Ingredient (%)	commercial-like diet (C)	Plant-based diet (V)
Fish meal	27.0	0.0
Extruded whole wheat	22.5	17.0
Corn gluten	8.7	17.0
Wheat gluten	7.0	17.0
Peas meal	3.0	6.0
Extruded Peas	7.0	9.5
White lupin seed meal	2.5	4.5
Rapeseed meal	0.8	3.0
Soy lecithin	0.0	2.0
L-Lysine	0.5	1.7
L-methionine	0.0	0.4
CaHPO <sub>4</sub> ·2H <sub>2</sub> O	0.0	1.4
Mineral premix <sup>a</sup>	1.0	1.0
Vitamin premix <sup>b</sup>	1.0	1.0
Fish oil	4.8	0.0
Palm oil	2.0	5.0
Sunflower oil	4.9	2.7
Linseed oil	0.0	10.8
DHA marine oil (from Polaris) <sup>c</sup>	7.3	0.0
Composition (% of DM)		
Dry matter (%)	96.77	92.11
Crude protein	43.94	44.08
Crude lipid	23.15	23.74
Starch	13.87	13.63
Ash	7.12	4.1
Energy (kJg-1 DM)	24.71	25.27

<sup>a</sup> Mineral premix: (g or mg kg<sup>-1</sup> 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 sulfate (36 % Zn), 0.4 g; copper sulfate (25 % Cu), 0.3 g; manganese sulfate (33 % Mn), 0.3 g; dibasic calcium phosphate (20 % Ca, 18 % P), 5 g; cobalt sulfate, 2 mg; sodium selenite (30 % Se), 3 mg; KCl, 0.9 g; NaCl, 0.4 g (UPAE, INRAE).

<sup>b</sup> Vitamin premix: (IU or mg kg<sup>-1</sup> diet): DL- $\alpha$ -tocopherol acetate, 60 IU; sodium menadione bisulfate, 5 mg; retinyl acetate, 15,000 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; B12, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium pantothenate, 50 mg; choline chloride, 2000 mg (UPAE, INRAE)

<sup>c</sup> Omegavie® DHA 70 (Polaris, Quimper, France): concentrated anchovy and sardine oil that contains DHA omega-3 fatty acids in triglyceride form.

1990). FAMES were then analyzed in a Varian 3900 gas chromatograph (Varian, les Ulis, France) 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 1 μL, using helium as carrier gas (1 mL/min). The temperatures of the injector and the flame ionization 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. FA were identified with reference to known standard mixture (Sigma, St Louis, MO, USA) and peaks were integrated using Varian Star Chromatography Software (Star Software, version 5). Individual FAs were expressed as a percentage of total FAME identified.

#### 2.4. Reproduction, fertilization, and incubation

During the spawning season, females were anesthetized with MS-222 (50 mg/L) and checked for ovulation once a week by applying manual pressure on the abdomen of the fish. Feeding was stopped for all animals when the first ovulation was detected. Ovulating females were weighed and manually stripped. The ovarian fluid and eggs produced by each female were weighed and eggs were sampled during the reproductive period.

Approximately 500 eggs from each batch were individually fertilized with a pool of sperm collected from neomales from the fish farm that were fed a commercial diet ad libitum. Three microlitres of a pool of semen obtained from three males with the highest sperm motility were pre-diluted to 1/10 in storFish solution (IMV technologies, L'Aigle, France) and added to each group of eggs. Fifteen mL of Actifish solution (sperm motility activating saline solution, IMV technologies, L'Aigle, France) were added to the eggs. Five minutes later, the sperm motility activating solution was drained and egg batches were transferred into individual incubators in recirculating water units. The water temperature remained constant (8 °C) throughout the incubation period. Dead eggs and embryos were counted and removed manually at regular intervals. Survival at the eyed stage and at completion of yolk-sac resorption was monitored and calculated as a percentage of the initial number of eggs used for the fertilization. The occurrence of noticeable morphological malformations (spinal cord torsion, head or caudal fin malformations, etc.) at yolk-sac resorption was also recorded.

#### 2.5. Measure of somatic index and sampling

After spawning, all females were sedated by immersion in MS-222 solution at a concentration of 50 mg/L and then euthanized by immersion in a MS-222 solution at a concentration of 400 mg/L (anesthetic overdose) for 5 min. The liver, viscera and the remaining eggs and gonads in the cavity were collected from each female and weighed. The hepato-somatic index (HSI), viscero-somatic index (VSI) and gonado-somatic index (GSI) were calculated as follows: (liver weight/total female body weight) × 100; (viscera weight/total female body weight) × 100 and ((egg+ovary) weight/total female body weight). A section of muscle was sampled 2 cm wide along the length of the dorsal muscle and frozen at -20 °C until further analysis.

#### 2.6. Egg phenotyping and egg and fry sampling

Stripped unfertilized eggs (approximately 400 eggs, about 25 to 30 g) were placed in 150 mL containers and water was added to hydrate the eggs. After 24 h of hydration, a picture of the sampled eggs was obtained using the VisEGG shooting system consisting of a light tablet and digital SLR camera (canon EOS 1000D, resolution: 10.1 M pixels) equipment, as previously described (Cardona et al., 2021). Pictures were analyzed with Fiji software (Schindelin et al., 2012) which allow automatic measurements to be made (number and size of the eggs and white egg percentage). The total number of ovulated eggs per female could be back-calculated from the number of eggs measured and the total weight of

the egg batch analyzed. The presence of white egg was interpreted as an indirect measure of egg integrity, the “whitening” being the result of water entry into the egg and through the vitelline membrane (Cardona et al., 2022). These white eggs were considered non-viable due to their lack of physical integrity.

The total number of ovulated eggs per female corresponded to the individual absolute fecundity. The individual fecundity was divided by the weight of the fish to give the value for relative fecundity in number of eggs/kg of female.

Unfertilized eggs (approximately 50 g per fish) were sampled and frozen at  $-20^{\circ}\text{C}$  until further analysis. Moreover 100 fry at yolk-sac resorption were euthanized using the method described above for females and were sampled and frozen at  $-20^{\circ}\text{C}$  until further analysis.

## 2.7. Enzymatic metabolite lipid mediators analysis

For extraction of enzymatic lipid mediators, each frozen female's muscle, eggs and fry tissue were crushed with a FastPrep®-24 Instrument (MP Biomedical) in 1 mL of HBSS (Invitrogen). After 2 crush cycles (6.5 m/s, 30 s) 1000  $\mu\text{L}$  of homogenate (around 15 mg of tissue) were withdrawn for oxylipin analyses. 300  $\mu\text{L}$  of cold methanol and 5  $\mu\text{L}$  of internal standard (Deuterium labeled compounds) were added. After centrifugation at 900g for 15 min at  $4^{\circ}\text{C}$ , supernatants were transferred into 2 mL 96-well deep plates and diluted in  $\text{H}_2\text{O}$  to 2 mL. Samples were then submitted to solid phase extraction (SPE) using OASIS HLB 96-well plate (30 mg/well, Waters) pretreated with MeOH (1 mL) and equilibrated with 10 % MeOH (1 mL). After sample application, extraction plate was washed with 10 % MeOH (1 mL). After drying under aspiration, lipids mediators were eluted with 1 mL of MeOH. Prior to LC-MS/MS analysis, samples were evaporated under nitrogen gas and reconstituted in 10  $\mu\text{L}$  on MeOH. LC-MS/MS analyses of oxylipins were performed as described (Le Faouder et al., 2013). Briefly, enzymatic lipid mediators were separated on a ZorBAX SB-C18 column (2.1 mm, 50 mm, 1.8  $\mu\text{m}$ ) (Agilent Technologies) using Agilent 1290 Infinity HPLC system (Technologies) coupled to an ESI-triple quadruple G6460 mass spectrometer (Agilent Technologies). Data were acquired in Multiple Reaction Monitoring (MRM) mode with optimized conditions (ion optics and collision energy). Peak detection, integration and quantitative analysis were done using Mass Hunter Quantitative analysis software (Agilent Technologies) based on calibration lines built with commercially available oxylipin standards (Cayman Chemicals).

## 2.8. Non-enzymatic metabolite lipid mediators analysis

After having carried out a suitable extraction of the lipids, levels of non-enzymatic lipid mediators were measured in females muscle, eggs and fry by micro-LC-MS/MS (Yonny et al., 2016). Briefly, after a lipid extraction step (Folch extraction) of samples with added Internal Standards (IS), an alkaline hydrolysis was performed. Then lipid mediators were concentrated thanks to a solid phase extraction (SPE) step conducted on weak-anion exchange materials. Therewith lipid mediators were analyzed by micro-LC-MS/MS. Mass spectrometry analyses were performed in a Sciex QTRAP 5000 (Sciex Applied Biosystems). The ionization source was electrospray (ESI) in negative mode. Detection of the fragmentation ion products from each deprotonated molecule  $[\text{M}-\text{H}]^{-}$  was performed in the multiple reaction-monitoring mode (MRM). Concentration of the non-enzymatic lipid mediators was obtained by calibration curves calculated by the area ratio of analytes and IS. Data processing was achieved using the MultiQuant 3.0 software (Sciex Applied Biosystems).

## 2.9. Statistical analysis

Statistical analyses were performed using R software (v3.5.2)/R Commander package. Data were analyzed by *t*-test (student) to assess the differences between diets. All data are expressed as mean  $\pm$  S.E. Diet

effect were considered statistically significant at  $P < 0.05$ . Analyses were carried out on untransformed raw data since criteria for normality and homogeneity of variances were fulfilled (Shapiro-Wilk's and Levene's test respectively).

## 3. Results

### 3.1. Biometrics parameters and spawning performances of females

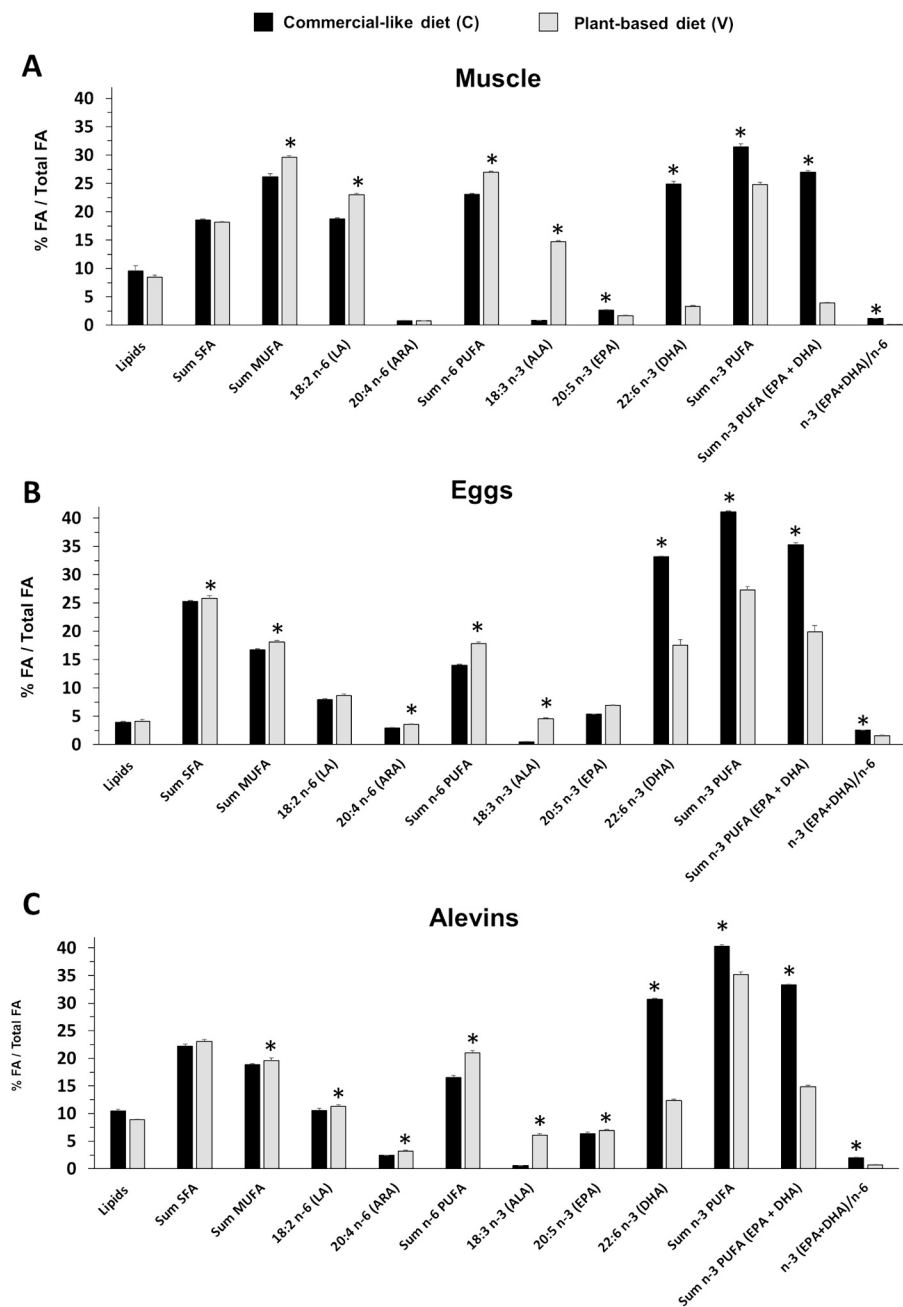
Data on biometric parameters and spawning performances are reported in Table 3. Survival rate after broodstock rearing was high for all treatments (i.e. superior to 92 %). At the end of the feeding trial, trout fed C-diet presented significant higher body weight (+23 %) and higher spawn egg weight (+33 %) compared to trout fed the V diet. The absolute fecundity was higher (+9 %) for trout fed C diet than fish fed V diet but relative fecundity was higher (+14 %) for fish fed C-diet than fish fed V-diet. The GSI was higher (+18 %) for fish fed C diet than fish fed V-diet. In terms of egg quality, C diet led to bigger eggs (4.52 mm vs. 4.19, respectively for eggs from C-fed females and V-fed females). The percentage of white eggs 24 h after hydration, used as a proxy for poor egg integrity (and therefore non viability), is not impacted by dietary treatments. No significant impact of diets was observed on survival at eyed stage, hatching rate and malformation rate at yolk resorption. However, the fry weight at yolk-sac resorption were negatively impacted by the V-diet ( $-12\%$ ).

### 3.2. Fatty acid profile of diets, broodstock muscle, egg and fry

As expected, differences in raw material composition resulted in different FA profiles in the two diets (refer to Fig. 1 and Supplemental Table 1). The most significant differences were attributed to the C-diet having a higher proportion of  $\omega$ -3 and a lower proportion of  $\omega$ -6 compared to the V-diet. Notably, the V diet lacked DHA, EPA, and arachidonic acid (AA). Conversely, the V diet exhibited a higher proportion of ALA (18:3  $\omega$ -3) and linoleic acid (LA, 18:2  $\omega$ -6) than the C-diet (27.2 % vs. 0.7 % for ALA and 25.6 % vs. 20.0 % for LA, for V and C-diets respectively). ALA and LA accounted for almost all of the total  $\omega$ -3 and  $\omega$ -6 in the V diet. The proportions of major FAs in muscle, eggs, and fry are shown in Fig. 1, while the proportions of all FAs are detailed in Supplemental Table 1. The FA profiles of female muscle, eggs, and fry exhibited substantial differences between the diets ( $n = 22$  for C-diet and 19 for V-diet). Overall, the effects of the diets on the distribution of different FAs were relatively consistent in muscle, eggs, and fry. Similar to the diet, higher proportions of monounsaturated fatty acids, the sum of  $\omega$ -3 and  $\omega$ -6, ALA, and LA were observed in the muscle, eggs, and fry of females fed the V-diet compared to those fed the C-diet. As expected, as EPA was abundant in the C diet and absent in the V diet, its levels were significantly higher in the muscle of females fed the C-diet (2.65 % compared to 1.69 % for the V-diet). Conversely, eggs from females on the V diet had similar levels of EPA to those from females on the C diet (6.96 % versus 6.95 %, for the V and C-diets, respectively), but fry from the V-diet showed significantly higher levels of EPA than those from the C-diet (6.41 % compared to 5.96 %). The AA content was significantly higher in fry on the V-diet at 3.58 %, compared to 2.97 % for those on the C-diet. This trend was also observed in eggs, where the AA concentration was 3.18 % for the V-diet, higher than 2.42 % for the C-diet.

### 3.3. Selected non-enzymatic metabolites of fatty acid profile of broodstock muscle, eggs and fry

The analysis of selected non-enzymatic metabolites of FA (ALA, AA, EPA and DHA) is detailed in Fig. 2 and in Table 2 (supplemental information). When examining the muscle composition of the broodstock (Fig. 2A), it was observed that the non-enzymatic metabolites of AA, 15-F<sub>2t</sub>-IsoP, 15-*epi*-15-F<sub>2t</sub>-IsoP, 5-F<sub>2c</sub>-IsoP, exhibited higher levels in rainbow trout fed the V-diet. Conversely, all eight selected non-



**Fig. 1.** Selected fatty lipid in female muscle, eggs and fry proportion (% of total FA) of the main FA.

Representative summary (saturated, mono-insaturated,  $\omega$ -6,  $\omega$ -3,  $\omega$ -3(EPA + DHA) and  $\omega$ -3(EPA + DHA)/ $\omega$ -6) of selected main FA proportion (LA, AA, ALA, DHA and EPA; % of total FA) and lipids level in female muscle (A) eggs (B) and fry (C) according to C and V diet. Values are expressed as group mean  $\pm$  SEM. *p*-values were produced by Welch's *t*-test followed by Tukey post hoc; differences between treatments are represented by \* (*p* < 0.05). Replicates (*n* = 8) correspond to different individual fish.

enzymatic metabolites of ALA were found to be higher in rainbow trout fed the V-diet. Interestingly, no significant differences were detected in the non-enzymatic metabolites of EPA between the two dietary groups. However, non-enzymatic metabolites of DHA, namely 10(*R*)-F<sub>4t</sub>-NeuroP, 13B(*RS*)-13-F<sub>4t</sub>-NeuroP, and 14(*S*)-14-F<sub>4t</sub>-NeuroP, were higher in rainbow trout fed the C-diet.

In the analysis of egg composition (Fig. 2b), it was observed that only one non-enzymatic metabolite of AA, 15-*epi*-15-F<sub>2t</sub>-IsoP, exhibited elevated levels in rainbow trout fed the C-diet. Additionally, ent-16-A-9-*epi*-ST- $\Delta^{14}$ -10-PhytoF and ent-16-B-9-*epi*-ST- $\Delta^{14}$ -10-PhytoF, two selected non-enzymatic metabolites of ALA, were found to be higher in rainbow trout fed the V-diet. Notably, all selected non-enzymatic

metabolites of EPA and DHA were higher in rainbow trout fed the C diet, except for one out of the nine selected non-enzymatic metabolites of DHA, 20(*R*)-20-F<sub>4t</sub>-NeuroP, which did not show significant differences between the diets (supplemental Table 3).

For fry composition (Fig. 2C), it was observed that the non-enzymatic metabolite of AA, 15-F<sub>2t</sub>-IsoP, exhibited higher levels in the fry from female broodstock fed the C and V-diet respectively. Additionally, 9-*epi*-9-F<sub>1t</sub>-PhytoP, 9-L<sub>1t</sub>-PhytoP, ent-16-A-9-*epi*-ST- $\Delta^{14}$ -10-PhytoF, ent-16-B<sub>1t</sub>-PhytoP, ent-16-F<sub>1t</sub>-PhytoP, ent-16-*epi*-16-F<sub>1t</sub>-PhytoP which are selected non-enzymatic metabolites of ALA, were found to be higher in the fry from female broodstock fed the V-diet. Only, one non-enzymatic metabolite of EPA, 8(*R*)-8-F<sub>3t</sub>-IsoP, showed higher levels in the fry from

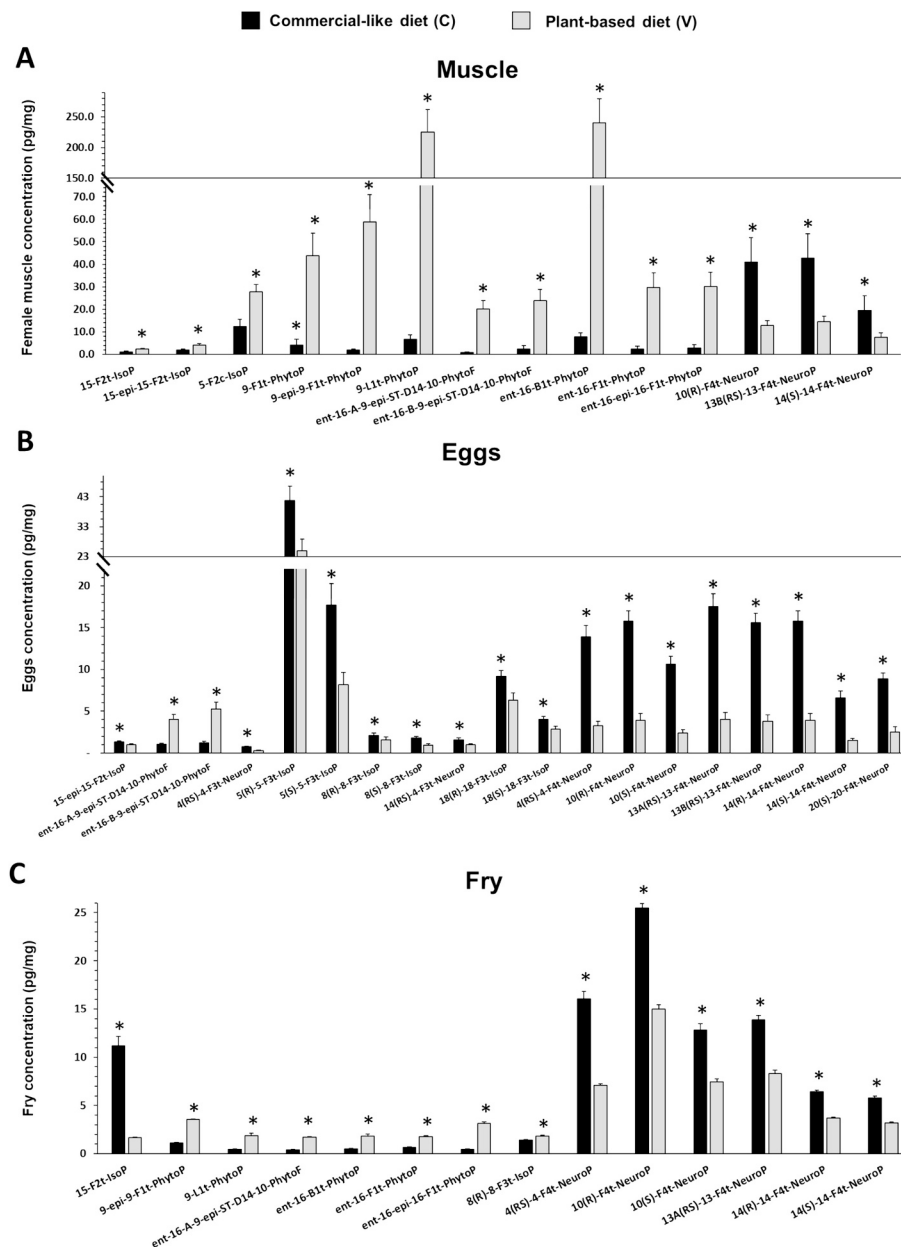


Fig. 2. Proportion of selected non-enzymatic lipid oxidation mediators (pg/mg) in female muscle, eggs and fry.

Representative selected of non-enzymatic lipid oxidation mediators (15-F<sub>2t</sub>-IsoP, 9-F<sub>1t</sub>-PhytoP, 9-epi-9-F<sub>1t</sub>-PhytoP, 9-L<sub>1t</sub>-PhytoP, ent-16-A-9-epi-ST- $\Delta^{14}$ -10-PhytoF, ent-16-B-9-epi-ST- $\Delta^{14}$ -10-PhytoF, ent-16-B<sub>1t</sub>-PhytoP, ent-16-F<sub>1t</sub>-PhytoP, ent-16-epi-16-F<sub>1t</sub>-PhytoP, 4(RS)-4-F<sub>3t</sub>-NeuroP, 5(R)-5-F<sub>3t</sub>-IsoP, 5(S)-5-F<sub>3t</sub>-IsoP, 8(R)-8-F<sub>3t</sub>-IsoP, 8(S)-8-F<sub>3t</sub>-IsoP, 14(RS)-4-F<sub>3t</sub>-NeuroP, 18(R)-18-F<sub>3t</sub>-IsoP, 18(S)-18-F<sub>3t</sub>-IsoP, 4(RS)-4-F<sub>4t</sub>-NeuroP, 10(R)-4-F<sub>4t</sub>-NeuroP, 10(S)-4-F<sub>4t</sub>-NeuroP, 13A(RS)-13-F<sub>4t</sub>-NeuroP, 13B(RS)-13-F<sub>4t</sub>-NeuroP, 14(R)-14-F<sub>4t</sub>-NeuroP, 14(S)-14-F<sub>4t</sub>-NeuroP, 20(R)-20-F<sub>4t</sub>-NeuroP, 20(S)-20-F<sub>4t</sub>-NeuroP; pg/mg) in female muscle (A) eggs (B) and fry (C) according to C and V diet. Values are expressed as group mean  $\pm$  SEM. p-values were produced by Welch's t-test followed by Tukey post hoc; differences between treatments are represented by \* (p < 0.05). Replicates (n = 8) correspond to different individual fish.

female broodstock fed the C diet. Additionally, 4(RS)-4-F<sub>4t</sub>-NeuroP, 10(R)-F<sub>4t</sub>-NeuroP, 10(S)-F<sub>4t</sub>-NeuroP, 13A(RS)-13-F<sub>4t</sub>-NeuroP, 14(R)-14-F<sub>4t</sub>-NeuroP, 14(S)-14-F<sub>4t</sub>-NeuroP, selected non-enzymatic metabolites of DHA, were found to be higher in the fry from female broodstock fed the C diet.

The sum of non-enzymatic metabolites of ALA was higher in broodstock muscle, eggs and fry from V-fed females than those from C-fed females (Supplemental Table 2). The sum of non-enzymatic metabolites of DHA was higher in the muscle and eggs of C-fed females than in those from V-fed females.

### 3.4. Selected enzymatic metabolites of fatty acids profile of broodstock muscle, eggs and fry

The analysis selected enzymatic metabolites of FAs (ALA, AA, EPA and DHA) is detailed in Fig. 3 and in Table 3 (supplemental information). For muscle composition (Fig. 3a), the 14-HoDE and 17-HoDE, two enzymatic metabolites of DHA from lipoxygenase (LOX), were lower for female fed the V-diet. No significant changes (or detection) occurred in the other enzymatic metabolites of FA (Table 3).

For eggs composition (Fig. 3B), the proportion of PGD<sub>2</sub>, an enzymatic metabolite of AA from cytochrome P450 (Cyto-P450), was lower in the eggs from fish fed the C-diet. For the enzymatic metabolite of AA from lipoxygenase, the proportion of 5-HETE was lower in the eggs from

**Table 2**  
Selected fatty acid composition of experimental diet (% total fatty acid).

Diets	Commercial-like diet (C)	Plant-based diet (V)
12:00	0.19	0.30
14:00	1.85	0.40
15:00	0.19	0.04
16:00	13.37	16.13
17:00	0.23	0.07
18:00	2.90	3.59
20:00	0.30	0.24
22:00	0.27	0.23
0.24:00	0.08	0.13
Sum of saturated fatty acids	<b>19.38</b>	<b>21.12</b>
16:1 n-7	1.92	0.17
18:1 n-9	17.54	25.05
20:1 n-9	1.21	0.28
22:1 n-9	1.27	0.05
Sum of MUFAs	<b>22.42</b>	<b>25.65</b>
18:2 n-6	20.02	25.57
18:3 n-6	0.13	0.0
20:2 n-6	0.14	0.0
20:3 n-6	0.01	0.0
20:4 n-6	0.75	0.0
22:2 n-6	0.10	0.08
22:4 n-6	0.24	0.0
22:5 n-6	1.74	0.0
Sum of n-6 LC-PUFAs	<b>23.81</b>	<b>25.80</b>
16:4 n-3	0.11	0.0
18:3-3	0.69	27.22
n-3 (DHA + EPA) / n-6	0.41	0.0
20:3 n-3	0.10	0.0
20:4 n-3	0.29	0.0
20:5 n-3	3.61	0.0
21:5 n-3	0.21	0.0
22:4 n-3	0.0	0.0
22:5 n-3	1.69	0.0
22:6 n-3 (DHA)	26.37	0.0
Sum of n-3 LC-PUFAs	<b>34.00</b>	<b>27.23</b>
Sum of n-3 (EPA + DHA)	<b>29.98</b>	<b>0.0</b>
n-3 (DHA + EPA) / n-6	<b>1.26</b>	<b>0.0</b>

female fed the V-diet, 8-HETE was higher in females fed the C-diet and 8isoPGA<sub>2</sub> was higher in females fed the V-diet. For TBX<sub>2</sub>, an enzymatic metabolite of AA from lipoxygenase, the proportion was higher in rainbow trout fed the V-diet compared to the C-diet. The 14-HoDE and 17-HoDE, two enzymatic metabolites of DHA from lipoxygenase were lower in females fed the V-diet. No significant changes (or detection) occurred in the other enzymatic metabolites of FA (supplemental Table 3).

For fry composition (Fig. 3C), two prostaglandins (PG); PGE<sub>2</sub> and PGF<sub>2α</sub>, two enzymatic metabolites of AA via cytochrome P450 and for 5oxoETE, 8-HETE and 8isoPGA<sub>2</sub>, 12-HETE, 15-HETE and TBX<sub>2</sub>, six enzymatic metabolites of AA via lipoxygenase, the proportion were higher in the fry from maternal broodstock fed the C diet. This is also the case for 9-, 13-HoDE and 18-HETE, enzymatic metabolites of LA and EPA from lipoxygenase respectively. The enzymatic metabolites of DHA and EPA from lipoxygenase, PGE<sub>3</sub> was higher in fry from female fed the C-diet. The two enzymatic metabolites of DHA from lipoxygenase, 7(R)-MaR1 and 4-HoDE were higher in fry from female fed the C-diet. This is also the case for the 5,6-EET, an enzymatic metabolite of DHA from cytochrome P450. There were no significant changes (or detections) in the other enzymatic metabolites of FA (Table 9). The sum of enzymatic metabolites of AA was higher in muscle and fry from C-fed females than those from V-fed females but higher in eggs from V-fed females than those from C-fed females (Supplemental Table 3). The sum of enzymatic metabolites of EPA was higher in eggs from V-fed females than those from C-fed females but higher in fry from C-fed females than those from V-fed females (Supplemental Table 3). Finally, the sum of enzymatic metabolites of DHA was higher in muscle, eggs and fry from C-fed females than those from V-fed females (Supplemental Table 3).

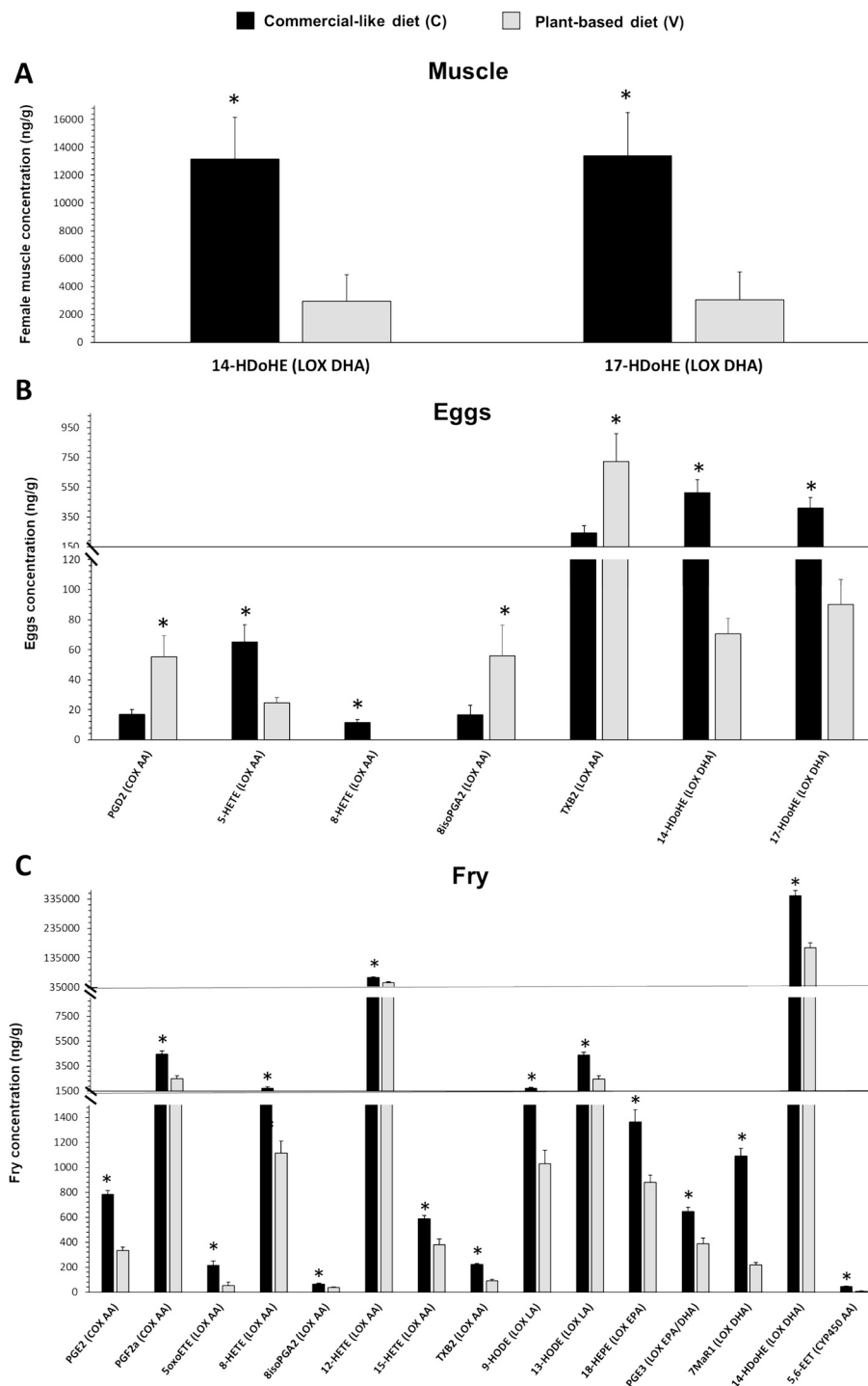
#### 4. Discussion

Considering the significant shift towards plant-based feed formulations, this discussion delves into the intricate relationship between altered PUFA content in these diets and the resulting metabolic responses in rainbow trout. Recognizing the pivotal role of egg lipids as the primary source of essential FA for fry, any deficiencies or imbalances in eggs could potentially impact tissue PUFA incorporation, lipid metabolism, and the overall growth and development during embryogenesis. Our objective is to explore how changes in dietary PUFA profiles, stemming from the transition to plant-based ingredients, influence the FA profile of fry and the synthesis of key enzymatic and non-enzymatic lipids metabolites. This investigation is crucial for understanding the repercussions of such dietary shifts on reproductive performance and provide a comprehensive perspective on metabolic adaptations and their potential influence on fish reproduction and development. To the best of our knowledge, this study represents the first attempt to elucidate the effects of a V-diet compared to a conventional C-diet on enzymatic and non-enzymatic lipid metabolites in female broodstock eggs and fry over a two-year feeding period, starting from the first meal in trout.

Adaptive synthesis of LC-PUFAs in rainbow trout on plant-based diet: Implications for reproductive outcomes.

The reduced reproductive performance observed in trout on the V-diet, characterized by reduced female body weight, lower fecundity, and smaller egg size, presents an intriguing contrast to the otherwise unimpaired egg and embryo quality. The resilience in egg quality and embryonic development, with similar rates of viable unfertilized eggs, survival to the eyed stage, hatching, and malformation between the C and V diets, suggests a remarkable biological adaptation. In particular, female rainbow trout show an extraordinary capacity to biosynthesize PUFA such as AA, EPA and DHA from available dietary precursors (LA and ALA). The FA composition of fish muscle serves as a reliable proxy for diet, supporting the findings of previous studies in fish (Izquierdo et al., 2015; Mazorra et al., 2003). Notably, despite the complete absence of these three FA (AA, EPA, DHA) in the V-diet, they were present in significant proportions in the eggs and subsequently in the fry. For EPA and AA, these proportions were even higher in the eggs and fry from females fed the V-diet compared to the C-diet. This compelling evidence suggests that the neosynthesized PUFA were selectively channeled into the eggs, as previously shown by Lazzarotto et al. (in 2015) (Lazzarotto et al., 2015) and Cardona et al. (in 2022) (Cardona et al., 2022). However, this study is the first to observe this effect during the first reproduction of female rainbow trout after 2 years of feeding from the first meal. In fish, eggs serve as the critical nutrient reservoir for the embryo until it begins to feed externally (Mazorra et al., 2003). The embryonic phase is classified as endogenous feeding, with essential nutrients for processes such as cell division and organogenesis being obtained from the yolk. Despite substantial differences in diets (V or C), fish eggs in the lecithotrophic stage consistently provide all essential nutrients for larval development, ensuring homeostasis and growth until the larvae begin to consume external nutrients (Mourete and Vázquez, 1996). Thus, the biochemical synthesis pathways of female trout compensated for dietary deficiencies and ensured the transfer of essential FA to the offspring. This adaptive mechanism highlights the potential for a V diet to support critical life stages of trout without compromising critical aspects of reproductive success and offspring development. In summary, our results suggest a compensatory physiological mechanism in response to the V-diet to maintain reproductive functions from the first reproductive cycle. This suggests that the absence of fish-derived ingredients in the V-diet did not adversely affect the essential quality and developmental potential of eggs and fry. Given the observed differences in reproductive performance in trout fed a V-diet, it is pertinent to investigate how dietary FA profiles affect the formation of enzymatic and non-enzymatic lipid metabolites in eggs and fry. These metabolites, directly derived from PUFA, play a critical role in physiological processes and may be the key to linking dietary FA





**Fig. 3.** Proportion of selected enzymatic lipid oxidation mediators (ng/g) in female muscle, eggs and fry. Representative selected of enzymatic lipid oxidation mediators (PGE<sub>2</sub>, PGF<sub>2α</sub>, 5oxoETE, 8-HETE, 8-isoPGA<sub>2</sub>, 12-HETE, 15-HETE, 9-HoDE, 13-HoDE, 18-HEPE, TBX<sub>2</sub>, PG<sub>3</sub>, 7(R)-MaR1, 14-HoDE, 5-6-EET; ng/g) in female muscle (A) eggs (B) and fry (C) according to C and V diet. Values are expressed as group mean ± SEM. p-values were produced by Welch's t-test followed by Tukey post hoc; differences between treatments are represented by \* (p < 0.05). Replicates (n = 8) correspond to different individual fish.

composition to reproductive outcomes. Understanding the nuances of how these metabolites, formed by enzymatic and non-enzymatic reactions, are influenced by dietary FA composition is essential. This approach will allow a more comprehensive assessment of the effects of V-diets on trout reproductive performances of trout and provide valuable insights into potential changes in lipid metabolism underlying the observed differences in reproductive performance.

Impact of an entire two-year breeding cycle on non-enzymatic

metabolites level of broodstock muscle, eggs and fry from females fed the C and V-diets.

The analysis of non-enzymatic metabolites in the tissues studied has provided groundbreaking insights. For the first time, it has been shown that all 29 metabolites quantified here are present in the muscle and eggs of breeding females, with 21 of them also found in the fry. Our data consistently reveal significant differences in metabolite levels across the three tissues. In eggs, the metabolite composition aligns with the dietary

**Table 3**

Reproductive performances of females, egg quality and fingerling biometric parameters according to diet treatment during reproductive Cycle 1.

Diets	Commercial-like diet (C)	Plant-based diet (V)	Statistical results
<b>FEMALE BIOMETRIC PARAMETERS AND SPAWING PERFORMANCES</b>			
Survival (%)	100	92.1	na
Egg spawn weight (g)	236.06 ± 17.11	159.26 ± 12.36	***
Absolute fecundity	5365.52 ± 338.09	4897.62 ± 402.57	*
Relative fecundity	2583.07 ± 96.40	3014.38 ± 243.83	*
HSI	1.22 ± 0.35	1.20 ± 0.20	n.s
VSI	6.22 ± 1.26	6.51 ± 1.60	n.s
GSI	14.22 ± 2.73	11.68 ± 2.46	*
<b>EGG QUALITY</b>			
Egg diameter (mm)	4.52 ± 0.05	4.19 ± 0.07	***
Variation Coefficient of egg diameter	3.05 ± 0.07	3.32 ± 0.16	n.s
White eggs (%)	0.64 ± 0.21	0.57 ± 0.24	n.s
<b>EMBRYO DEVELOPMENT</b>			
Survival at eyed stage (%)	87.31 ± 5.58	84.54 ± 4.02	n.s
Hatching rate (%)	84.721 ± 6.39	81.63 ± 3.42	n.s
Malformation rate at resorption stage (%)	5.26 ± 1.80	11.42 ± 3.42	n.s
Fry weight (mg)	78.2 ± 7.59	69.1 ± 7.31	***

trend, indicating a higher abundance of metabolites derived from DHA and EPA in the C diet compared to the V diet. Notably, two phytoprostanes derived from ALA are more prevalent in eggs from the V diet. This suggests that the diet provided to the females strongly influences the composition of non-enzymatic metabolites in the egg, reflecting what occurs in the ovaries of the female, directly impacted by the intake of PUFA from the diet. In fry, the influence of the diet is still evident, although not necessarily with the same non-enzymatic metabolites that differ from those in the eggs based on the diet received by the female. Upon closer examination, metabolites derived from EPA are much less affected by the diet received by the female, and in some cases, there is a reversal. For instance, 8(R)-F<sub>31</sub>-isoP are present at higher amounts in fry from females fed the V diet than in fry from females fed the C-diet. In contrast, in the eggs, the proportion of EPA was higher with the V diet. This could indicate that non-enzymatic metabolites continue to be formed during embryogenesis from the PUFAs present in the egg. With regard to the various metabolites produced, it is difficult to determine their specific benefits for embryonic development, as there are few or no studies on this subject. Over the past two decades, numerous studies investigated the complex biological roles of these metabolites, particularly in mammals (Galano et al., 2017). It is noteworthy that neuroprostanes derived from DHA, as well as EPA-derived isoprostanes, which are found at higher levels in eggs from females fed the C-diet, are known for their anti-inflammatory role in mammals (Gabbs et al., 2015). However, few studies have addressed their role in female reproduction.

In the field of reproduction, several studies have highlighted the significant impact of F<sub>4</sub>-Neuroprostanes and F<sub>2</sub>-Isoprostanes (no study to date on the role of F<sub>1</sub>-phytoprostanes and F<sub>3</sub>-isoprostane role in reproduction), which have biological activity in sperm function. These compounds, when added to sperm suspensions, promote capacitation, thereby increasing the number of sperm capable of fertilization (Castellini et al., 2019; Moretti et al., 2023; Signorini et al., 2021). If, as has been suggested in mammalian studies (Castellini et al., 2019), the significant presence of the neuroprostane family in the diet does indeed play a key role in fertility and, consequently, successful reproduction, further research is needed to validate these findings. A recent human study showed that low levels of placental F<sub>4</sub>-neuroprostanes from DHA may indicate impaired fetal development and offspring health (Barden et al., 2012). Interestingly, in our study, numerous F<sub>4</sub>-neuroprostanes (−4, −10, −13, 14- -20-F<sub>4</sub>-neuroprostanes) are more abundant in the muscle and eggs of the female and ultimately in the fry. Conversely, they

are on average three to four times less abundant in the muscle and eggs of females fed a V diet for two years. Another study reported that in normal pregnant women, maternal plasma F<sub>4</sub>-neuroprostanes and F<sub>2</sub>-isoprostanes were negatively related to birth weight (Ferreira et al., 2023). The authors observed an inverse relationship between maternal F<sub>2</sub>-isoprostanes and F<sub>4</sub>-neuroprostanes and birth weight, suggesting their potential as predictors of low birth weight in normal pregnancy. In our study, similar to F<sub>4</sub>-neuroprostanes, we observed that F<sub>2</sub>-isoprostanes are also more abundant in females fed a C-diet compared to a V-diet, although this pattern is less evident in eggs and fry, which are nevertheless smaller at hatch. Although further investigation is required, significantly lower levels of these metabolites may play a role in smaller eggs and ultimately smaller fry, as demonstrated in our studies.

Impact of an entire two-year breeding cycle on enzymatic metabolites level of broodstock muscle, eggs and fry from females fed the C and V diets.

The importance of enzymatic oxylipins in fish reproduction is frequently emphasized. However, the term “eicosanoids” is often used interchangeably with oxylipins, which may not be optimal, as it is intended to include only those oxylipins derived from EPA or AA (Bruce et al., 1999; Henrotte et al., 2011; Knight and Van Der Kraak, 2015a). Analysis of the enzymatic metabolites in the tissues studied shows, for the first time, that among those quantified, 16 are present in the muscle of females, 14 in eggs, and 21 in fry. To the best of our knowledge, this is the first measurement of these enzymatic metabolites of FA in these tissues after two years of feeding, starting from the first meal in trout.

There is a stark contrast between the quantities present in eggs and fry. There are over 160 times more metabolites in fry than in eggs, suggesting the formation of enzymatic metabolites of PUFAs present in the egg during embryogenesis (Ugwuagbo et al., 2019). The oxylipin family, which includes eicosanoids, comprises several inflammatory mediators., PGs have been extensively studied in relation to fish maturation, ovulation and spawning (Criscuolo-Urbini et al., 2012; Fujimori et al., 2011; Knight and Van Der Kraak, 2015a; Lister and Van Der Kraak, 2008, 2009; Norambuena et al., 2012). Oxylipins also include other subclasses such as leukotrienes, hydroxyeicosatetraenoic acids, thromboxanes, and prostacyclins, each of which plays a distinct role in regulating physiological processes related to ovulation. However, little or no research has been carried out in teleosts in a reproductive context. The involvement and control of eicosanoids in teleost oocyte maturation, ovulation, and spawning remains a subject of ongoing debate. The specific oxylipins involved, their mechanisms of action, and their position in the physiological cascade appear to differ between species (Knight, 2014; Knight and Van Der Kraak, 2015b). In the muscles of female broodstock, only two DHA metabolites, 14- and 17-HDoHE, show a higher abundance in those fed the C-diet compared to those fed the V-diet. This limited variation suggests an efficient mobilization of most enzymatic metabolites from muscle to eggs, a phenomenon commonly observed for PUFA (Cleveland et al., 2012; Nassour and Léger, 1989; Tocher, 2003). Consequently, a relatively similar profile within the muscle is maintained between the two diets. Given that these two metabolites are the most abundant enzymatic metabolites in muscle, it is plausible that egg requirements have reached a saturation point, especially considering their high quantities in both eggs and fry. These dietary effects extend to the eggs for both metabolites and to the fry for 14-HDoDE only. In particular, these metabolites are recognized for their anti-inflammatory role in mammals, as demonstrated by previous studies investigating the biological activity of the hydroxydocosahexaenoic acid family (Bento et al., 2011; González-Pérez et al., 2006; Lima-García et al., 2011; Weylandt et al., 2011).

The increased levels EPA and DHA in the C-diet resulted in a significant increase in enzymatic metabolites from EPA and DHA in eggs, particularly for DHA metabolites, and in fry of females fed the C-diet, consistent with the PUFA composition of the diet. In contrast, there was a divergence between eggs and fry for enzymatic metabolites of AA.

While eggs from females on the V-diet showed a higher presence of enzymatic metabolites of AA compared to those from females on the C-diet, despite the fact that the V-diet contained less AA than the C-diet, the opposite trend was observed in the fry. Fry from females on the C-diet had higher levels of enzymatic metabolites of AA. Examination of PGs, the most studied oxylipins in fish, revealed that fry from females fed the V-diet had higher levels of PGD<sub>2</sub> and PGF<sub>2A</sub>. Conversely, PGE<sub>2</sub> was more abundant in eggs from females on the C-diet. AA serves as the major eicosanoid precursor in fish cells (Bell et al., 1994; Tocher et al., 1997), and these compounds play a crucial role in the control of ovulation (Ann Sorbera et al., 2001; Mustafa and Srivastava, 1989) and are likely to be involved in embryogenesis, immune system development, hatching, and early larval performance. However, the excess of enzymatic metabolites of AA in eggs may be related to the increased inflammation induced by the V-diet. Excessive enzymatic metabolites of AA have been associated with inflammation and oxidative stress (Bao et al., 2023; Tian et al., 2019). Furthermore, prostaglandins, especially PGE<sub>2</sub>, are known to be produced by early embryos and play a role in promoting embryonic vascular maturation and angiogenesis in zebrafish (Ugwuagbo et al., 2019). The observed changes in enzymatic metabolites of AA, also in terms of quantity, between eggs and fry in this study highlight that the fish embryo already has a highly active system capable of catalyzing eicosanoid formation. This may explain why, even with an excess of enzymatic metabolites of AA in the eggs, the V-diet does not result in a reduction in hatchability and malformation rates in newly hatched larvae compared to the C-diet. In general, oxylipins have been studied little or not at all in teleosts in a reproductive context. In mammals, research suggests that the ovulation period is associated with cyclic tissue remodelling processes leading to changes in the synthesis of enzymatic metabolites. In particular, enzymatic metabolites of AA and LA through the cytochrome P450 and LOX pathways play a critical role in the late stages of pregnancy. HETEs and HODEs are integral components of a normal reproductive cycle and pregnancy (Kikut et al., 2020) and are found in the placenta, amniotic fluid and myometrium of mammals (Pearson et al., 2009; Sato et al., 2008; Zhang et al., 2007). They have the ability to modulate the contractile activity of reproductive tissues. Certain metabolites, such as 12-15-HETE and 13-HODE, may influence the ovulation process. It is plausible that 12/15-LOX enzymes may act as important modulators of uterine function during implantation (Corriveau et al., 2014). These enzymatic metabolites, found at higher levels in both eggs and juveniles from broodstock fed C-diet, could have a significant impact on fecundity rates and reproductive success in farmed fish. Therefore, although this study does not allow an assessment of the effect of these metabolites on the reproductive response of the two diets, we do not overlook the possible influence of these metabolites on the results. Further investigation of their role in this event would be appropriate.

## 5. Conclusion

In conclusion, the results of this experiment highlights the significant impact of maternal diet in the first reproductive performance cycle and the PUFA profile of both eggs and hatched fry. The study highlights the critical role of maternal diet in shaping the production of both non-enzymatic and enzymatic lipid metabolites in eggs and fry. In particular, females on the V diet appear to induce an excessive production of AA-derived oxylipins, which may induce inflammation. However, the fish embryo has an active system capable of catalyzing eicosanoid formation, allowing it to mitigate the negative effects of this overproduction. This may explain why, despite the excess of AA-derived oxylipins in the eggs, the V diet does not result in a reduction in hatching and malformation rates in newly hatched fry compared to the C diet. Therefore, FA metabolites have the potential to serve as markers for poor reproductive performance in females and may affect fry. Thus, the supplementation of diets with appropriate purified vegetable oils, fish oil, or a combination of both may generate local anti-inflammatory,

antioxidant, and anti-proliferative metabolites. These metabolites may contribute to the successful management of reproduction in fish farming.

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## Ethics approval and consent to participate

Experiments were conducted at the INRAE facilities of Léés-Athas, France (permit no. A64.104.1, Pyrenees Atlantiques, France). All fish were reared and handled in strict accordance with the French (Decree No 2013-118, 1 February 2013) and European (i.e. Directive 2010/63/EU) animal welfare policies, followed ARRIVE guidelines.

## Consent for publication

Not applicable.

## CRediT authorship contribution statement

**Emilie Cardona:** Software, Methodology, Formal analysis, Data curation, Writing – original draft. **Elodie Baranek:** Data curation. **Claire Vigor:** Software, Formal analysis, Data curation. **Valérie Gros:** Software, Formal analysis, Data curation. **Guillaume Reversat:** Software, Formal analysis, Data curation. **Anne Surget:** Formal analysis, Data curation. **Laurence Larroquet:** Formal analysis, Data curation. **Patrick Maunas:** Methodology, Data curation. **Nicolas Turrone:** Methodology, Data curation. **Camille Oger:** Methodology, Conceptualization, Writing – review & editing. **Jean-Marie Galano:** Methodology, Conceptualization, Writing – review & editing. **Thierry Durand:** Methodology, Conceptualization, Writing – review & editing. **Jérôme Roy:** Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization, Writing – original draft.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2024.741602>.

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