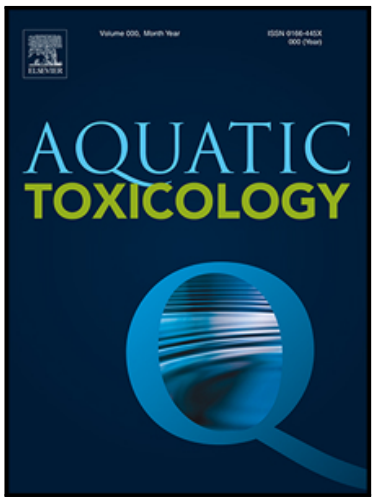

Dietary methylmercury and fatty acids affect the lipid metabolism of adipose tissue and liver in rainbow trout

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HIGHLIGHTS

- Whole-body lipid content was decreased by MeHg and increased by LA.
- Adipocytes were larger after MeHg exposure and feeding with plant-derived oils.
- Fatty acid composition reflected retention and bioconversion of dietary fatty acids.
- MeHg decreased n-6 PUFA amounts in hepatic membranes of fish rich in n-6 PUFA.

Dietary methylmercury and fatty acids affect the lipid metabolism of adipose tissue and liver in rainbow trout

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ABSTRACT

Methylmercury (MeHg) is a pervasive environmental contaminant in aquatic ecosystems that can reach elevated concentrations in fish of high trophic levels, such as salmonids. The present study aims at investigating the individual and combined impacts of dietary MeHg and fatty acids on lipid metabolism in juvenile rainbow trout (*Oncorhynchus mykiss*) with a focus on two key organs, adipose tissue and liver. MeHg and fatty acids are both known to act on energy homeostasis although little is known about their interplay on lipid metabolism in fish. Fish were fed diets enriched in linoleic acid (LA, 18:2 n-6), α-linolenic acid (ALA, 18:3 n-3), eicosapentaenoic acid (EPA, 20:5 n-3) or docosahexaenoic acid (DHA, 22:6 n-3) for ten weeks, with the addition of MeHg to the diets during the last six weeks (0, 2.4 or 5.5 mg MeHg/kg dry matter). LA and ALA are polyunsaturated fatty acids (PUFA) typical of plant-derived oils whereas EPA and DHA are n-3 long chain PUFA largely found in fish oil, all used in feed formulation in aquaculture. The results showed that the LA-enriched diet induced a higher whole-body lipid content compared to the three other diets. On the contrary, the addition of MeHg led to a significant reduction of the whole-body lipid content, regardless of the diet. Interestingly, the adipocytes were larger both in presence of LA, compared to EPA and DHA,

or MeHg, indicating a lipogenic effect of these two compounds. No effect was, however, observed on lipid accumulation per gram of adipose tissue. The fatty acid composition of adipose tissue and liver was significantly modified by the dietary lipids, reflecting both the fatty acid composition of the diets and the high bioconversion capacity of the rainbow trout. Exposure to MeHg selectively led to a release of n-6 PUFA from the hepatic membranes of fish fed the LA-enriched diet, showing a disruption of the pathways using n-6 PUFA. This study highlights the significant impact of MeHg exposure and dietary fatty acids on lipid metabolism in fish. Further investigation is needed to elucidate the underlying mechanisms and to explore the potential involvement of other organs.

KEYWORDS

- Methylmercury
- Polyunsaturated fatty acids
- Adipose tissue
- Liver
- Rainbow trout

ABBREVIATIONS

AA, arachidonic acid (20:4 n-6); ALA, α-linolenic acid (18:3 n-3); DHA, docosahexaenoic acid (22:6 n-3); DM, dry matter; EPA, eicosapentaenoic acid (20:5 n-3); HSI, hepatosomatic index; LA, linoleic acid (18:2 n-6); MeHg, methylmercury; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; THg, total mercury.

1 INTRODUCTION

Methylmercury (MeHg) is a toxic and ubiquitous organo-metal compound (Clarkson, 2002) that is produced via the methylation of inorganic mercury by bacteria in aquatic sediments (Selin, 2009). Inorganic mercury mainly comes from legacy and current anthropogenic emissions and, to a lesser extent, from natural emissions (UN Environment, 2019). MeHg bioaccumulates and biomagnifies throughout the aquatic food chain (Lavoie et al., 2013)

resulting in high levels of contamination in aquatic species, especially those at high trophic levels (Schwindt et al., 2008). Total mercury (THg) concentrations range between 17 and 411 ng/g of wet weight in salmonids while concentrations reach up to 1327 ng/g in top predators such as sharks and swordfish (Mahaffey et al., 2004; Schwindt et al., 2008). Depending on the location and the past and current human activities, some areas can be more contaminated with MeHg levels in fish exceeding 2000 ng/g (Durrieu et al., 2005; Wiener et al., 2002). These concentrations are at levels of concern for animal and human health (Bourdineaud et al., 2008; Bourdineaud et al., 2011; Cambier et al., 2018; Okpala et al., 2018).

MeHg present in the diet is absorbed by the gastrointestinal tract and distributed in the whole body via the blood stream (Syversen and Kaur, 2012). MeHg is a well-known neurotoxicant in mammals and fish but other organs like liver, muscle and kidney can also be affected (Cambier et al., 2009; Farina et al., 2011; Kidd and Batchelar, 2011). Elevated oxidative stress often results from MeHg exposure due to the production of reactive oxygen species and the reduction of antioxidant defences (Kidd and Batchelar, 2011). Hepatic transcriptomic and proteomic analyses in fish revealed that pathways such as fatty acid biosynthesis and β-oxidation, electron transport chain and phospholipid metabolism, were among the most affected by mercury exposure (Klaper et al., 2008; Olsvik et al., 2021; Ung et al., 2010; Yadetie et al., 2016). Similarly, dyslipidemia (abnormal levels of lipids in the blood) has also been observed in mice exposed to MeHg (Leocádio et al., 2020). These results illustrate that MeHg could impair lipid metabolism and cellular energy homeostasis (reviewed by Zhou et al. (2022)). Fatty acid composition can also be affected by MeHg, for example n-6 polyunsaturated fatty acids (PUFA) content in phospholipid membranes was reduced in rainbow trout hepatocytes and Atlantic salmon brain following MeHg exposure (Amlund et al., 2012; Ferain et al., 2018). Adipose tissue also appears as a potential target of heavy metals including MeHg (reviewed by Tinkov et al. (2021)). For instance, MeHg induced a major intracellular lipid accumulation in rainbow trout preadipocytes (Tinant et al., 2021). In contrast, *in vivo*, adipose tissue mass was reduced in male mice exposed to MeHg in drinking water (Ferrer et al., 2018).

Lipids, and their constitutive fatty acids, are key organic molecules that fulfil crucial functions to maintain fish health. They are (i) a source of energy in the form of neutral lipids, (ii) constituents of cellular membranes in the form of phospholipids, (iii) precursors of cell

signaling molecules such as eicosanoids, and (iv) nuclear receptor ligands in the form of free fatty acids (Tocher, 2003). Perivisceral adipose tissue, liver and muscle are the major sites of lipid deposition in fish (Weil et al., 2013). The main cell type in adipose tissue is represented by adipocytes that contain a large lipid droplet filled with neutral lipids. The adipose tissue is able to expand through hyperplasia (i.e., increased number of cells) and/or hypertrophy (i.e., increased cell size) in case of positive energy balance, and to shrink during lipid mobilisation through lipolysis (Weil et al., 2013). The liver is a central organ in lipid metabolism and fatty acid bioconversion. In particular, rainbow trout and other salmonids possess relatively high fatty acid bioconversion capacity. Two essential fatty acids, α-linolenic acid (ALA, 18:3 n-3) and linoleic acid (LA, 18:2 n-6), are desaturated and elongated to produce long chain PUFA such as eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) for the n-3 pathway and arachidonic acid (AA, 20:4 n-6) for the n-6 pathway (Tocher, 2003). However, despite high bioconversion capacity, fish fed a diet poor in long chain PUFA do not produce these fatty acids at concentrations as high as those found in fish fed diets rich in long chain PUFA such as EPA and DHA (Francis et al., 2014; Mellery et al., 2017; Turchini and Francis, 2009). This is of particular interest in aquaculture in the context of fish oil replacement by more sustainable oils from economic and environmental perspectives, mainly plant-derived oils. Contrary to fish oil rich in EPA and DHA, plant-derived alternatives are rich in PUFA with eighteen carbons such as LA and ALA (Glencross, 2009; Leaver et al., 2008; Tocher, 2015).

If lipid and, in particular, fatty acid metabolism can be disrupted by MeHg, fatty acids can in turn modulate the biological responses to MeHg. As an example, the transcriptional response to MeHg was shown to differ in the liver, brain and white muscle of Atlantic salmon fed a diet containing fish oil compared to soybean oil (Olsvik et al., 2011). It has also been shown using *in vitro* and *in vivo* approaches in fish and mammals that PUFA can modulate MeHg uptake, oxidative stress and cell sensitivity to MeHg (Ferain et al., 2016; Kaur et al., 2007; Nøstbakken et al., 2012). Currently, little is known about the interplay between MeHg and fatty acids on lipid metabolism at the organ level and on the resulting effect on energy homeostasis at body level in fish.

This study aims to assess the individual and combined impacts of MeHg and four dietary fatty acids on whole-body energy homeostasis and fatty acid composition of adipose tissue and liver in juvenile rainbow trout. The fatty acids under investigation are EPA and DHA, which are typical of fish oil, as well as LA and ALA, which are largely found in plant-derived oils frequently studied as alternative lipid sources for salmonids in aquaculture. To our knowledge, it is the first *in vivo* investigation of the interplay between MeHg and these four fatty acids on lipid metabolism in rainbow trout. This study should be of great interest to the aquaculture in regards to the feed formulations that promote fish and human health.

2 MATERIAL & METHODS

2.1 Experimental design

The experiment was carried out with juvenile rainbow trout (*Oncorhynchus mykiss*) for ten weeks. Four experimental diets, differing only in their fatty acid composition, were prepared. Diets were enriched in either LA (18:2 n-6), ALA (18:3 n-3), EPA (20:5 n-3) or DHA (22:6 n-3). For the initial 4-week period, fish (50 fish/tank, randomly distributed) were fed one of the four diets at apparent satiation, with no exposure to MeHg (12 tanks per diet, randomly allocated). From week 5 to 10, fish (20 fish/tank) received a fixed amount (2% of tank biomass/day) of the same diet treated or not with MeHg (0, 2.4 or 5.5 mg MeHg/kg of dry matter (DM)) (4 tanks per experimental condition, randomly allocated) (Figure 1). The MeHg concentrations were chosen to fall within the range found in lightly and heavily contaminated food webs (Durrieu et al., 2005; Wiener et al., 2002). At the end of week 10, fish were euthanized and sampled to determine their body proximate composition and mercury accumulation. Furthermore, mercury concentration and adipocyte area were quantified in fish liver and perivisceral adipose tissue, respectively. Fatty acid composition of phospholipids, neutral lipids and free fatty acids was determined in both tissues.

2.2 Ethics statement and fish husbandry

The experiment was approved by the Animal Care and Use Committee of the Université catholique de Louvain (Permit number: 133201) and was carried out at the 'Plateforme technologique en biologie aquicole Marcel Huet' (Université catholique de Louvain, Louvain-la-Neuve, Belgium) (Permit number for animal facilities: 1220034). Female rainbow trout eggs were obtained from the 'Pisciculture Charles Murgat' (Beaufort, France) and, after hatching, rainbow trout fry were fed a commercial diet (Skretting, Stavanger, Norway) until the

beginning of the study. The experiment started with 50-day old fish with an initial mean body weight of 0.8 ± 0.2 g that were maintained in a flow-through system (1 L/min) at 12.2 ± 0.4°C under a 16h:8h light:dark photoperiod cycle. Fish were fed experimental diets twice a day, six days a week.

2.3 Diets

Four isolipidic, isoenergetic and isoproteic diets differing only by their fatty acid composition were prepared. Diet formulation was based on Rollin et al. (2003) (Table 1). All diets contained a common basal diet devoid of fish meal but including 2.4 g/kg cod liver oil and were supplemented with 117.6 g/kg of one specific oil, i.e., either sunflower oil (Bio-time, Colruyt, Belgium), linseed oil (Vandeputte, Mouscron, Belgium), EPA-rich fish oil (Omegavie EPA 70 TG, Polaris, Quimper, France) or DHA-rich fish oil (Omegavie DHA 70 TG, Polaris) corresponding to LA-, ALA-, EPA- and DHA-enriched diets, respectively. The fatty acid composition of the four diets is presented in Table 2. The amount of vitamin E added in each diet was determined based on the endogenous amount in each specific oil in order to reach the same content in every diet. From week 5, oils were treated with MeHg (CH_3HgCl; CAS 115-09-3) (Sigma-Aldrich, Saint-Louis, MO, USA) before being added to the diets.

2.4 Sampling

At the end of the 10-week experiment and after a 24-h fast, fish were euthanized with MS-222 (ethyl 3-aminobenzoate methanesulfonate, Sigma-Aldrich) (0.5 g/L) buffered with $NaHCO_3$. Thirteen fish per tank were lyophilised, homogenised and kept at -20°C until analyses (body proximate composition and mercury accumulation). Perivisceral adipose tissue and liver were collected on three fish per tank, lyophilised (livers only) and pooled together (samples of equal weight) before being stored at -80°C until analyses (fatty acid profiles and hepatic mercury accumulation). A sample of adipose tissue from one fish per tank was collected and stored in 4% (v:v) paraformaldehyde at 4°C for histological investigations.

2.5 Fish proximate composition

The DM, crude ash and crude lipid content in fish were quantified based on analytical methods from the Association of Official Analytical Chemists (A.O.A.C., 1995). Briefly, samples were dried at 105°C for 16 h to determine the DM content (%) followed by an incineration at 550°C for 16 h to measure the crude ash content. Crude lipid content was quantified following diethyl ether extraction according to Soxhlet method. Crude protein content was estimated as follows: crude protein (% DM) = 100 – crude ash (% DM) – crude lipid (% DM).

2.6 THg quantification

THg (i.e., both organic and inorganic mercury) was quantified by atomic absorption spectrophotometry with Direct Mercury Analyzer 80 (DMA-80) (Milestone, Sorisole, Italy) (Habran et al., 2013) in lyophilised whole fish body and fish liver collected at week 10. Mercury concentrations in the diets were also quantified. Repeatability of measurements was verified with technical duplicates. Hg standards and blanks were included in the analyses.

2.7 Determination of fatty acid composition and concentration

Total lipids were extracted from homogenized samples of fresh adipose tissue (approximately 25 mg) and lyophilised liver (approximately 7 mg) with methanol:chloroform:water (2:2:1.8, v:v:v) (Bligh and Dyer, 1959). An internal standard composed of tridecanoic acid (Larodan, Solna, Sweden), 1,2-dipentadecanoyl-sn-glycero-3-phosphatidylcholine (Larodan), and triheptadecanoin (Larodan) was added to each sample. Extracted lipids were separated into neutral lipid, free fatty acid and phospholipid fractions through solid phase extraction columns (Bond Elut-NH_2, 200 mg, 3 mL) (Agilent Technologies, Santa Clara, CA, USA) using chloroform:2-propanol (2:1, v:v), diethylether:acetic acid (98:2, v:v) and methanol, respectively (Schneider et al., 2012). Fatty acids were then methylated in a KOH solution in methanol (0.1 M) at 70°C for 1 h and in a HCl solution in methanol (1.2 M) at 70°C for 15 min (Schneider et al., 2012). Fatty acid methyl esters (FAMEs) were extracted with hexane. An injection standard (i.e., methyl-undecanoate (Larodan)) was added to all samples. FAMEs were separated by gas chromatography. All technical details are available in Appendix A. Results are expressed in µmol of fatty acids per gram of wet adipose tissue sample and per gram of lyophilised liver sample.

2.8 Histological sections

After being fixed in 4% (v:v) paraformaldehyde and embedded in paraffin, sections (5 µm of thickness) of perivisceral adipose tissue were processed and stained with hematoxylin and eosin (Sigma-Aldrich). The slices were analysed using Visiopharm 2017.1 software to determine the area of each adipocyte.

2.9 Statistical analyses

Statistical analyses were performed using JMP® Pro 15.2.0 software. Before parametric tests, homoscedasticity and normality of residuals were checked for all conditions. Data were transformed (log10 or root square) if necessary. The diet and MeHg exposure effects, as well as the interaction diet x MeHg were analysed by a two-way generalized linear model (GLM)

for all parameters, except fatty acid composition. In order to evaluate specifically the effects of the diet for each MeHg level and the effects of the MeHg level for each diet on the fatty acid composition, two one-way GLM analyses were performed. Non-parametric Kruskal-Wallis test was performed when required. Significant effects were followed by Tukey (parametric) or Dunn (non-parametric) *post hoc* tests. A Bonferroni correction was applied in case of multiple tests. The effects were considered statistically significant if the corrected p-value was below 0.05. Biological unit was the tank and four tanks per condition were used, unless stated otherwise in figure or table legends.

3 RESULTS

Juvenile rainbow trout were fed LA-, ALA-, EPA-, or DHA-enriched diets for ten weeks and were concomitantly exposed to MeHg (0, 2.4 or 5.5 mg MeHg/kg DM (i.e., dry diet)) during the last six weeks (Figure 1). The three MeHg levels are referenced below as the control, low and high MeHg doses, respectively. All results refer to week 10 sampling.

3.1 Fish body composition

At week 10, the fish body mass (mean value: 6.2 ± 0.1 g per fish) and the hepatosomatic index (HSI) (mean value: 1.4 ± 0.0%) were similar between experimental conditions (Table 3).

In contrast, the proximate composition of the fish was highly affected by the diet. The LA-enriched diet induced significantly higher mean amounts of DM, crude ash, crude protein (Table 3) and crude lipid (Figure 2) compared to other diets. In contrast, the mean amounts of crude ash and crude protein were significantly decreased in fish fed the DHA-enriched diet compared to other diets (Table 3). The high MeHg dose (as well as the low dose in case of crude ash) significantly reduced the mean amounts of DM, crude ash (Table 3) and crude lipid (Figure 2) compared to untreated controls, irrespective of the diet.

3.2 THg accumulation in fish

The present study revealed a clear dose-dependent accumulation of THg in both the whole fish and the liver (+195% and +214% in the body and liver of fish exposed to the high MeHg dose compared to the low MeHg dose, irrespective of the diet) (Figure 3A and B). A significant effect of the dietary lipids on THg accumulation was observed in the whole fish, but not in the liver. Specifically, the fish fed the DHA-enriched diet exposed to the high MeHg dose

accumulated significantly more THg than those fed the other three diets (Figure 3A). The liver stored on average 1.6 times more THg per unit of lyophilised mass than the whole fish.

3.3 Adipocyte area

Adipocytes from perivisceral adipose tissue were significantly larger in fish fed with plant-derived oils (i.e., diets enriched in LA or ALA) compared to fish fed with fish oils (i.e., diets enriched in EPA or DHA) (Figure 4). Additionally, MeHg exposure induced a significant increase of the mean adipocyte area compared to control (+30% for the low MeHg dose and +41% for the high MeHg dose, irrespective of the diet) (Figure 4). This increase was particularly noted in fish fed the LA-enriched diet treated with the high MeHg concentration (+73% compared to the control diet), followed by the fish fed the ALA-enriched diet (+49% in fish fed with the high MeHg dose compared to the corresponding control diet). In contrast, mean adipocyte area increased to a lesser extent in fish fed any of the fish oil-enriched diets (+19% and +23% in fish fed EPA- and DHA-enriched diets with the high MeHg dose, respectively, compared to the corresponding control diets).

3.4 Fatty acid composition in adipose tissue and liver

3.4.1 Organ-specific proportion and amount of lipid classes

Adipose tissue was primarily composed of neutral lipids (98.1-98.6% of total adipose lipids, irrespective of the condition) whereas free fatty acids and phospholipids accounted for 1.0-1.5% and 0.3-0.4%, respectively. In liver, phospholipids were the most represented lipid class with 68.4-81.3% of total hepatic lipids followed by neutral lipids (9.5-23.2%) and free fatty acids (7.6-12.8%). The adipose tissue collected from the fish contained more total lipids (1990-2564 μmol fatty acids/g wet matter, Figure 5A) than the liver (53-67 μmol fatty acids/g wet matter, data not shown).

3.4.2 Significant modulation of fatty acid composition by dietary lipids in adipose tissue and liver

Crude lipid amount in whole fish was significantly higher in fish fed the LA-enriched diet. In contrast, the total fatty acids content within the adipose tissue (for the three lipid classes) did not vary between the four diets. Similarly, the dietary lipids did not influence the hepatic total fatty acids content in phospholipids and free fatty acids. On the other hand, the fish fed the DHA-enriched diet stored significantly more neutral lipids in their liver than the fish fed the EPA-enriched diet. The fatty acid composition of the two tissues was, however, affected by the diets. The results about n-3 and n-6 PUFA are detailed below (Figures 5 and 6) whereas the

effects on saturated and monounsaturated fatty acids are described in supplementary data (Figures A.1 and A.2). These effects being similar between the three MeHg doses, the description of the results correspond only to the unexposed control fish. All the data are, however, presented in the figures.

3.4.2.1 Distinct n-3 PUFA composition between fish fed the different n-3 PUFA-enriched diets

Feeding the fish with a n-3 PUFA (i.e., ALA, EPA or DHA)-enriched diet significantly increased the total n-3 PUFA content in all lipid classes and both tissues compared to fish fed the LA-enriched diet (Figures 5A and 6A). Overall, fish fed the diets supplemented in fish oil rich in n-3 PUFA exhibited the highest n-3/n-6 ratio, followed by fish fed the ALA-enriched diet. Unlike phospholipids, the total n-3 PUFA content in neutral lipids was influenced by the nature of the n-3 PUFA used to supplement the diet. Adipose tissue collected from fish fed the EPA-enriched diet contained the highest amount of n-3 PUFA in neutral lipids followed by fish fed the DHA- and then ALA-enriched diets. In contrast, fish fed the DHA-enriched diet contained the highest amount of n-3 PUFA in neutral lipids in the liver. Regarding the free fatty acid fraction, the EPA-enriched diet induced a significantly higher amount of total n-3 PUFA in the liver but not in the adipose tissue. The fish fed the LA-enriched diet contained a low amount of total n-3 PUFA compared to fish fed the other diets and the diversity in n-3 PUFA was reduced. The most represented n-3 PUFA in fish fed the LA-enriched diet was DHA.

When fatty acid composition was analysed individually, the n-3 PUFA composition was modified to the greatest extent in both tissues and all lipid classes in fish fed with the different n-3 PUFA-enriched diets (Figures 5B and 6B).

The adipose tissue and liver of fish fed the ALA-enriched diet were significantly richer in ALA and its direct desaturation (i.e., 18:4 n-3) and elongation (i.e., 20:3 n-3) products compared to fish fed other diets in all lipid classes except in adipose membranes in which 20:3 n-3 was not detected. A similar pattern was observed for the elongation product of 18:4 n-3 (i.e., 20:4 n-3) in hepatic phospholipids and neutral lipids. This fatty acid was also quantified in adipose neutral lipids and adipose and hepatic free fatty acids although its amount was not significantly different than in fish fed the EPA- and DHA- (in case of the adipose neutral lipids) enriched diets. Longer and more desaturated n-3 PUFA were lower in fish fed the ALA-enriched diet,

except in hepatic membranes and adipose neutral lipids that contained significant amounts of EPA, 22:5 n-3, 24:6 n-3 and DHA.

Fish fed the EPA-enriched diet were significantly richer in EPA and its direct elongation product (i.e., 22:5 n-3) than fish fed the other diets except in adipose free fatty acid and hepatic neutral lipid fractions in which similar amounts of 22:5 n-3 were found in fish fed the EPA- and DHA-enriched diets. In hepatic phospholipids and free fatty acids in both organs, the elongation product of 22:5 n-3 (i.e., 24:5 n-3) was only present in fish fed the EPA-enriched diet. In neutral lipids, 24:5 n-3 was also quantified in fish fed the DHA-enriched diet in a similar and lower amount in liver and adipose tissue, respectively. Fish fed the diets enriched in fish oils (i.e., containing EPA or DHA) were significantly richer in the desaturation product of 24:5 n-3 (i.e., 24:6 n-3) than fish fed the diets enriched in plant-derived oils (i.e., containing LA or ALA) in adipose tissue (neutral lipids and free fatty acids). This result was similar in regard to hepatic neutral lipids. On the other hand, in the hepatic phospholipids, fish fed the ALA-enriched diet contained a similar content of 24:6 n-3 to fish fed the EPA- and DHA-enriched diets.

Finally, the DHA content was significantly higher in adipose tissue of fish fed the DHA-enriched diet compared to other diets. Moreover, fish fed the EPA- and ALA-enriched diets contained higher amounts of DHA in adipose tissue compared to fish fed the LA-enriched diet. In liver, the same trend was observed in neutral lipids, whereas in phospholipids and free fatty acids the DHA amount did not differ between fish fed the DHA-, ALA- and EPA- (only in the case of free fatty acids) enriched diets but was higher (although not significantly in the free fatty acids) than in the fish fed the LA-enriched diet.

3.4.2.2 Strong difference of n-6 PUFA composition in fish fed the LA-enriched diet compared to fish fed the n-3 PUFA-enriched diets

In both organs, the LA-enriched diet markedly increased the total n-6 PUFA in all lipid classes (Figures 5A and 6A). Consequently, the n-3/n-6 ratio was close to zero in fish fed the LA-enriched diet. In adipose tissue, the content of LA and the first desaturation (i.e., 18:3 n-6) and elongation (i.e., 20:2 n-6 and 20:3 n-6) products was higher in fish fed the LA-enriched diet in all lipid classes (Figure 5C). This was followed by fish fed the ALA-enriched diet and finally, the lowest amount of LA was found in fish fed the EPA- and DHA-enriched diets. In the phospholipids, 18:3 n-6, 20:2 n-6, and 20:3 n-6 were present only in fish fed the LA-enriched diet, whereas in the two other lipid classes they could be found in fish fed the other diets. The

amount of AA in fish fed the LA- and EPA-enriched diets was similar in adipose phospholipids whereas it was significantly higher in the neutral lipids and free fatty acids of fish fed the EPA-enriched diet. The elongation product of AA (i.e., 22:4 n-6) as well as 22:5 n-6 were significantly higher in fish fed the DHA-enriched diet in both neutral lipids and free fatty acids. In phospholipids, the former was not detected, and the latter had similar amounts in fish fed the LA- and DHA-enriched diets.

The livers of fish fed the LA-enriched diet were significantly richer in LA and in subsequent bioconversion products (i.e., 18:3 n-6, 20:2 n-6, 20:3 n-6, AA, 22:4 n-6 and 22:5 n-6) compared to fish fed the three other diets (Figure 6C). Some exceptions were, however, noticed in hepatic neutral lipids. For example, LA and 20:2 n-6 contents were significantly higher in fish fed the LA-enriched diet only compared to those fed the EPA-enriched diet. Also, similar amounts of 22:4 n-6 and 22:5 n-6 were quantified in fish fed the LA-, EPA- (only for 22:4 n-6) and DHA-enriched diets. In phospholipids of fish fed the diets enriched in n-3 PUFA, n-6 PUFA were mainly represented by LA, and 20:3 n-6, AA, 22:5 n-6 in fish fed the ALA-, EPA-, and DHA-enriched diets, respectively. Similar results were found in free fatty acids apart from the fish fed the DHA-enriched diet in which AA was the second most represented fatty acid. In addition, comparable amounts of 20:2 n-6, the elongation product of LA, was found in fish fed the n-3 PUFA-enriched diets.

3.4.3 Reduced content of n-6 PUFA by MeHg in hepatic cell membranes

Contrary to its effect on total lipids, MeHg did not induce any significant impact on fatty acid accumulation in either liver or adipose tissue (all lipid classes), except for PUFA in hepatic membranes. In that case, the high MeHg dose induced a significant decline of the total n-6 PUFA and total PUFA in hepatic cell membranes in fish fed the LA-enriched diet (-12% and -13%, respectively, compared to the fish fed the corresponding control diet) (Figure 6A). These effects were mainly explained by the non-significant decrease of all n-6 fatty acids (Figure 6C).

4 DISCUSSION

The present 10-week experiment was designed to investigate the effects of four dietary fatty acids widely present in fish and plant-derived oils used in aquaculture and MeHg exposure on lipid metabolism in juvenile rainbow trout. The diets used to modify the fatty acid composition were supplemented with either sunflower oil (rich in LA), linseed oil (rich in ALA), EPA-rich

fish oil or DHA-rich fish oil. During the last six weeks, MeHg was added to the diets (0, 2.4 or 5.5 mg MeHg/kg DM) to simulate different environmental exposures to MeHg (Durrieu et al., 2005; Wiener et al., 2002). The impacts were studied at organismal and organ levels, with a focus on perivisceral adipose tissue and liver, two major sites of lipid deposition (Weil et al., 2013) and key organs in lipid metabolism, playing important roles in energy homeostasis.

4.1 Effects of dietary fatty acids on fish growth

At the end of the study, the fish body mass was not affected by dietary lipids, as previously observed (Amlund et al., 2012; Francis et al., 2014; Mellery et al., 2016). This result is in line with the diet formulation that meets the nutritional requirements of rainbow trout, including the supply in essential fatty acids through the incorporation of small amounts of cod liver oil to all diets (Francis et al., 2014). Even if no impact of the diet was noticed on fish body mass, the LA-enriched diet produced fish with higher DM (ash, proteins and lipids) and lower water content. The HSI was not impacted by the diet in the present study, which is in agreement with previously acquired results for rainbow trout (Ferain et al., 2021) and Atlantic salmon (Menoyo et al., 2005) fed similar diets.

4.2 Effects of dietary fatty acids on fish lipid deposition

As mentioned above, the LA-enriched diet induced the highest lipid accumulation in fish whereas no significant difference was observed between the fish fed the three n-3 PUFA-enriched diets. This partially contrasts with Mellery et al. (2016), who reported that crude lipid content was decreased in rainbow trout fed a diet enriched in linseed oil compared to fish oil.

The changes in fat deposition could also be visible at organ level. Notably, in adipose tissue, it was observed that the diets supplemented in plant-derived oils, and especially in sunflower oil, led to larger adipocytes. Similarly, gilthead sea bream (*Sparus aurata L.*) fed a diet containing a blend of plant-derived oils (rapeseed, linseed, and palm oils) (66%) and fish oil (34%) for 14 months presented bigger adipocytes in perivisceral adipose tissue than fish fed a diet supplemented only with fish oil, although no difference on adipose tissue mass and mesenteric fat index was observed (Cruz-Garcia et al., 2011). The enlargement of the cells by plant-derived oils could be related to metabolic changes in adipocytes. On one hand, the two dietary 18-carbon fatty acids (i.e., LA and ALA) could have enhanced the number of fatty acid transporters as observed *in vitro* and *in vivo*, at mRNA or protein level, in salmonid preadipocytes (Huang et al., 2010; Riera-Heredia et al., 2020). On the other hand, EPA and DHA from fish oil-enriched diets could have downregulated the expression of transcription factors involved in

adipogenesis as observed *in vitro* for CCAAT/enhancer binding protein α, β and δ (Huang et al., 2010).

Despite the higher body lipid content and adipocyte size observed in the present study in fish fed the plant-based diets, no change of lipid content per unit of adipose tissue mass was measured. This may be explained by a difference in the number of cells sampled per unit of tissue weight. Indeed, one gram of adipose tissue should be made of a lower number of larger cells in the case of plant-based diets.

Fish oil replacement by plant-derived oils has been demonstrated to also lead to an increased adipose tissue lipolysis in fish (Albalat et al., 2005; Cruz-Garcia et al., 2011; Riera-Heredia et al., 2020). A positive correlation between adipocyte size and basal lipolysis has been reported in obese humans (Arner, 2005). Lipolysis leads to an elevated level of circulating fatty acids released from adipose tissue, which can in turn induce lipid accumulation in the liver, as shown in fish fed a diet supplemented in plant-derived oils (Benedito-Palos et al., 2008; Cruz-Garcia et al., 2011) and humans (Browning and Horton, 2004). Previous studies specifically revealed that plant-derived oils rich in LA or ALA increased the fat content in the liver compared to fish oil (Li et al., 2016; Menoyo et al., 2005; Peng et al., 2014). Contrary to what was expected, the plant-based diets did not induce an increase of hepatic lipids in the present study. The only difference observed at the end of the experiment was the higher accumulation of neutral lipids in the fish fed the DHA- compared to the EPA-enriched diet, although the effect was significant only in the absence of mercury.

Lipid accumulation could also occur in tissues other than liver and adipose tissues. For example, soybean oil was shown to produce a higher number of lipid droplets in enterocytes compared to fish oil (Olsen et al., 2003). The diet could also modify fat inclusion in muscle. The LA-induced increase of the body lipid content could thus be explained by cumulative effects in various organs.

4.3 Effects of dietary fatty acids on fish fatty acid composition

Individual fatty acid concentrations in adipose tissue and liver after the 10-week feeding trial reflected each specific diet, as previously described (Ferain et al., 2021; Todorčević et al., 2009; Turchini et al., 2018). The fatty acid composition of neutral lipids in adipose tissue was particularly comparable to the specific diet, highlighting the storage function of the lipid droplet for the fatty acids present in excess. The current fatty acid profiles of both tissues supported the efficient fatty acid bioconversion capacity of salmonids reported previously, as

elongation and/or desaturation products of ALA (i.e., 18:4 n-3, 20:3 n-3 and 20:4 n-3), EPA (i.e., 22:5 n-3 and 24:5 n-3) and LA (i.e., 18:3 n-6, 20:2 n-6 and 20:3 n-6) were quantified in both tissues although they were not present in the diet (Francis et al., 2014; Menoyo et al., 2005; Tocher, 2003). Fish fed a diet rich in ALA also contained more EPA and DHA than fish fed the LA-enriched diet, highlighting the bioconversion of ALA with a selective retention of the biotransformation products. The phenomenon was particularly notable in the hepatic membranes where the concentrations of DHA were equally high in fish fed the ALA- and the DHA-enriched diets. Similar results were noticed in fish fed the EPA-enriched diet in which EPA was converted to 24:6 n-3 and DHA. The selective retention of DHA in fish fed a n-3 PUFA-enriched diet combined with the fact that DHA was the predominant n-3 PUFA in fish fed the LA-enriched diet highlights the importance of this fatty acid within membranes (Ferain et al., 2021). On the other hand, the amount of DHA in neutral lipids of both fish fed ALA- and EPA-enriched diets was not as high as in fish fed the DHA-enriched diet, as previously observed after a 60-day feeding trial (Mellery et al., 2016).

Similarly, for the n-6 pathway, LA was converted to longer and more desaturated fatty acids (i.e., AA, 22:4 n-6 and 22:5 n-6) absent from the diet but detected within the hepatic membranes. Nevertheless, in neutral lipids, more AA was stored in adipose tissue in fish fed the EPA- compared to the LA-enriched diet. A comparable situation was observed with 22:4 n-6 and 22:5 n-6 in adipose and hepatic neutral lipids in fish fed the DHA-enriched diet. This can be explained by the presence of these fatty acids in the EPA- and DHA-enriched diets.

4.4 Accumulation of THg in fish

The dose-dependent accumulation of THg observed in both the fish and liver in this study is consistent with previous findings (Ferain et al., 2018; Liu et al., 2013). Interestingly, our results also suggest that dietary fatty acid composition may influence the bioaccumulation of THg. Specifically, fish fed the DHA-enriched diet exhibited higher THg concentration compared to those fed the other three diets, which is in agreement with a previous study in which the THg content in Atlantic salmon exposed to mercury (5 mg MeHg/kg, 3 months) tended to be higher in brain and muscle of fish fed a diet enriched in fish oil compared to soybean oil (Amlund et al., 2012). Our observation that fish fed the EPA-enriched diet had lower THg concentrations may be due to a slightly lower effective concentration in the corresponding diet, as daily food intake was monitored throughout the study. In contrast to the whole fish, no significant difference in THg accumulation was observed in the liver among the four dietary conditions,

which is consistent with previous *in vitro* findings using rainbow trout liver cells cultured with various PUFA (Ferain et al., 2018).

4.5 Effects of MeHg on fish growth

The 6-week dietary exposure to MeHg did not modify fresh body weight and HSI of the juvenile rainbow trout. A similar result was observed for the body weight in Atlantic salmon after 3-month exposure to MeHg (5 mg/kg) (Amlund et al., 2012). On the other hand, in the present study, MeHg exposure resulted in reduced fish DM, regardless of the diet, suggesting a higher proportion of water in fish exposed to MeHg.

4.6 Effects of MeHg on fish lipid deposition

The decrease of fish DM content induced by MeHg was not associated with a lower body protein level but with a lower body lipid content, irrespective of the diet.

In adipose tissue, dietary MeHg exposure had no effect on total fatty acids content in neutral lipid, phospholipid and free fatty acid fractions. However, this result does not preclude that the total amount of perivisceral adipose tissue could have been affected by MeHg. Indeed, the fatty acid analysis determines the total amount of fatty acids per gram of tissue but does not provide information on the extent of perivisceral adipose tissue deposition, which remains unknown in the present study. Supporting results describe a reduction of visceral and subcutaneous adipose tissue in male mice exposed to MeHg, without any change in body weight (0.5 or 5 ppm MeHg in drinking water, 30 days) (Ferrer et al., 2018). Although MeHg exposure did not affect adipose tissue lipid content (per unit of tissue weight), it increased the size of the adipocytes. This result suggests an impact of MeHg on adipose metabolism associated with an induction of adipocyte hypertrophy, which has been reported in a recent *in vitro* study where rainbow trout preadipocytes accumulated significantly more neutral lipids when incubated with MeHg (3.8 μM, 6 days) (Tinant et al., 2021). In the present study, the extent of adipocyte size increase depended on the diet. MeHg led to larger adipocytes in fish fed the diets supplemented in plant-derived oils whereas the increase remained limited in fish fed the fish oil-enriched diets. The effects of MeHg and fatty acids typical of plant-derived oils seem thus to be cumulative.

Similar to adipose tissue, MeHg had no impact on the total amount of phospholipids, neutral lipids and free fatty acids in the liver. However, in the literature, several studies showed that hepatic lipid metabolism could be affected by mercury exposure. On the one hand, lipogenic effects of mercury were reported in the liver. Transcriptome analysis in tusk (*Brosme brosme*) liver revealed that genes necessary for adipogenesis were among the most affected by mercury

(both organic and inorganic forms), with an enhanced expression of peroxisome proliferator-activated receptor γ and lipoprotein lipase that could be implicated in hepatic steatosis (Olsvik et al., 2021). On the other hand, hepatic transcriptome and proteome analyses indicated that fatty acid β-oxidation was upregulated following mercury exposure in tusk (Olsvik et al., 2021) and Atlantic cod (0.5 or 2 mg MeHg/kg body weight, 14 days) (Yadetie et al., 2016; Yadetie et al., 2013). The increase of energy production capacity could compensate the energetically costly adaptive response to mercury exposure and the potential increased oxidative stress. In mice, MeHg exposure (30 and 15 days) resulted in reduced hepatic triglycerides after exposure to MeHg in drinking water (5 ppm and 20 mg/L, respectively) (Ferrer et al., 2018; Leocádio et al., 2020). This could be linked to increased β-oxidation or reduced hepatic lipid synthesis. The fact that we did not observe any impact on hepatic lipids in the present study may be due to differences linked to the species or experimental protocol (exposure duration, mercury concentration).

Muscles can efficiently accumulate MeHg (Amlund et al., 2007) and store significant amounts of lipids in the adipocytes found in myosepta (Nanton et al., 2007). MeHg could impair the mitochondrial respiration in muscle as described by Cambier et al. (2009). More lipids could therefore be required to compensate for the lower ATP synthesis, which could explain the drop of the whole-body lipid content in the present study.

Taken together, the results indicate that MeHg can impair lipid and energy metabolism, with effects that may be diet-dependent, even if it remains complex to predict the resulting phenotype at the organ and organismal level. Decreased levels of whole-body lipids could result from a direct effect on various cellular pathways and a concurrent indirect effect due to a higher need of organic molecules to cope with the deleterious effects of MeHg.

4.7 Effects of MeHg on fish fatty acid composition

From a qualitative point of view, MeHg exposure led to a reduction of n-6 PUFA in the hepatic phospholipids of fish fed the LA-enriched diet. This result suggests that MeHg disrupts the n-6 PUFA pathway in cells containing high amounts of these fatty acids. Similar results have been previously reported. For example, MeHg treatment (0.15 μM, 24 h) reduced the relative amount of 20:3 n-6 in phospholipids of AA-enriched RTL-W1 cells (Ferain et al., 2018) while it reduced the amount of AA in brain phosphatidylinositol in Atlantic salmon fed a vegetable oil-based diet rich in n-6 PUFA (5 mg MeHg/kg, 3 months) (Amlund et al., 2012). This observation might be explained by an increased activity of the phospholipase A2 by MeHg,

leading to a significantly higher release of n-6 PUFA from the phospholipid membranes (Shanker et al., 2004; Shanker et al., 2002) to provide precursors of eicosanoids involved in biological pathways such as inflammation (Kremmyda et al., 2011). This result is further evidence that phospholipid membranes are a target of mercury toxicity, as recently highlighted by transcriptomic analysis (Olsvik et al., 2021).

Adipose fatty acid profile was not modified following MeHg exposure in the present study in contrast to previous *in vitro* results which described that MeHg led to a release of AA from phospholipids in rainbow trout preadipocytes as well as to an intracellular storage of n-3 PUFA (3.8 μM, 6 days) (Tinant et al., 2021). This discrepancy could be explained by differences in the protocol of exposure (short-term exposure in the *in vitro* study vs. long-term in the present study). It should also be taken into account that adipose tissue does not represent an important site of mercury accumulation, contrary to the liver (Wagemann et al., 1998). The low exposure of adipocytes *in vivo* could be at the origin of the lack of effect on fatty acid profile, as opposed to what is observed in the liver.

5 CONCLUSION

Diets enriched in n-6 (i.e., LA) or n-3 (i.e., ALA, EPA or DHA) PUFA significantly influenced the lipid metabolism in juvenile rainbow trout and the fatty acid composition of the adipose tissue and liver, primarily reflecting the dietary fatty acid composition. However, the efficient bioconversion capacity of rainbow trout caused differences in all experimental dietary conditions. The 6-week dietary MeHg exposure led to a dose-dependent accumulation of mercury in the whole organism and in the liver. Although MeHg exposure reduced whole-body lipid content, it induced adipocyte hypertrophy, especially in the fish fed LA- and ALA-enriched diets. Further studies should explore the effects of dietary lipids and MeHg on the total amount of perivisceral adipose tissue and on the lipid content of other organs such as muscle. MeHg reduced the n-6 PUFA content in the hepatic membranes, but only in fish fed a high amount of n-6 PUFA. This study confirms that dietary MeHg and fatty acids can significantly affect lipid metabolism in adipose tissue and liver. Our results offer new insights regarding the development of new feed formulations that optimize fish health and nutritional value while reducing the accumulation of toxic contaminants in farmed fish.

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.

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APPENDIX A. SUPPLEMENTARY DATA

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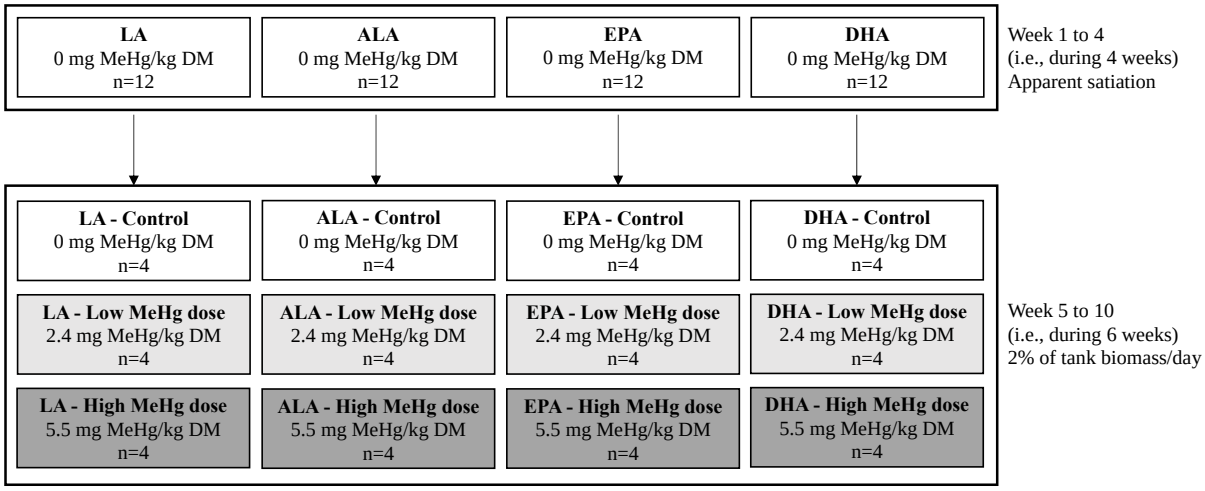
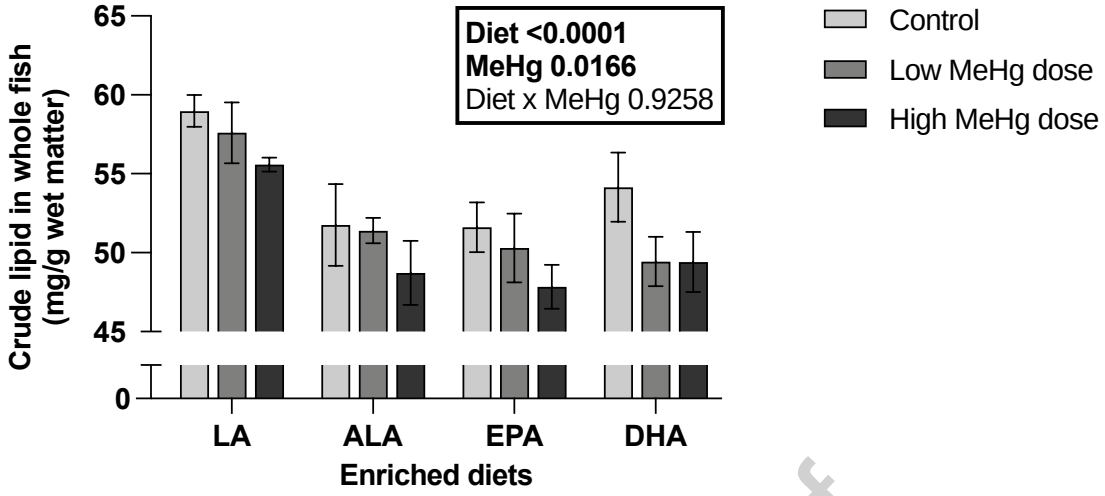


Figure 1. Experimental design. The biological unit was the tank and is represented by "n" in the figure. Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; DM, dry matter; EPA, eicosapentaenoic acid; LA, linoleic acid; MeHg, methylmercury.



	Diet effect				MeHg effect		
	LA	**ALA**	**EPA**	**DHA**	**Control**	**Low MeHg dose**	**High MeHg dose**
Crude lipid	a	b	b	b	a	a,b	b

Figure 2. Crude lipid amount (mg/g wet matter) in juvenile rainbow trout after a 10-week feeding with one of the four diets enriched in one specific fatty acid (LA, ALA, EPA or DHA) and exposure to MeHg (control, low and high MeHg doses corresponding to 0, 2.4 and 5.5 mg MeHg/kg DM, respectively) during the last six weeks. The p-values related to two-way GLM are reported in the box at the top of the figure. Significant effects are shown in bold ($p < 0.05$). Results of relevant *post hoc* tests are detailed in the table. Conditions with different letters indicate significant effect of the diet (irrespective of the MeHg dose) or the MeHg (irrespective of the diet). Data are presented as mean ± standard error of the mean (n = 4 except for the condition "ALA-Control" where n = 2, and for the condition "DHA-Control" where n = 3). Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; DM, dry matter; EPA, eicosapentaenoic acid; LA, linoleic acid; MeHg, methylmercury.

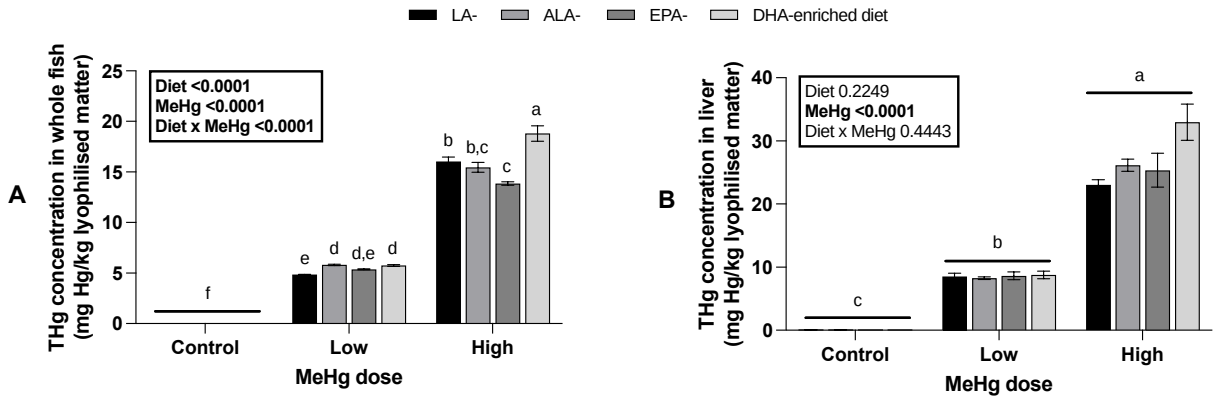
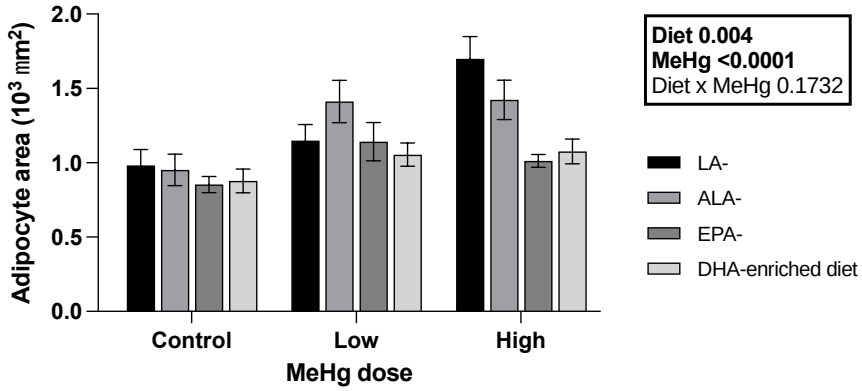
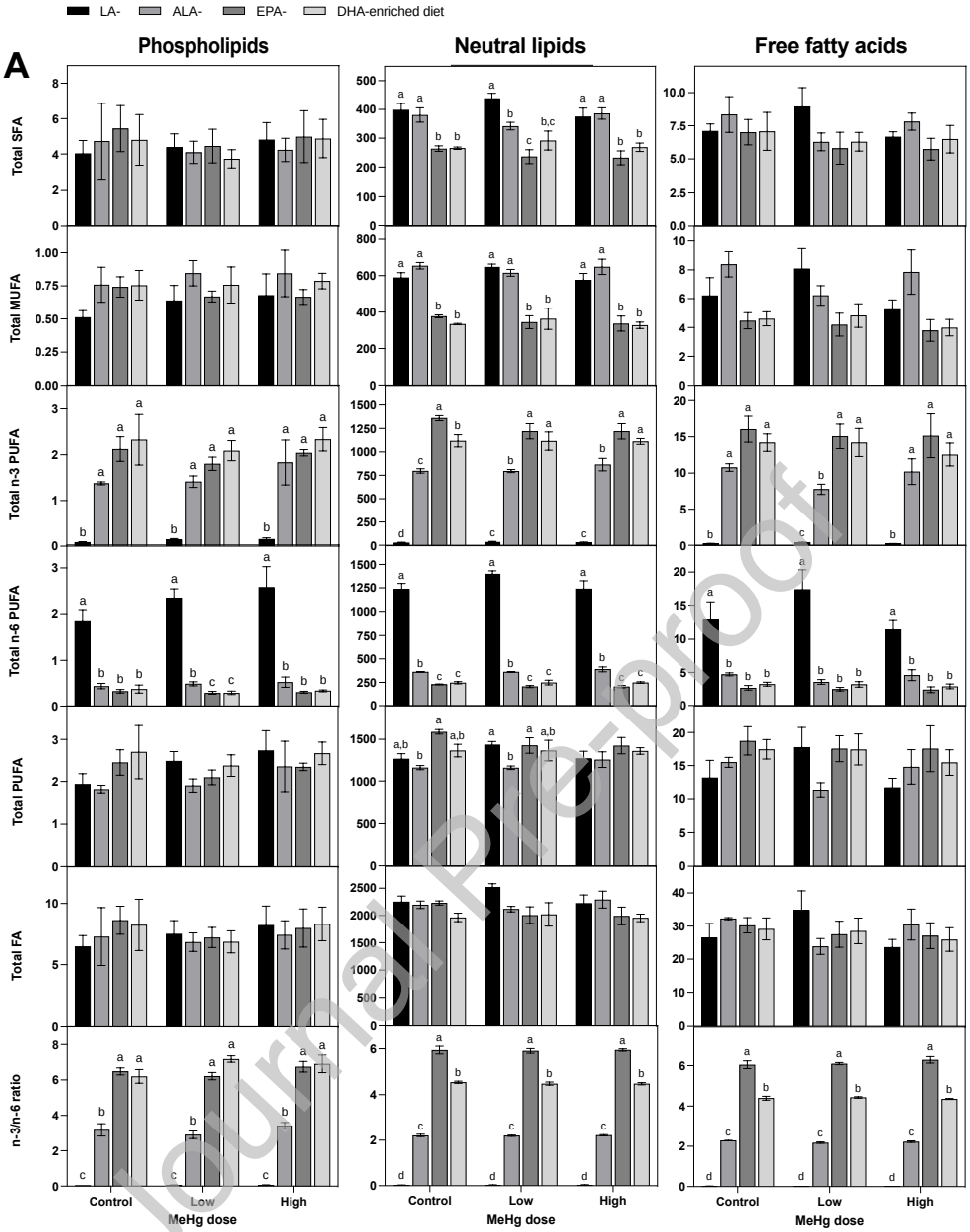


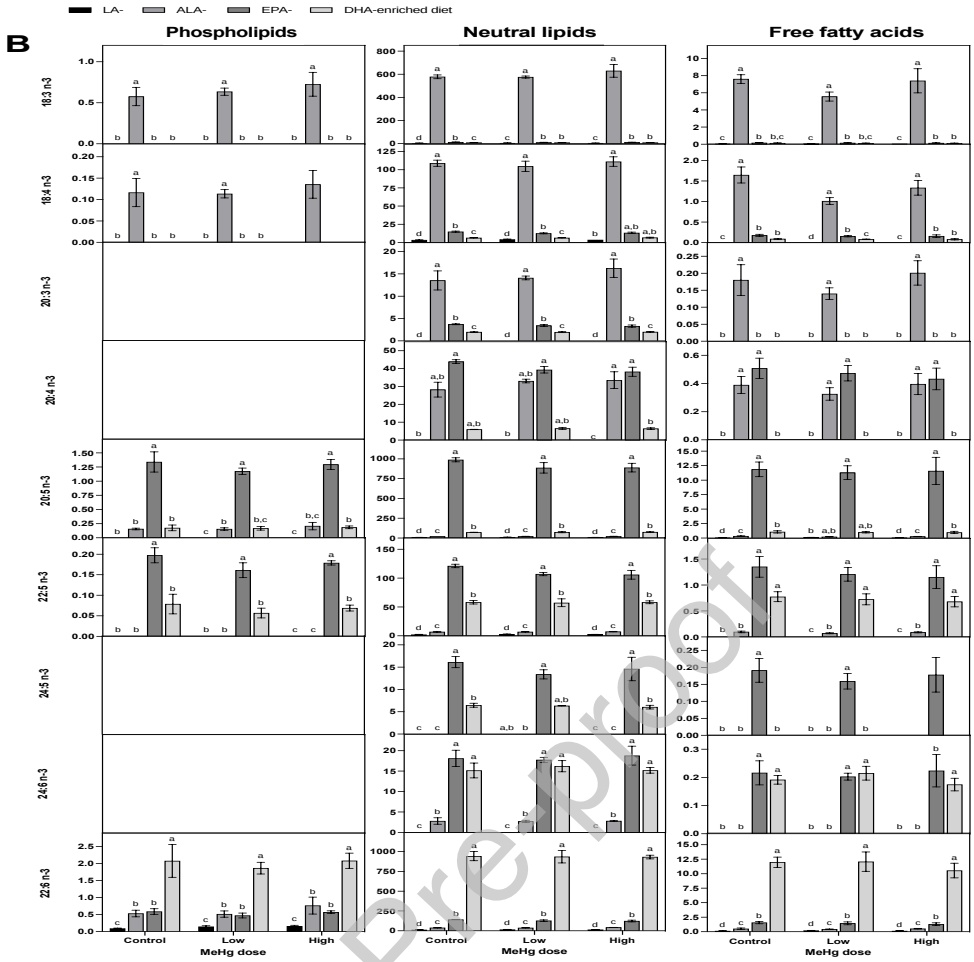
Figure 3. THg concentration (mg Hg/kg lyophilised matter) in (A) the whole body and (B) the liver of juvenile rainbow trout after a 10-week feeding with one of the four diets enriched in one specific fatty acid (LA, ALA, EPA or DHA) and exposure to MeHg (control, low and high MeHg doses corresponding to 0, 2.4 and 5.5 mg MeHg/kg DM, respectively) during the last six weeks. The p-values related to two-way GLM are reported in the box at the top of the figures. Significant effects are shown in bold ($p < 0.05$). In Figure 3(A), the conditions with different letters are significantly different. In Figure 3(B), irrespective of the diet, the conditions with different letters indicate significant effect of the MeHg, whereas no significant effect of the diet was observed. Data are presented as mean ± standard error of the mean (n = 4 except for the condition "ALA-Control" where n = 2). Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; DM, dry matter; EPA, eicosapentaenoic acid; LA, linoleic acid; MeHg, methylmercury; THg, total mercury.



	Diet effect				MeHg effect		
	LA	**ALA**	**EPA**	**DHA**	**Control**	**Low MeHg dose**	**High MeHg dose**
Adipocyte area	a	a	b	b	b	a	a

Figure 4. Area (μm^2) of adipocytes in perivisceral adipose tissue of juvenile rainbow trout after a 10-week feeding with one of the four diets enriched in one specific fatty acid (LA, ALA, EPA or DHA) and exposure to MeHg (control, low and high MeHg doses corresponding to 0, 2.4 and 5.5 mg MeHg/kg DM, respectively) during the last six weeks. The p-values related to two-way GLM are reported in the box at the top of the figure. Significant effects are shown in bold ($p < 0.05$). Results of relevant *post hoc* tests are detailed in the table. Conditions with different letters indicate significant effect of the diet (irrespective of the MeHg dose) or the MeHg (irrespective of the diet). Data are presented as mean ± standard error of the mean (n = 4 except for the condition "ALA-Control" where n = 2, and for the condition "LA-High" where n = 3). Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; DM, dry matter; EPA, eicosapentaenoic acid; LA, linoleic acid; MeHg, methylmercury.





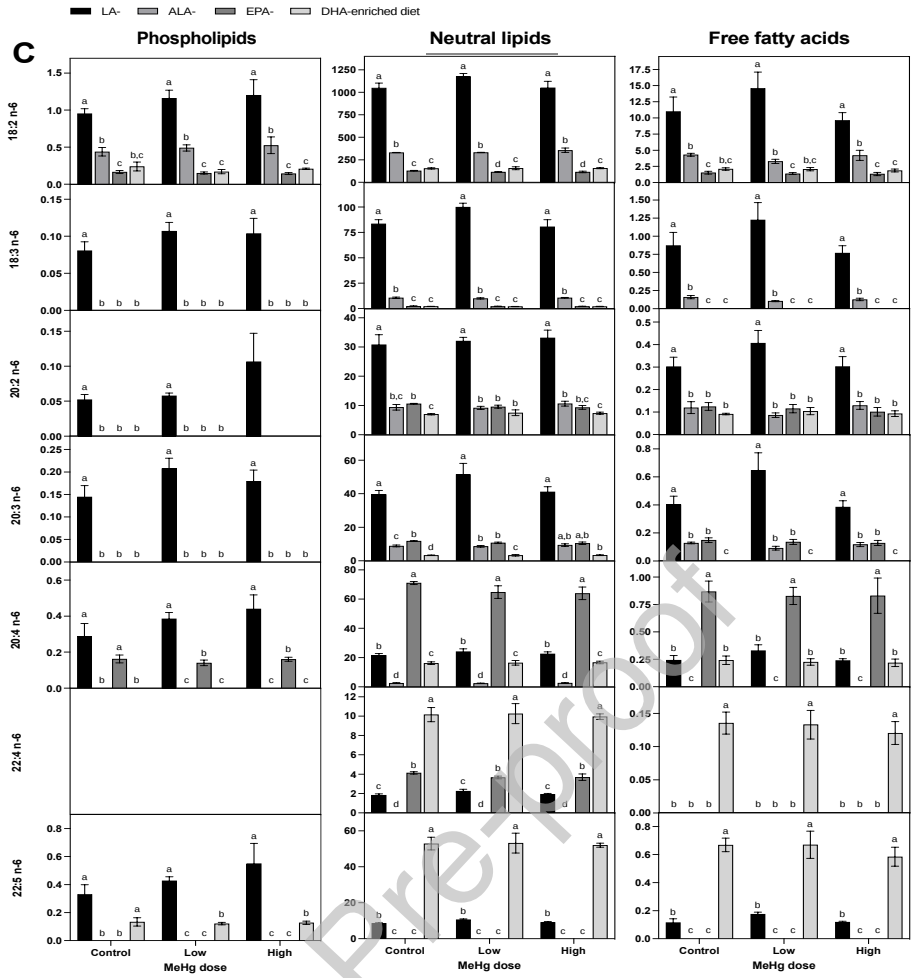
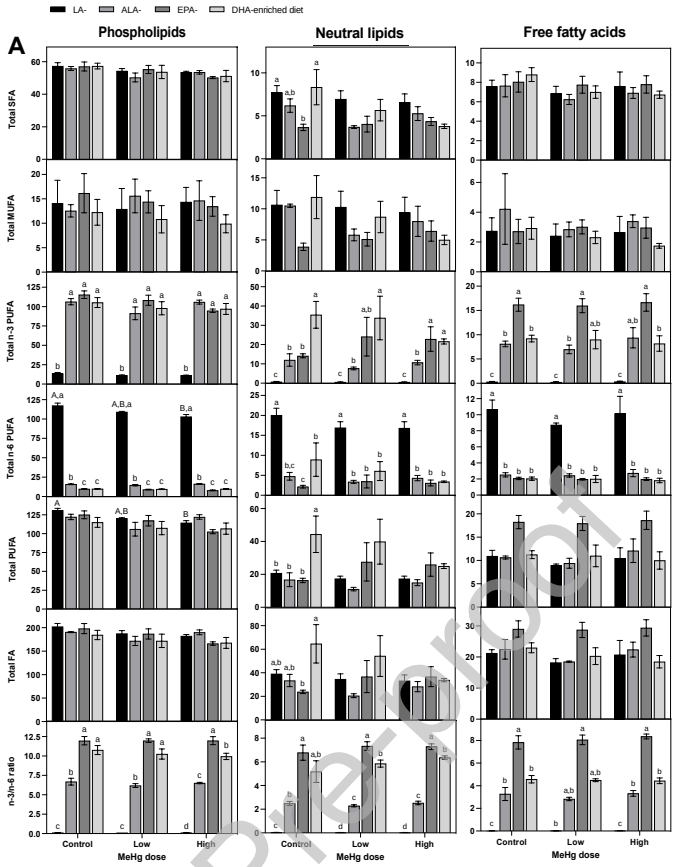
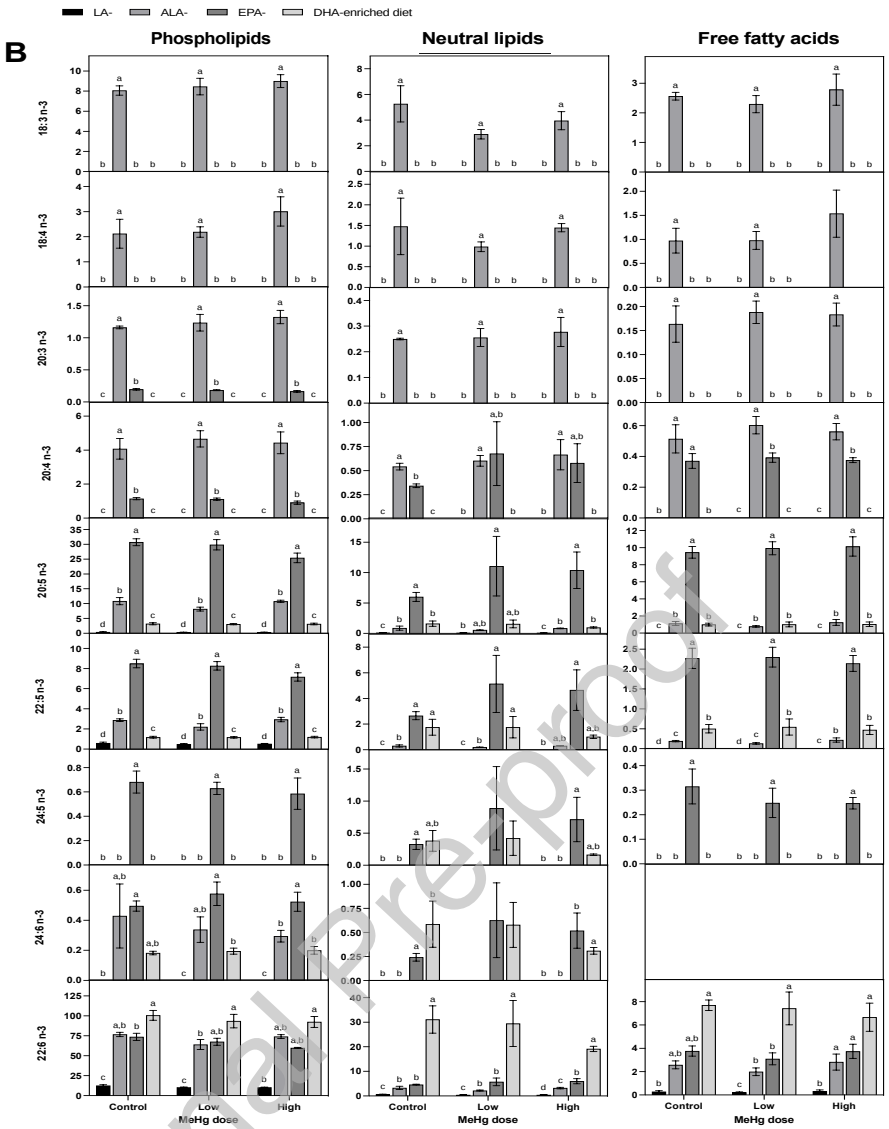


Figure 5. Concentration (μmol of fatty acids per g of adipose tissue) of (A) total fatty acids in the lipid classes and of individual (B) n-3 PUFA and (C) n-6 PUFA in phospholipids, neutral lipids, and free fatty acids in adipose tissue of juvenile rainbow trout after a 10-week feeding with one of the four diets enriched in one specific fatty acid (LA, ALA, EPA or DHA) and exposure to MeHg (control, low and high MeHg doses corresponding to 0, 2.4 and 5.5 mg MeHg/kg DM, respectively) during the last six weeks. For each MeHg level, values with different lowercase letters indicate significant effect of the diet ($p < 0.05$). No significant effect of the MeHg was observed in adipose tissue. Data are presented as mean ± standard error of the mean (n = 3 or 4 except for the conditions "ALA-Control" and "DHA-Low" where n = 2). Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; DM, dry matter; EPA, eicosapentaenoic acid; FA, fatty acids ; LA, linoleic acid; MeHg, methylmercury; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.





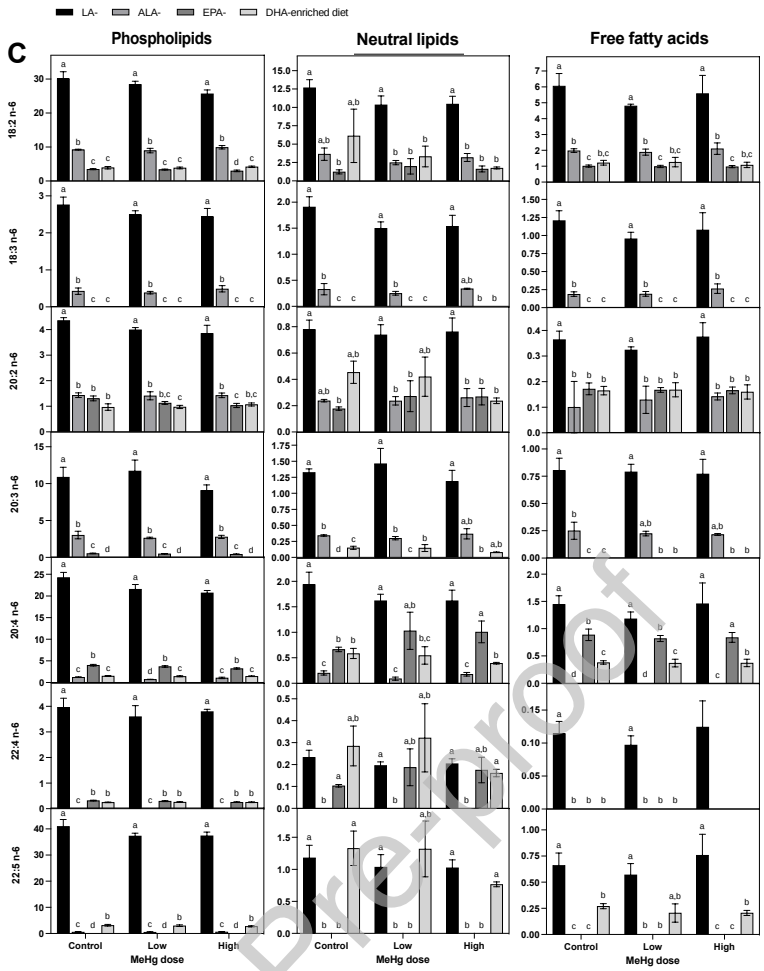


Figure 6. Concentration (μmol of fatty acids per g of lyophilised liver) of (A) total fatty acids in the lipid classes and of individual (B) n-3 PUFA and (C) n-6 PUFA in phospholipids, neutral lipids, and free fatty acids in liver of juvenile rainbow trout after a 10-week feeding with one of the four diets enriched in one specific fatty acid (LA, ALA, EPA or DHA) and exposure to MeHg (control, low and high MeHg doses corresponding to 0, 2.4 and 5.5 mg MeHg/kg DM, respectively) during the last six weeks. For each MeHg level, values with different lowercase letters indicate significant effect of the diet ($p < 0.05$). For each diet, values with capital letters indicate significant effect of the MeHg ($p < 0.05$). Data are presented as mean ± standard error of the mean (n = 4 except for the condition "ALA-Control" where n = 2). Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; DM, dry matter; EPA, eicosapentaenoic acid; FA, fatty acids ; LA, linoleic acid; MeHg, methylmercury; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Table 1. Composition (g/kg DM) of the four experimental diets enriched in LA, ALA, EPA or DHA.

Component	**LA**	**ALA**	**EPA**	**DHA**
Casein	276.8	276.8	276.8	276.8
Gelatin	50.0	50.0	50.0	50.0
Wheat gluten	223.0	223.0	223.0	223.0
Dextrin	100.0	100.0	100.0	100.0
Sucrose	50.0	50.0	50.0	50.0
Cellulose	80.0	80.0	80.0	80.0
Carboxymethylcellulose	30.0	30.0	30.0	30.0
Mineral premix[a]	63.4	63.4	63.4	63.4
Vitamin premix[b]	5.8	5.8	5.8	5.8
Cod liver oil	2.4	2.4	2.4	2.4
Sunflower oil	117.6	0.0	0.0	0.0
Linseed oil	0.0	117.6	0.0	0.0
EPA-rich fish oil	0.0	0.0	117.6	0.0
DHA-rich fish oil	0.0	0.0	0.0	117.6

[a] Mineral premix (g/kg premix): $CaHPO_4$ 239.41, $Ca(H_2PO_4)_2.H_20$ 222.33, $NaHCO_3$ 96.82, $Na_2SeO_3.5H_2O$ 0.01, KCl 102.45, NaCl 176.63, KI 0.20, $MgCl_2$ 65.26, $MgSO_4.7H_2O$ 71.95, $MnSO_4.H_2O$ 1.55, $FeSO_4.7H_2O$ 12.72, $CuSO_4.5H_2O$ 0.41, $ZnSO_4.7H_2O$ 10.25

[b] Vitamin premix (g/kg premix) including hydrosoluble vitamins: ascorbic acid (Vit C) 56.00, thiamin (Vit B1) 6.27, riboflavin (Vit B2) 13.44, pyridoxine (Vit B6) 5.04, calcium pantothenate (Vit B5) 15.79, p-aminobenzoic acid (Vit H1) 44.79, cyanocobalamin (Vit B12) 0.03, niacin (Vit B3) 33.59, biotin (Vit H) 0.11, choline chloride 391.94, folic acid (Vit M) 1.68, inositol 55.99, canthaxanthin (E161g) 11.20, and liposoluble vitamins: retinyl acetate (Vit

A) 0.63, cholecalciferol (Vit D3) 0.16, menadione (Vit K3) 3.59, butylated hydroxyanisole 2.45, butylated hydroxytoluene 2.45 and, tocopherol acetate (Vit E) 10.46/21.01/10.29/9.00 in LA-/ALA-/EPA-/DHA-enriched diet, respectively.

Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; DM, dry matter; EPA, eicosapentaenoic acid; LA, linoleic acid.

Table 2. Fatty acid composition (g/kg DM (% of total identified fatty acids)) of the four experimental diets enriched in LA, ALA, EPA or DHA.

Fatty acid	**LA**	**ALA**	**EPA**	**DHA**
14:0	0.2 (0.2)	0.1 (0.1)	0.2 (0.2)	0.3 (0.3)
16:0	6.7 (6.6)	6.1 (6.1)	1 (1)	2.2 (2.4)
18:0	3.3 (3.2)	4.3 (4.3)	3.1 (3.1)	0.9 (1)
20:0	0.2 (0.2)	0.2 (0.2)	0.5 (0.5)	0.2 (0.3)
22:0	0.6 (0.6)	0.2 (0.2)	0 (0)	0.3 (0.3)
24:0	0.2 (0.2)	0 (0)	0.5 (0.5)	0 (0)
Total SFA	11.1 (11)	11 (10.9)	5.2 (5.4)	4 (4.4)
16:1 n-7	0.3 (0.2)	0.3 (0.3)	0.2 (0.2)	0.8 (0.8)
18:1 n-7	0.7 (0.7)	0.8 (0.8)	1.5 (1.6)	0.5 (0.6)
18:1 n-9	18.5 (18.3)	19.8 (19.7)	3.5 (3.6)	3.7 (4.1)
20:1 n-9	0.4 (0.3)	2.7 (2.7)	3.3 (3.4)	1 (1.1)
22:1 n-9	0 (0)	0 (0)	0 (0)	0.5 (0.5)
24:1 n-9	0 (0)	0 (0)	0 (0)	1.2 (1.3)
Total MUFA	19.8 (19.6)	23.6 (23.5)	8.5 (8.7)	7.6 (8.4)
18:3 n-3 (ALA)	0.3 (0.3)	49.8 (49.7)	0.4 (0.4)	0.2 (0.2)
18:4 n-3	0 (0)	0 (0)	0.6 (0.6)	0.2 (0.2)
20:3 n-3	0 (0)	0 (0)	0.3 (0.3)	0 (0)
20:4 n-3	0 (0)	0 (0)	2.5 (2.6)	0.3 (0.3)
20:5 n-3 (EPA)	0.2 (0.2)	0.2 (0.2)	72.3 (74.2)	3.6 (4)
22:5 n-3	0 (0)	0 (0)	0 (0)	4.5 (4.9)

22:6 n-3 (DHA)	0.2 (0.2)	0.2 (0.2)	0.2 (0.2)	61.1 (67)
24:5 n-3	0 (0)	0 (0)	0 (0)	0 (0)
24:6 n-3	0 (0)	0 (0)	0 (0)	0.3 (0.3)
Total PUFA n-3	0.6 (0.6)	50.2 (50.1)	76.2 (78.3)	70.3 (77)
18:2 n-6 (LA)	69.4 (68.8)	15.3 (15.3)	1.8 (1.9)	3.8 (4.2)
18:3 n-6	0 (0)	0.2 (0.2)	0 (0)	0 (0)
20:2 n-6	0 (0)	0 (0)	0.4 (0.4)	0 (0)
20:3 n-6	0 (0)	0 (0)	0.6 (0.6)	0 (0)
20:4 n-6	0 (0)	0 (0)	4.5 (4.7)	0.7 (0.8)
22:4 n-6	0 (0)	0 (0)	0 (0)	0.9 (0.9)
22:5 n-6	0 (0)	0 (0)	0 (0)	4 (4.3)
Total PUFA n-6	69.4 (68.8)	15.5 (15.5)	7.3 (7.5)	9.4 (10.3)
Total PUFA	70.1 (69.4)	65.7 (65.5)	83.6 (85.9)	79.6 (87.3)
Total FA	100.9 (100)	100.2 (100)	97.3 (100)	91.2 (100)
n-3/n-6 ratio	0	3.2	10.4	7.5

Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; DM, dry matter; EPA, eicosapentaenoic acid; FA, fatty acids; LA, linoleic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Table 3. Fish parameters and proximate composition (mg/g wet matter) of juvenile rainbow trout after a 10-week feeding with one of the four diets enriched in one specific fatty acid (LA, ALA, EPA or DHA) and exposure to MeHg (control, low and high MeHg doses) during the last six weeks.

(A)	LA			ALA			EPA			DHA			p-value		
	Control	Low MeHg dose	High MeHg dose	Control	Low MeHg dose	High MeHg dose	Control	Low MeHg dose	High MeHg dose	Control	Low MeHg dose	High MeHg dose	Diet effect	MeHg effect	Diet x MeHg
Fish mass (g)	6.1 ± 0.4	6.1 ± 0.2	5.9 ± 0.3	6.0 ± 0.2	6.1 ± 0.5	6.0 ± 0.4	6.9 ± 0.2	6.2 ± 0.2	6.2 ± 0.3	6.2 ± 0.1	6.1 ± 0.3	6.5 ± 0.3	0.4364	0.6895	0.7207
HSI (%)	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	1.3 ± 0.1	1.3 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.0	1.4 ± 0.1	1.3 ± 0.0	1.3 ± 0.0	0.1178	0.5105	0.5393
Fish DM	216.2 ± 0.5	216.1 ± 2.1	213.5 ± 1.2	203.3 ± 0.8	207.5 ± 0.7	200.4 ± 2.9	209.4 ± 4.5	203.2 ± 2.4	199.5 ± 2.0	205.2 ± 2.9	196.1 ± 3.1	195.2 ± 1.8	**<0.001**	**0.0051**	0.2213
Fish crude ash	19.7 ± 0.4	19.5 ± 0.1	18.8 ± 0.2	18.4 ± 0.3	18.4 ± 0.2	17.7 ± 0.1	19.6 ± 0.7	17.9 ± 0.1	17.7 ± 0.4	18.0 ± 0.2	16.5 ± 0.4	16.8 ± 0.1	**<0.001**	**<0.001**	0.1558
Fish crude protein	137.5 ± 1.2	139.1 ± 0.5	139.1 ± 1.1	133.1 ± 1.5	137.7 ± 0.3	134.0 ± 0.9	138.1 ± 2.8	135.0 ± 0.9	134.0 ± 1.0	132.8 ± 1.8	130.1 ± 1.4	129.0 ± 0.8	**<0.001**	0.2369	0.0694

(B)	Diet effect				MeHg effect		
	LA	**ALA**	**EPA**	**DHA**	**Control**	**Low MeHg dose**	**High MeHg dose**
Fish DM	a	b	b	b	a	a,b	b
Fish crude ash	a	b	b	c	a	b	b
Fish crude protein	a	b	b	c	-	-	-

The control, low and high MeHg doses correspond to 0, 2.4 and 5.5 mg MeHg/kg DM, respectively. The p-values related to two-way GLM are reported in the last three columns of the Table (A). Significant effects are shown in bold ($p < 0.05$). Results of relevant *post hoc*

tests are detailed in Table (B). Conditions with different letters indicate significant effect of the diet (irrespective of the MeHg dose) or the MeHg (irrespective of the diet). Crude lipid results are presented in Figure 2. Data are presented as mean ± standard error of the mean (n = 4 except for the condition "ALA-Control" where n = 2, and for the condition "DHA-Control" where n = 3 for the Crude protein parameter). Abbreviations: ALA, α-linolenic acid; DHA, docosahexaenoic acid; DM, dry matter; EPA, eicosapentaenoic acid; HSI, hepatosomatic index; LA, linoleic acid; MeHg, methylmercury.

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Cathy Debier : Conceptualization, Methodology, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: