Interaction between dietary selenium and methylmercury on growth performance, deposition and health parameters in rainbow trout fed selenium-rich tuna-based diets or selenium-poor plant-based diets

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Highlights

- Se addition in plant diets prevented growth reduction induced by MeHg at 21 days
- SeMet in tuna diets hampered MeHg-induced pro-inflammatory response at 21 days
- Se(IV) in tuna diets reduced Hg body level in fish fed MeHg at 21 days
- MeHg addition in tuna diets limited growth reduction by Se(IV) at 168 days
- MeHg addition in plant and tuna diets increased body Se content at 168 days

Abstract

This study evaluated the effect of dietary mercury (Hg) and selenium (Se) sources and levels on growth performance, Hg and Se accumulation, pro-inflammatory cytokine levels and global health parameters in rainbow trout juveniles. A 6-month feeding trial was carried out with a control plant-based diet (containing analyzed Se and Hg levels: 0.3 and 0 mg/kg diet, respectively) or a control tuna-based diet (containing analyzed Se and Hg levels: 7.5 and 0.3 mg/kg diet, respectively) supplemented with 0 or 1.5 mg Se/kg diet supplied either as sodium selenite (Se(IV)) or selenomethionine (SeMet) combined with 0, 1.6 or 2 mg Hg/kg diet supplied as methylmercury (MeHg). Fish sampling was carried out at 2 times: after a short (21 days) and a long term dietary exposure (168 days). In the short term, a temporarily reduced growth was noticed in fish fed MeHg-supplemented plant-based diet in absence of dietary Se supplementation. MeHg supplementation increased pro-inflammatory cytokine TNF-α and IL-1β levels in plant and tuna-based diets. MeHg supplementation also affected short-term fish global health parameters with an increase of glucose and albumin levels in tuna-based diets. The addition of SeMet in MeHg-supplemented tuna-based diets protected against the increase of TNF-α level in the long term whereas the addition of Se(IV) in MeHg-supplemented tuna-based diets decreased whole-body Hg level and retention in the short term. In the long term, a reduced growth, a higher feed conversion ratio and a lower protein efficiency ratio were recorded in fish fed Se(IV)-supplemented tuna-based diets in absence of MeHg supplementation. The addition of Se(IV) or SeMet in tuna-based diets increased plasma IL-6 level. The addition of MeHg in plant and tuna-based diets increased Se accumulation and retention. This work underlines that different forms of Se supplementation, in two dietary background context (low basal Se level in plant-based diets and high basal Se level in tuna-based diets), have specific effects on metabolism and biological consequences of dietary MeHg. Dietary inorganic Se (but not organic Se) affected MeHg metabolism by reducing Hg

accumulation in fish. However, dietary organic Se displayed better ability to afford protection against MeHg pro-inflammatory effects.

Keywords (between 4 and 6 words maximum):

Methylmercury; sodium selenite; selenomethionine; tuna by-products; inflammation; rainbow trout.

1. Introduction

Global concerns of public opinion rise on aquaculture production and its impact on the environment, due to the use of fishmeal (FM) and fish oil (FO) produced from wild fish catch (FAO, 2022). Plant and other alternative ingredients are increasingly used in aquafeed formulation to replace FM and FO (FAO, 2022). Another strategy is to increase the part of fish by-products: in 2020, 27% of the global production of FM and 48% of the total production of FO came from by-products (IFFO, 2021). Tunas represent one of the most high-value fish group with their high catch volume and important economic value. In 2020, catches of tuna and tuna-like species represented 7.8 million tonnes (FAO, 2022). Tuna cannery industry generates high amount of by-products made of viscera, head, bones, skin and fins, representing up to 70% of processed fish (FAO, 2022). Reports are available concerning the use of tuna by-products as aquafeed ingredient for FM replacement. A substitution up to 30% of FM by tuna by-products during 7 weeks in olive flounder juveniles (Kim et al., 2014) and a substitution up to 75% during 12 weeks in Korean rockfish juveniles (Kim et al., 2018) had no adverse effect on growth compared to fish receiving a non FM-substituted commercial diet. On the other hand, a FM replacement by 55% of tuna by-products decreased final body weight and increased feed conversion ratio of rainbow trout, compared to fish fed a commercial diet with anchovy meal as FM after 12 weeks of feeding (Tekinay et al., 2009). Tunas accumulate contaminants like mercury (Hg), lead and arsenic (Ormaza-González et al., 2020) and Hg levels in tunas vary widely depending on factors such as species and tissues (Ruelas-Inzunza et al., 2018).

Therefore, contrary to plant feedstuffs that present other nutritional limitations (Gatlin et al., 2007), the presence of Hg in tuna by-products raises some concern with regards to fish welfare and consumer safety as World Health Organization has classified Hg in the top ten chemicals of major concern for public health. Hg toxicity depends, among others on chemical Hg form (Jang et al., 2020). Methylmercury (MeHg) is the predominant chemical form found

in the environment and threatens human health to a higher extent than Hg inorganic forms (Honda et al., 2006). In salmonids, toxicity threshold is also reported to be lower for MeHg than for inorganic Hg (Berntssen et al., 2004) and a dietary level of 2 mg/kg MeHg has been shown to induce a pro-inflammatory response in juvenile Nile tilapia (Abu Zeid et al., 2021).

In addition to Hg, tunas accumulate high amount of selenium (Se) (Yamashita et al., 2011). Thus, tuna by-products are expected to contain high Se concentration, probably above rainbow trout and salmonid Se requirement, defined between 0.15 and 0.65 mg/kg diet (NRC, 2011; Antony Jesu Prabhu et al., 2020). On the contrary, plant-based aquafeeds are known to contain low Se level, below salmonid requirements (Antony Jesu Prabhu et al., 2020). This essential element plays also a great role in the protection against Hg toxicity as demonstrated in goldfish and zebrafish (Bjerregaard et al., 2011; Amlund et al., 2015). These studies underlined also the importance of dietary Se concentration and chemical forms on Hg accumulation and detoxification.

The aim of this study was to investigate the impact of dietary MeHg and various Se chemical forms in alternative aquafeeds containing different basal Hg and Se concentrations. The Hg and Se deposition linked with growth performance, pro-inflammatory and overall health status after a short and long-term dietary exposure was assessed in rainbow trout juveniles.

2. Material and methods

2.1. Ethical statement

The feeding trial was conducted in accordance with the European directive 2010/63/EU and the French decree n° 2013-118 on the protection of animals used for scientific purposes. The protocol was approved by the Ethical Committee C2EA-73 and the French Ministry of Higher Education and Research (reference number APAFIS#27846-2020102812241350 v2).

2.2. Experimental diets

Diets were manufactured in the INRAE experimental facilities of Donzacq (Landes, France, https://doi.org/10.15454/GPYD-AM38), using a twin-screw extruder (BC 45, Clextral, Firminy, France). Twelve feeds were formulated with two different basal ingredient compositions (Table 1a). Plant-based diets were formulated with plant-derived proteins and tuna-based diets with FM derived from tuna by-products (Marine Biotechnology Products, Port-Louis, Mauritius). Contrary to tuna-based diets, plant-based diets were MeHg-free and known to contain low basal Se level. Control plant (PC) and control tuna (TC) diets were unsupplemented with MeHg and Se(IV) or SeMet either, containing thus only basal levels of Hg and Se (Table 1b). Two diets were supplemented with methylmercury(II) chloride (Sigma-Aldrich, Saint-Quentin-Fallavier, France): 2 mg Hg/kg diet for plant-based diet (PH) and 1.6 mg Hg/kg diet for tuna-based diet (TH). Four other diets were supplemented with 1.5 mg Se/kg diet, using either inorganic Se source (sodium selenite (Se(IV)), Sigma-Aldrich, Saint Louis, Missouri, USA) in diets PI and TI or organic Se source (L-selenomethionine (SeMet), Excential Selenium 4000, Orffa, Breda, Netherlands) in diets PO and TO, respectively. Two diets were supplemented with both Se(IV) and MeHg (PHI and THI diets) and two others with both SeMet and MeHg (PHO and THO diets).

2.3. Experimental design

A 6-month feeding trial was carried out in the INRAE experimental fish farm at Donzacq. All-female diploid rainbow trout (*Oncorhynchus mykiss*) from the same parental stock (n = 1800) with an initial body weight of 26 ± 1 g were randomly allocated to 50-L fiberglass tanks with 50 fish per tank for the first two months, then to 150-L fiberglass tanks with 26 fish per tank for the last four months of the trial. Water flow was set to ensure an oxygen

concentration above 90% saturation. Tanks were supplied with flow-through (0.1 L/s, renewal 4.5 times/h at the beginning, then 0.25 L/s, renewal 7 times/h) spring water at 17 ± 1 °C. Fish were hand-fed twice a day to visual satiation under natural light regimen. Each diet was distributed to three replicate groups of fish over a 6-month growth trial. Mortality was recorded daily. Fish from each tank were bulk weighed every three weeks.

2.4. Sampling and data collection

Before every weighing and sampling (after 21 and 168 days), fish were overnight starved during 16 h. At the start, 21 fish in common, then 8 fish from each replicate tank (n = 24 fish per diet) at day 21 and day 168 were anaesthetized with benzocaine (30 mg/L) then killed with an overdose of benzocaine (60 mg/L). Fish were weighed and blood samples were collected from the caudal vein. Plasma was recovered from half of the centrifuged (5500 × g for 10 min) blood samples, immediately frozen and stored at -80 °C prior to analysis (n = 12 per diet). Three fish collected at the start and two fish per tank at day 21 and day 168 (n = 6 per diet) were frozen and stored at -20°C prior to proximate analysis. The liver of other collected fish (n = 18 at the start and n = 18 per diet at day 21 and day 168) were weighed for calculating hepatosomatic index (HSI). At the end of the feeding trial, remaining fish (from 3 to 10 per tank) were fed for another week, anaesthetized and killed 16 h after the last meal for faeces collection in distal gut for the determination of apparent digestibility coefficient (ADC) of Se and Hg. The samples of faeces were collected over ice, pooled per tank, frozen immediately and stored at -20 °C prior to Hg and Se analysis.

2.5. Proximate and mineral analyses in diets, whole fish and faeces

Dry matter content was determined by drying directly faeces samples or after grinding fish and diet samples at 105 °C for 24 h. Whole fish and faeces samples were freezed-dried

before further analyses. Proximate composition of diets and freeze-dried fish was determined according to following procedures: ash by incineration at 550 °C for 10 h, protein (N × 6.25) by the Kjeldahl method after acid digestion, total lipid according to Folch et al. (1957) using dichloromethane instead of chloroform and gross energy in an adiabatic bomb calorimeter. Total Se and Hg concentrations in diet, freeze-dried fish and faeces samples were measured by inductively coupled plasma mass spectrometry (ICP-MS) according to Silva et al. (2019).

2.6. Determination of apparent digestibility coefficient (ADC) of minerals

The apparent digestibility coefficient (ADC) of Se and Hg was measured using vanadium (V) as an internal marker in faeces and diets according to Witkowski et al. (2019).

2.7. Determination of plasma and blood metabolites

Plasma metabolites were determined in plasma of fish, $n = 3$ pooled samples per dietary treatment representing 4 fish each. Fish pro-inflammatory cytokines: tumor necrosis factor α (TNF-α), interleukine 1β (IL-1β) and interleukine 6 (IL-6) were determined with ELISA kit tests (Cusabio Biotech Co., Wuhan, Hubei, China) according to manufacturer's instructions using zebrafish TNF-α (cat. No. CSB-E13254Fh), zebrafish IL-1β (cat. No. CSB-E13254Fh) and Atlantic salmon (*Salmo salar*) IL-6 (cat. No. CSB-E13258Fh). TNF-α and IL-6 ELISA kits used a competitive inhibition enzyme immunoassay technique whereas IL-1β ELISA kit used a quantitative sandwich enzyme immunoassay technique. Kits provided cytokines biotin-conjugated, avidin conjugated horseradish peroxidase and a substrate solution. Horseradish peroxidase transformed the substrate into a coloured product. In TNF-α and IL-6 ELISA kits, colour developed in opposite to the amount of pro-inflammatory cytokines in samples. In IL-1β ELISA kit, the colour developed in proportion to the amount of IL-1β in samples. The absorbance was measured at 450 nm with a microplate reader and converted into concentrations

using the standard curve formula. Plasma lysozyme activity was determined according to Ellis et al. (1990) using a turbidimetric assay with lyophilized particles of *Micrococcus lysodeikticus* (Merck, Saint-Quentin-Fallavier, France). Alanine amino transferase (ALAT) and aspartate amino transferase (ASAT) activities were assessed in plasma by an end-point colorimetric test (AST GOT-ALT GPT Biolabo, France) at 505 nm with 2-oxoglutarate and 2,4-dinitrophenylhydrazine according to the manufacturer's instructions using 40 µL of fish plasma. Total protein content in plasma was determined through the colorimetric Biuret method using bicinchoninic acid (BCA) solution and copper(II) sulfate solution (Interchim uptima, France), using 10 µL of plasma sample diluted in distilled water (1:100) mixed with 200 µL of working reagent (50 volumes of BCA with 1 volume of copper(II) sulfate solution). Absorbance was measured at 562 nm. Total immunoglobulin level in plasma was determined using 10 µL of plasma samples diluted in distilled water (1:100), mixed with an equal volume of polyethylene glycol 70 % and centrifuged at 10 000 g, for 30 min at 15 °C for immunoglobulin precipitation. Precipitated samples (10 µL) were mixed with 200 µL of a solution containing BCA and copper(II) sulfate (1:50) into a microplate and then incubated for 30 min at 37 °C. The absorbance was measured at 562 nm and converted into concentration using a standard curve as for total protein determination. Albumin level in plasma samples was assessed by end-point colorimetric test using bromocresol green method. A volume of 10 µL of plasma was used and absorbance was measured at 630 nm. Glycaemia was assessed at 504 nm using 5 µL of fish plasma and colorimetric method with glucose oxidase, following manufacturer's instructions (Glucose GOD-POD, Sobioda S.A.S, Montbonnot St Martin, France). Haemoglobin was measured in blood samples (n = 3 per dietary treatment) using a colorimetric method (Hb-Randox kit, Ref HG1539, United Kingdom) following the manufacturer's instructions with 20 µL of blood.

2.8. Statistical analyses

Data are expressed as mean ± standard error (SEM). Differences between dietary groups were analysed using a two-way ANOVA to test the effect of dietary Hg supplementation and dietary Se supplementation and their interaction. Prior to the two-way ANOVA, normality and homogeneity of variance of data were checked with a Shapiro-Wilk and a Levene test, respectively. When appropriate, analysis was followed with a Tukey *post hoc* test. Statistical analyses were performed using R software (version 3.6.1, R development Core Team, 2008). Differences were considered significant when *p-value* was < 0.05.

3. Results

3.1. Dietary MeHg impact on growth performance, proximate body composition of rainbow trout juveniles and interactive effects of dietary Se

After 21 days of feeding, fish fed MeHg-supplemented plant-based diet PH displayed decreased body weight compared to fish fed control plant-based diet PC (Fig. 1A). Such effect of MeHg was not noticed in the presence of Se in diets PHI and PHO compared to control diets PI and PO (Fig. 1A). No significant effect of dietary MeHg supplementation was noticed on feed intake (FI) whereas a significant interaction was detected between MeHg and dietary Se forms on feed conversion ratio (FCR) and protein efficiency ratio (PER), but Tuckey's *post hoc* test did not allow to discriminate between plant-based dietary groups (Table 2). The highest FCR and the lowest PER were displayed in PH group and the lowest FCR and highest PER were noticed in PC group (Table 2). After 168 days of feeding, fish fed PH diet displayed decreased body ash retention compared to fish fed PC diet (Table 2). No other significant effect of MeHg supplementation in plant-based diets was observed on body weight (Fig. 1B), FI, FCR, PER, survival or proximate body composition (Table 2).

After 21 days of feeding, fish fed MeHg-supplemented tuna-based diets TH, THI and THO exhibited decreased body gross energy content compared to fish fed control diets TC, TI and TO (Table 3) without significant effect on growth (Fig. 1A). After 168 days of feeding, fish fed MeHg-supplemented diets TH, THI and THO displayed higher FI and reduced hepatosomatic index compared to fish fed TC, TI and TO (Table 3). A higher body weight was recorded in fish fed the MeHg and Se(IV)-supplemented diet THI compared to the control group TI (Fig. 1B) without significant difference on FCR and PER (Table 3). No significant effect of dietary MeHg was recorded on survival rate of fish fed tuna-based diets (Table 3).

3.2. Dietary Se impact on growth performance, proximate body composition of rainbow trout juveniles and interactive effect of dietary MeHg

After 21 days of feeding, fish fed Se-supplemented plant-based diets PI, PHI, PO and PHO displayed no significant differences in growth performance (Fig. 1A and Table 2) or proximate body composition compared to fish fed control diets PC and PH (Table 2). After 168 days of feeding, higher body dry matter, total lipid, gross energy content and lipid retention were noticed in fish fed Se-supplemented diets PI and PO compared to the control group PC (Table 2), but no significant effect of dietary Se supplementation was noticed on fish body weight (Fig. 1B) or other growth and survival parameters (Table 2).

After 21 days of feeding, fish fed Se-supplemented tuna-based diets TI, THI, TO and THO displayed decreased body weight compared to fish fed diets TC and TH (Fig 1A). After 168 days of feeding, reduced body weight was recorded in fish fed Se(IV)-supplemented diet TI compared to fish fed diets TC and TO (Fig. 1B). Fish fed SeMet-supplemented diets TO and THO displayed higher PER, protein retention and decreased FCR compared to Se(IV)-supplemented diets TI and THI (Table 3). Fish fed Se-supplemented diets TI, THI, TO and THO exhibited reduced ash retention compared to fish fed control diets TC and TH (Table 3).

No significant effect of dietary Se was recorded on survival rate of fish fed tuna-based diets (Table 3).

3.3. Dietary MeHg impact on Hg and Se body composition, retention and digestibility in rainbow trout juveniles and interactive effect of dietary Se

MeHg supplementation in plant-based diets PH, PHI and PHO increased both short-term and long-term body Hg content compared to fish fed control diets PC, PI and PO (Fig. 2A and Fig. 2B). After 168 days of feeding, fish fed diets PH, PHI and PHO displayed higher faecal Hg content compared to fish fed diets PC, PI and PO (Table 4). Fish exposed to diet PHO exhibited higher body Se content compared to fish receiving diet PO (Fig. 2D). Fish fed diets PHI and PHO presented reduced faecal Se content, increased Se digestibility and retention compared to fish fed diets PI and PO (Table 4).

In both the short and long term, higher body Hg content and lower Hg retention were recorded in fish fed MeHg-supplemented tuna-based diets TH, THI and THO compared to diets TC, TI and TO (Fig. 2A, Fig. 2B and Table 5). After 168 days of feeding, diets TH, THI and THO increased faecal Hg content and Hg apparent digestibility coefficient compared to diets TC, TI and TO (Table 5). After 21 days of feeding, fish fed THI diet exhibited reduced Hg retention compared to fish fed TI diet (Table 5). Fish fed diets TH, THI and THO displayed decreased Se retention (Table 5) without significant impact on body Se content (Fig. 2C) compared to fish fed control diets TC, TI and TO. However, in the long term, increased Se retention (Table 5) and body Se content (Fig. 2D) were recorded in fish exposed to diets TH, THI and THO compared to fish receiving diets TC, TI and TO.

3.4. Dietary Se impact on Hg and Se body composition, retention and digestibility in rainbow trout juveniles and interactive effect of dietary MeHg

In both the short and long term, fish exposed to Se-supplemented plant-based diets PI, PHI, PO and PHO displayed higher body Se content compared to fish fed control diets PC and PH (Fig. 2C and Fig. 2D). The highest body Se content was observed in fish fed SeMet-supplemented diets PO and PHO (Fig. 2C and Fig. 2D). These fish also exhibited higher Se digestibility compared to fish fed Se(IV)-supplemented diets PI and PHI and non Se-supplemented diets PC and PH with a higher Se retention and a reduced faecal Se content compared to fish fed PI and PHI (Table 4). Fish fed Se(IV)-supplemented diets PI and PHI displayed a higher short-term Se retention compared to fish fed PC and PH but a reduced long-term Se retention (Table 4). Se(IV) supplementation in MeHg-supplemented diet PHI increased faecal Hg content compared to diets PH and PHO (Table 4) without significant effect on Hg digestibility, retention (Table 4) or body Hg content (Fig. 2B).

Se supplementation in tuna-based diets TI, THI, TO and THO increased fish body Se content (Fig. 2C and Fig. 2D) and retention (Table 5) as early as 21 days of feeding with a superiority for SeMet, similarly to plant-based diets. No significant differences were observed on Se digestibility coefficient and faecal Se content between fish fed the Se(IV)-supplemented diets TI and THI and fish fed SeMet-supplemented diets TO and THO (Table 5). After 21 days of feeding, fish fed Se(IV)-supplemented diet THI displayed a reduced whole-body Hg content compared to fish fed diets TH and THO (Fig. 2A) with a significantly reduced Hg retention compared to fish fed diet THO (Table 5).

3.5. Dietary MeHg impact on plasma pro-inflammatory cytokines and global health parameters in rainbow trout juveniles and interactive effect of dietary Se

Short-term feeding with MeHg-supplemented plant-based diets PH, PHI and PHO induced higher plasma TNF-α and IL-1β levels compared to control diets PC, PI and PO (Fig. 3A and Fig. 3C). Long-term feeding with MeHg-supplemented plant-based diets also resulted

in higher plasma TNF-α levels, however only fish fed the diet PHO supplemented with both MeHg and SeMet, exhibited significantly higher TNF-α levels than those fed the control diet PO (Fig. 3B). A long-term dietary supplementation with MeHg increased plasma total protein content and decreased immunoglobulin proportion when fed with Se(IV) (Table 6).

Short-term feeding with MeHg-supplemented tuna-based diets TH, THI and THO induced higher plasma IL-1β (Fig. 3C), glucose and albumin levels (Table 7) compared to fish fed diets TC, TI and TO. Plasma TNF-α level was also increased in MeHg-supplemented, non Se-supplemented diet TH compared to control diet TC (Fig. 3A). When MeHg was added concomitantly with both Se compounds (Se(IV) and SeMet) in diets THI and THO, no more significant increase of TNF-α level was observed compared to non MeHg-supplemented diets TI and TO (Fig. 3A). On the contrary, MeHg intake increased lysozyme levels only when fed with SeMet in diet THO compared to control diet TO (Table 7). In the long term, no more significant effect of MeHg supplementation in tuna-based diets was noticed (Fig. 3B, Fig. 3D, Fig. 3F and Table 7).

3.6. Dietary Se impact on plasma pro-inflammatory cytokines and global health parameters in rainbow trout juveniles and interactive effect of dietary MeHg

Short-term feeding with Se(IV)-supplemented diets PI and PHI resulted in decreased plasma IL-1β levels compared to other plant-based diets PC, PH, PO and PHO (Fig. 3C). In fish fed non MeHg-supplemented diets for 168 days, SeMet increased blood haemoglobin levels (Table 6) compared to control diet PC but when added concomitantly with MeHg in diet PHO, SeMet increased plasma TNF-α levels compared to diet PHI (Fig. 3B). Long-term Se(IV) intake in plant-based diets PI and PHI increased blood haemoglobin levels compared to non-Se supplemented diets PC and PH (Table 6).

Short-term feeding with SeMet-supplemented diets TO and THO reduced plasma TNF-α and IL-1β levels compared to other tuna-based diets TC, TH, TI and THI (Fig. 3A and Fig. 3C). In absence of dietary MeHg supplementation, Se(IV)-supplemented diet TI led to higher TNF-α levels compared to control diet TC (Fig. 3A) and higher lysozyme levels compared to SeMet-supplemented diet TO (Table 7). Long-term SeMet supplementation decreased TNF-α level only when supplemented with MeHg in diet THO compared to diets TH and THI (Fig. 3B) whereas both Se(IV) and SeMet supplementation in diets TI, THI, TO and THO increased IL-6 levels compared to non Se-supplemented diets TC and TH (Fig. 3F).

4. Discussion

4.1. Short-term effect of dietary MeHg on growth, pro-inflammatory cytokines, overall health status and Hg deposition in rainbow trout juveniles and interactive effect of dietary Se

In MeHg-supplemented plant-based diet PH, containing low basal Se level (1.8 mg Hg/kg diet and 0.3 mg Se/kg diet), MeHg caused a transient growth reduction with higher FCR and plasma pro-inflammatory cytokine content observed only after 21 days of feeding without effect on survival. Such transient effect, after a 21-day exposition to dietary MeHg, is to our knowledge, not reported in the literature. Nile tilapia exposed to MeHg-supplemented FM-based diet (2 mg Hg/kg, 30 days of feeding) displayed higher pro-inflammatory cytokine transcript levels (TNF-α, IL-1β and IL-8) in the spleen (Abu Zeid et al., 2021). Our study confirm those observations but at a plasma metabolite level. Recently, a meta-analysis in Atlantic salmon underlined that fish growth performance decreased with increased severity of enteritis, a pathological state associated with important induction of pro-inflammatory parameters in intestines (Agboola et al., 2022). Thus, the inflammation noticed in our study could explain the observed growth reduction. However, fish fed MeHg-supplemented tuna-based diets displayed also higher pro-inflammatory cytokine levels and despite a decreased

gross energy content, those fish displayed no growth alteration. So the transient growth reduction observed in fish fed MeHg in low Se plant-based diets might not only be related to the induction of inflammatory response but also to a toxic effect of Hg that would be emphasized in absence of Se such as the induction of oxidative stress (Baldissera et al., 2020). Indeed we noticed increased transcript levels of antioxidant enzymes such as glutathione-S-transferase π and methionine sulfoxide reductase B3 in liver of trout fed the MeHg-supplemented plant-based diet PH for 21 days compared to the control diet PC (Bidon et al., 2021). An increased transcript level of the selenoprotein P and glutathione peroxidase 1 in Se-supplemented diets PI, PHI, PO and PHO was also noticed, suggesting a better antioxidant protection with dietary Se supplementation that deserves further investigation for a better characterization and understanding.

The effects observed after 21 days of feeding on growth and inflammatory status by dietary MeHg in low Se plant-based diets was not supported by plasma parameters. Short-term effects of dietary MeHg were recorded but only in fish fed tuna-based diets with an increase of glucose and albumin levels. A 3-month feeding trial on Atlantic salmon established at 5 mg MeHg/kg the threshold at which the lowest toxic effect of dietary MeHg could be observed with a reduction of haematocrit and an increase of plasma protein (Berntssen et al., 2004). This concentration is above our dietary MeHg levels and could explain our results in rainbow trout.

The addition of MeHg in plant and tuna-based diets increased significantly the whole-body Hg level after short and long-term feeding. Despite the same level of dietary MeHg in plant and tuna-based diets, higher body Hg content were noticed in fish fed plant-based diets, after short (1.8 fold increase in PH diet compared to TH) and long-term exposure (1.3 fold increase in PH diet compared to TH). A negative linear correlation have been underlined between dietary molar ratio of Se/Hg and total Hg levels in Sacramento splittail larvae (Deng et al., 2008). Molar ratio of Se/Hg in MeHg-supplemented tuna-based diets was higher than in

MeHg-supplemented plant-based diets (11.6 vs. 2.0, respectively) and might explain why fish fed MeHg-supplemented tuna-based diets displayed lower whole-body Hg level than fish fed MeHg-supplemented plant-based diets. However, despite the fact that dietary Se/Hg molar ratio was negatively correlated to whole-body Hg level in fish, it must be addressed that this parameter did not take into consideration chemical form of Se, another parameter that can influence Hg deposition. Indeed, despite a similar or even higher dietary Se/Hg molar ratio in THO diet compared to THI diet (13.0 vs. 11.9, respectively), fish fed THO displayed higher whole-body Hg level than fish fed THI in the short term (0.28 vs. 0.22 mg Hg/kg wet weight, respectively). However, this effect was no more significant after 168 days of feeding (0.78 vs. 0.91 mg Hg/kg wet weight). The reduced Hg digestibility (57% vs. 87% for MeHg-supplemented tuna and plant-based diets) associated with the reduced Hg retention after short (52% vs. 75% for MeHg-supplemented tuna and plant-based diets, respectively) and long term (54% vs. 64% for MeHg-supplemented tuna and plant-based diets, respectively) supports the reduced body Hg content for fish fed high Se tuna-based diets. It is noteworthy that, in fish fed MeHg-supplemented plant-based diets, Hg retention was higher after a short-term than a long-term exposure (75% after 21 days vs. 64% after 168 days of feeding). Similar trends were observed in muscle of zebrafish fed 5 and 10 mg MeHg/kg diet after 2 weeks by Amlund et al. (2015) and attributed to a variation in the uptake or in the elimination kinetic. It would have been interesting to also determine Hg digestibility after a short term exposure to know if there is variation in Hg uptake through time and describe better the Hg kinetic deposition in rainbow trout.

Interestingly, the short-term effect of dietary MeHg in diet PH containing low basal Se level on fish growth, was not observed in Se-supplemented diets PHI and PHO or in Se-rich tuna diets. Similar protective effect of high dietary Se on MeHg-induced growth reduction was

reported in zebrafish exposed during 153 days to 10 mg/kg diet of SeMet and 12 mg/kg diet of MeHg (Penglase et al., 2014).

4.2. Effect of dietary MeHg on Se deposition in rainbow trout juveniles

No short-term effect of dietary MeHg was found on Se whole-body level. However, higher Se retention and digestibility were noticed after long-term exposure to MeHg-supplemented plant-based diets. Similarly, fish fed MeHg-supplemented tuna-based diets displayed higher Se retention, highlighting the effects of dietary MeHg on whole-body Se deposition in both dietary groups. The increase of Se digestibility observed in fish fed MeHg-supplemented diets contradicts the decreased SeMet digestibility described in juvenile white sturgeon by Huang et al. (2013). The differences observed in both studies could be attributed to the dissimilar Hg and Se exposures. In the study of Huang et al. (2013), MeHg and SeMet were administered by intragastric gavage at lower concentration than in our study (0.85 mg Hg/kg body weight and 0.5 mg Se/kg body weight). On the other hand, the increase of whole-body Se level measured in fish fed MeHg-supplemented diets was reported in Sacramento spittail larvae exposed to dietary SeMet (0.64-35.0 mg/kg diet) and MeHg (0.01-11.7 mg/kg diet) with higher whole-body Se level when concomitantly exposed to SeMet and MeHg (Deng et al., 2008). The increase of body Se level in fish exposed simultaneously to MeHg and Se in comparison to the one only exposed to Se could be attributed to the formation of Se-Hg complexes such as mercury selenide recently identified in freshwater fish as Hg detoxification pathway (Manceau et al., 2021).

4.3. Effect of dietary Se on Hg deposition, pro-inflammatory cytokines, growth in rainbow trout juveniles and interactive effect of dietary MeHg

A short-term effect of Se(IV) was observed on Hg deposition with lower whole-body Hg level and retention measured in fish fed THI diet. This result highlights the superiority of Se(IV) over SeMet to reduce Hg level in agreement with data reported in rainbow trout and goldfish by Bjerregaard et al. (1999, 2011). The lack of dietary SeMet effect on Hg deposition has also been reported in muscle of rainbow trout fed concomitantly MeHg and SeMet by Ribeiro et al. (2022), stressing the importance of Se chemical form on Hg detoxification. The lowering effect of Se(IV) supplementation in Se-rich tuna-based diets on body Hg accumulation was not noticed in Se-low plant-based diets, supporting the idea of a minimal Se threshold concentration to detect effects on Hg accumulation, as reported in an earlier study (Bjerregaard et al., 2011).

A protective effect of dietary Se on the short-term pro-inflammatory effect of dietary MeHg was recorded in our study. Both Se(IV) and SeMet addition in tuna-based diets helped to alleviate the increase of TNF-α level by dietary MeHg addition after a short-term exposure whereas only SeMet reduced TNF-α level after a long-term exposure. This result highlights the superiority of SeMet to afford protection against MeHg pro-inflammatory effects in the long term in presence of high dietary basal Se level in tuna-based diets, as the lowering effect of SeMet on cytokine levels was not observed with plant-based diets. The greater capacity of SeMet over Se(IV) to reduce inflammatory response was also reported in head kidney leucocytes isolated from Atlantic salmon post-smolts after a 9-week feeding trial (Antony Jesu Prabhu et al., 2020). As described earlier, inflammation could be responsible for a reduction of fish growth (Agboola et al., 2022). In fish fed plant-based diets, Se supplementation had no influence on the inflammatory state of fish, despite an improvement of growth in fish fed the Se-supplemented diets PHI and PHO compared to fish fed the MeHg-supplemented diet PH. Thus, as mentioned before, inflammation alone cannot explain effects on fish growth.

On the other hand, despite the anti-inflammatory effect of Se discussed above, too high dietary Se level could also be detrimental. After a short-term feeding with the Se(IV)-supplemented tuna-based diet TI, plasma TNF-α content was increased. Evidences of pro-inflammatory effects in presence of high dietary Se levels are scarce in fish. To our knowledge, only the study of Pérez-Valenzuela et al. (2021) reported mononuclear cell infiltration in rainbow trout liver, a sign of early inflammation after an 8-week feeding trial using 10 mg Se/kg diet supplied as Se-yeast, an organic chemical form of Se. IL-6 levels were increased after a long-term exposure with Se-supplemented tuna-based diets compared to fish fed non Se-supplemented tuna-based diets, strengthening the possibility of pro-inflammatory response occurring after a Se supplementation in presence of high dietary Se level.

The growth reduction noticed with TI after 6 months of feeding suggests the supplementation with Se(IV) in tuna-based diet containing high basal Se level might be toxic for rainbow trout but not when supplemented as SeMet. Se toxicity threshold in fish depends on chemical form of Se (Berntssen et al., 2018) and Se(IV) is considered more toxic than SeMet (Berntssen et al., 2017) with a lower toxicity threshold (Berntssen et al., 2018). These results are in accordance with our study as we did not observe adverse effects on growth with SeMet, despite the similar dietary Se level assessed in our tuna-based diets (9 mg Se/kg diet). However, it is important to note that, in tuna-based diets, basal Se level represents about 80% of the total Se concentration found in Se-supplemented tuna-based diets. The suspected principal chemical form of Se found in tuna is selenoneine, an organic chemical form of Se (Yamashita and Yamashita, 2010). Thus our results might rely on the chemical form added in the diet but also on the high basal Se level naturally present in tuna-based diets. It might be of great interest to perform a speciation analysis on tuna by-products and fish fed with these tuna-based diets to precisely characterize chemical form of Se.

When MeHg was added to TI diet, detrimental effects on fish growth performance were alleviated, underlying an antagonist effect of MeHg, maybe through the formation of Hg-Se compounds that are potentially associated with a reduction of Se bioavailability (Raymond and Ralston, 2020).

4.4. Tuna by-products an interesting source of selenium for aquafeeds?

Tuna-based diets used in the present study displayed high Se levels, far above salmonid requirement (Antony Jesu Prabhu et al., 2020). Despite Se concentration in the TC diet equal to 7.5 mg Se/kg diet and thus below levels reported to reduce fish growth performance: 13 mg Se/kg diet supplied as Se(IV) for rainbow trout (Hilton et al., 1982) and 11 mg Se/kg diet for Atlantic salmon (Berntssen et al., 2018), it might be safer to reduce dietary Se level. Tuna, like other fish, accumulate Se mainly in liver and kidney (Ruelas-Inzunza et al., 2018; Belmonte et al., 2021). These organs are parts of tuna by-products, thus adjusting their level in by-products might be interesting to control Se level. Another possibility to reduce dietary Se level is to decrease the level of tuna by-products inclusion, in our study, we used an inclusion level of 60%, lower level might be suitable.

However, the main concern of FM prepared from tuna by-products used in aquaculture comes from the presence of contaminants, especially MeHg (Kim et al., 2019). In Europe, the limit for Hg concentration in rainbow trout muscle must not exceed 0.3 mg/kg for human consumption (European Union, 2022). This threshold was not exceeded at whole-body level in fish fed the control tuna-based diet, even after 6 months of feeding, with whole-body Hg level concentration of 0.13 mg Hg/kg that might comply with the existing EU regulation, highlighting the potential of this ingredient for aquafeeds. However, Hg is known to accumulate in muscle (Giblin and Massaro, 1973) and so the Hg level in muscle is requested to conclude about the potential use of tuna by-products as aquafeed ingredient for rainbow trout aquaculture.

5. Conclusion

In conclusion, the present work highlights the importance of dietary chemical forms and concentration of Se on the understanding of MeHg and Se effects. MeHg accumulation was reduced by Se(IV) in high Se tuna-based diets after a short-term exposure and MeHg-induced pro-inflammatory response was hampered by SeMet in high Se tuna-based diets. On the other hand, growth performance and inflammatory status were impaired by Se(IV) in Se-rich tuna-based diets, and paradoxically, antagonist effect by MeHg supplementation was displayed. Se digestibility, body content and retention were also higher in fish exposed to dietary MeHg, that deserves further investigation.

Abbreviations

ALAT, alanine amino transferase; ASAT, aspartate amino transferase; Hg, mercury; IL-1β, interleukine 1β; IL-6, interleukine 6; MeHg, methylmercury; PC, control plant-based diet; PH, methylmercury-supplemented plant-based diet; PHI, methylmercury and selenite-supplemented plant-based diet; PHO, methylmercury and selenomethionine supplemented plant-based diet; PI, selenite-supplemented plant-based diet; PO, selenomethionine-supplemented plant-based diet; Se, selenium; Se(IV), selenite; SeMet, selenomethionine; TNF-α, tumor necrosis factor α; TC, control tuna-based diet; TH, methylmercury-supplemented tuna-based diet; THI, methylmercury and selenite-supplemented tuna-based diet; THO, methylmercury and selenomethionine-supplemented tuna-based diet; TI, selenite-supplemented tuna-based diet; TO, selenomethionine-supplemented tuna-based diet.

Author's contributions

MB: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, review & editing, Visualization; **AJPP:** Conceptualization, Formal analysis, Investigation, Writing – review & editing, Funding acquisition; **AB:** Formal analysis, Data curation; **AH:** Formal analysis, Data curation; **JR:** Writing – review & editing, Supervision; **ZPZ:** Conceptualization, Writing – review & editing, Funding acquisition; **SFD:** Conceptualization, Formal analysis, Investigation, Data curation, Writing –review & editing, Supervision.

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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Table 1a

Experimental diet formulation and composition.

Diet	Plant-based diets	Tuna-based diets
Ingredients (%)		
Plant-derived proteins[a]	73.8	25.8
Tuna by-products[b]	-	60
Crystalline amino acid premix[c]	3.2	-
Rapeseed lecithin[d]	2	-
Vegetable oils[e]	8	8
Fish oil[f]	8	4
Vitamin and mineral premixes[g]	5	2.2
Sodium selenite (mg Se/kg diet)	-	-
L-selenomethionine (mg Se/kg diet)	-	-
Methylmercury chloride (mg Hg/kg diet)	-	-
Analytical composition		
Dry matter (DM, %)	97.4	97.7
Crude protein (% DM)	49.3	51.2
Total lipid (% DM)	22.0	19.1
Gross energy (kJ/g DM)	24.8	24.1
Starch (% DM)	8.2	11.6
Ash (% DM)	6.7	7.8

[a] Plant-derived proteins (% diet in plant-based or tuna meal-based diets respectively): wheat gluten, 18 or 0 (Roquette, France); corn gluten, 17 or 0 (Inzo, France); soybean protein concentrate, 15 or 0 (Estril®75 Sopropêche, France); soybean meal, 5 or 0 (Sud-Ouest Aliment, France); white lupin meal, 5 or 0 (Farilup500 Terrena, France); rapeseed meal, 5 or 0 (Primor 00 Sud-Ouest Aliment, France); dehulled pea mea, 3.8 or 10.8 (Primatex Sotexpro, France), whole wheat, 5 or 15 (Sud-Ouest Aliment, France).

[b] Tuna meal HighPro68 (Port-Louis, Mauritius).

[c] Crystalline amino acid premix (% diet): L-lysine, 1.4; DL-methionine, 0.3; glucosamine, 0.5; taurine, 0.3; betaine, 0.3; glycine, 0.2; alanine, 0.2.

[d] Adivec (France).

[e] Vegetable oils (% diet): rapeseed oil, 4; linseed oil, 2.4; palm oil 1.6.

[f] Sopropêche (France).

[g] Vitamin and mineral premixes Se-free (per kg diet): retinyl acetate, 5000 IU; cholecalciferol, 2500 IU; DL-α-tocopheryl acetate, 50 IU; sodium menadione bisulfate, 10 mg, thiamin-HCl, 1

mg; riboflavin, 4 mg; niacin, 10 mg; D-calcium pantothenate, 20 mg; pyridoxine-HCl, 3 mg; myo-inositol, 0.3 g; D-biotin, 0.2 mg; folic acid, 1 mg; cyanocobalamin, 0.01 mg; L-ascorbyl-2-polyphosphate, 50 mg; choline-HCl, 1 g; $CaHPO_4.2H_2O$ (18% P; 22% Ca), 33 or 5 g in plant-based or tuna meal-based diets respectively; $CaCO_3$ (40% Ca),·2.15 g; $MgOH_2$ (42% Mg), 1.24 g; KCl (52% K), 0.9 g; NaCl (39% Na), 0.4 g; $FeSO_4·H_2O$ (33% Fe), 20 mg; $ZnSO_4·H_2O$ (36% Zn), 35 mg; MnO (77%); 10 mg; $CuSO_4·5H_2O$ (25% Cu), 5 mg; NaF (45% F), 10 mg; CaI_2 (86% I), 3 mg; $CoCO_3$ (50% Co), 0.05 mg; BHA, 0.75 mg; BHT, 0.75 mg; propyl gallate, 0.15 mg; sepiolite, 200 mg. All ingredients were diluted with α-cellulose.

Table 1b

Supplementation level for sodium selenite, L-selenomethionine and methylmercury chloride and elemental composition of tuna and plant-based diets.

Diet	Plant-based diets						Tuna-based diets					
	PC	PH	PI	PHI	PO	PHO	TC	TH	TI	THI	TO	THO
Ingredients												
Sodium selenite (mg Se/kg diet)	-	-	1.5	1.5	-	-	-	-	1.5	1.5	-	-
L-selenomethionine (mg Se/kg diet)	-	-	-	-	1.5	1.5	-	-	-	-	1.5	1.5
Methylmercury chloride (mg Hg/kg diet)	-	2	-	2	-	2	-	1.6	-	1.6	-	1.6
Elemental composition (mg/kg diet)												
Mercury	-	1.8	-	1.9	-	1.9	0.3	1.9	0.3	2.1	0.3	1.8
Selenium	0.3	0.3	3.2	2.3	2.3	2.0	7.5	7.2	9.0	9.8	9.0	9.0
Molar ratio of Se/Hg	-	0.4	-	3.1	-	2.6	62.7	9.9	84.7	11.9	83.2	13.0

Table 2

Growth performance, proximate body composition and retention of rainbow trout juveniles fed plant-based diets for 21 (D21) or 168 days (D168).

Plant based-diets	**PC**	**PH**	**PI**	**PHI**	**PO**	**PHO**	p_{Hg}	p_{Se}	$p_{Hg \times Se}$
Growth performance									
FI[1] D21	0.8 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	*0.434*	*0.441*	*0.131*
FI[1] D168	2.9 ± 0.1	2.7 ± 0.0	3.0 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	2.9 ± 0.0	*0.590*	*0.587*	*0.391*
FCR[2] D21	0.79 ± 0.01[a]	0.88 ± 0.02[a]	0.85 ± 0.03[a]	0.79 ± 0.01[a]	0.81 ± 0.03[a]	0.82 ± 0.01[a]	*0.400*	*0.630*	***0.023***
FCR[2] D168	0.89 ± 0.01	0.92 ± 0.04	0.94 ± 0.01	0.94 ± 0.01	0.90 ± 0.02	0.93 ± 0.03	*0.306*	*0.318*	*0.791*
PER[3] D21	2.6 ± 0.0[a]	2.3 ± 0.1[a]	2.4 ± 0.1[a]	2.6 ± 0.0[a]	2.5 ± 0.0[a]	2.5 ± 0.0[a]	*0.309*	*0.768*	***0.018***
PER[3] D168	2.3 ± 0.0	2.2 ± 0.1	2.2 ± 0.0	2.2 ± 0.0	2.3 ± 0.0	2.2 ± 0.1	*0.279*	*0.253*	*0.649*
HSI[4] D21	1.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.0	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.0	*0.937*	*0.072*	*0.373*
HSI[4] D168	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	*0.457*	*0.729*	*0.729*
SR[5] D21	99 ± 2	98 ± 2	97 ± 3	99 ± 1	100 ± 0	99 ± 1	*0.826*	*0.990*	*0.499*
SR[5] D168	95 ± 4	98 ± 2	97 ± 3	97 ± 3	96 ± 4	98 ± 2	*0.278*	*0.934*	*0.743*
Body composition[6]									
Dry matter D21	31 ± 0	31 ± 1	31 ± 1	31 ± 0	32 ± 1	31 ± 1	*0.455*	*0.511*	*0.933*
Dry matter D168	33 ± 1[b]	35 ± 0[ab]	37 ± 0[a]	35 ± 0[ab]	36 ± 0[ab]	37 ± 0[a]	*0.693*	***0.021***	***0.036***
Protein D21	15 ± 0	16 ± 0	15 ± 0	15 ± 0	16 ± 0	16 ± 0	*0.164*	*0.173*	*0.167*
Protein D168	17 ± 1	17 ± 0	17 ± 0	17 ± 0	17 ± 0	17 ± 0	*0.978*	*0.877*	*0.263*
Lipid D21	14 ± 0	13 ± 1	14 ± 0	13 ± 0	14 ± 1	13 ± 0	*0.190*	*0.827*	*0.892*
Lipid D168	15 ± 1[b]	16 ± 0[ab]	18 ± 0[a]	17 ± 1[ab]	17 ± 1[a]	18 ± 0[a]	*0.577*	***0.004***	***0.029***
Energy D21	9.1 ± 0.1	8.9 ± 0.2	9.0 ± 0.2	8.7 ± 0.1	9.1 ± 0.4	9.0 ± 0.3	*0.353*	*0.640*	*0.833*
Energy D168	9.7 ± 0.4[b]	10.5 ± 0.0[ab]	11.2 ± 0.1[a]	10.5 ± 0.2[ab]	10.7 ± 0.2[a]	10.9 ± 0.0[a]	*0.780*	***0.010***	***0.017***
Ash D21	1.9 ± 0.0	2.0 ± 0.1	1.9 ± 0.0	2.1 ± 0.2	2.0 ± 0.1	1.8 ± 0.1	*0.997*	*0.779*	*0.273*
Ash D168	2.1 ± 0.0	2.0 ± 0.1	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.1	2.0 ± 0.0	*0.958*	*0.522*	*0.345*
Body retention[7]									
Dry matter D21	41 ± 1	37 ± 2	36 ± 1	38 ± 2	42 ± 3	39 ± 3	*0.258*	*0.355*	*0.350*
Dry matter D168	38 ± 2	39 ± 1	41 ± 0	39 ± 0	41 ± 1	40 ± 1	*0.733*	*0.366*	*0.366*
Protein D21	40 ± 0	41 ± 2	38 ± 2	40 ± 1	42 ± 2	41 ± 1	*0.520*	*0.211*	*0.697*
Protein D168	39 ± 2	39 ± 1	38 ± 1	37 ± 0	38 ± 1	37 ± 1	*0.397*	*0.506*	*0.817*
Lipid D21	81 ± 7	65 ± 10	74 ± 2	67 ± 5	79 ± 11	74 ± 8	*0.165*	*0.754*	*0.771*
Lipid D168	78 ± 5	86 ± 2	93 ± 2	84 ± 3	89 ± 4	94 ± 2	*0.615*	***0.036***	*0.066*
Energy D21	51 ± 2	44 ± 3	46 ± 1	44 ± 2	50 ± 5	48 ± 4	*0.227*	*0.502*	*0.736*
Energy D168	45 ± 3	48 ± 1	50 ± 1	46 ± 1	49 ± 2	49 ± 1	*0.814*	*0.246*	*0.130*
Ash D21	39 ± 6	30 ± 5	35 ± 4	46 ± 10	43 ± 3	30 ± 5	*0.514*	*0.620*	*0.160*
Ash D168	38 ± 1[a]	28 ± 0[c]	34 ± 1[b]	34 ± 0[b]	34 ± 1[b]	35 ± 1[b]	***<0.001***	*0.183*	***<0.001***

PC, not supplemented; PH, supplemented with MeHg; PI, supplemented with Se(IV); PHI, supplemented with both MeHg and Se(IV); PO, supplemented with SeMet; PHO, supplemented with both MeHg and SeMet.

Values represent means ± SEM (n = 3). Means not sharing a common superscript letter are significantly different ($P_{Hg \times Se} < 0.05$) according to two-way ANOVA to test the effect of MeHg supplementation (P_{Hg}), Se supplementation (P_{Se}) and the interaction ($P_{Hg \times Se}$) followed by a Tukey's post hoc test. [1]FI, absolute feed intake expressed as g per fish per day = total dry feed intake per tank / (number of fish in tank × number of days over the experimental period). [2]FCR, feed conversion ratio = dry feed intake / wet weight gain. [3]PER, protein efficiency ratio = fish weight gain / crude protein intake. [4]HSI, hepatosomatic index expressed as % = liver weight / fish weight.[5]SR, survival rate expressed as %. [6]Body composition expressed as % for dry matter, crude protein, total lipid and ash and kJ/g for gross energy. [7]Body nutrient retention expressed as % = [(final weight × final body nutrient content) - (initial weight × initial body nutrient content)] / (total feed intake × feed nutrient content) × 100.

Table 3

Growth performance, proximate body composition and retention of rainbow trout juveniles fed tuna-based diets for 21 (D21) or 168 days (D168).

Tuna based-diets	TC	TH	TI	THI	TO	THO	p_{Hg}	p_{Se}	$p_{Hg\times Se}$
Growth performance									
FI[1] D21	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	*0.677*	*0.097*	*0.060*
FI[1] D168	2.6 ± 0.0	2.6 ± 0.2	2.3 ± 0.1	2.8 ± 0.0	2.7 ± 0.0	2.7 ± 0.1	***0.047***	*0.427*	*0.062*
FCR[2] D21	0.73 ± 0.01[a]	0.76 ± 0.01[a]	0.88 ± 0.09[a]	0.74 ± 0.02[a]	0.75 ± 0.01[a]	0.78 ± 0.02[a]	*0.878*	*0.427*	***0.028***
FCR[2] D168	0.84 ± 0.01[ab]	0.88 ± 0.01[a]	0.88 ± 0.01[a]	0.86 ± 0.01[ab]	0.85 ± 0.01[ab]	0.83 ± 0.00[b]	*0.960*	***0.012***	***0.010***
PER[3] D21	2.7 ± 0.0[a]	2.6 ± 0.0[a]	2.3 ± 0.2[a]	2.7 ± 0.1[a]	2.6 ± 0.0[a]	2.5 ± 0.1[a]	*0.460*	*0.255*	***0.046***
PER[3] D168	2.3 ± 0.0[ab]	2.2 ± 0.0[b]	2.2 ± 0.0[b]	2.3 ± 0.0[ab]	2.3 ± 0.0[ab]	2.4 ± 0.0[a]	*0.922*	***0.006***	***0.017***
HSI[4] D21	1.6 ± 0.1	1.6 ± 0.0	1.5 ± 0.0	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.0	*0.951*	*0.069*	*0.778*
HSI[4] D168	1.3 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	1.1 ± 0.1	1.2 ± 0.0	1.1 ± 0.0	***<0.001***	*0.406*	*0.287*
SR[5] D21	99 ± 1	99 ± 1	100 ± 0	100 ± 0	100 ± 0	99 ± 1	*0.990*	*0.138*	*0.398*
SR[5] D168	96 ± 2	92 ± 7	90 ± 1	90 ± 6	94 ± 0	84 ± 9	*0.073*	*0.298*	*0.266*
Body composition[5]									
Dry matter D21	30 ± 1	31 ± 0	31 ± 1	30 ± 0	30 ± 0	29 ± 0	*0.196*	*0.217*	*0.470*
Dry matter D168	33 ± 1	33 ± 0	34 ± 1	33 ± 0	34 ± 1	33 ± 1	*0.405*	*0.935*	*0.950*
Protein D21	16 ± 0	16 ± 0	16 ± 0	16 ± 0	16 ± 0	16 ± 0	*0.955*	*0.704*	*0.727*
Protein D168	17 ± 0	17 ± 0	17 ± 0	17 ± 0	17 ± 0	17 ± 0	*0.386*	*0.444*	*0.440*
Lipid D21	16 ± 0	16 ± 0	16 ± 0	16 ± 0	16 ± 0	16 ± 0	*0.955*	*0.704*	*0.727*
Lipid D168	17 ± 0	17 ± 0	17 ± 0	17 ± 0	17 ± 0	17 ± 0	*0.386*	*0.444*	*0.440*
Energy D21	8.4 ± 0.2	8.3 ± 0.2	8.7 ± 0.2	8.2 ± 0.1	8.5 ± 0.2	8.1 ± 0.1	***0.048***	*0.596*	*0.567*
Energy D168	9.7 ± 0.4	9.5 ± 0.1	9.9 ± 0.3	9.7 ± 0.1	9.7 ± 0.3	9.5 ± 0.3	*0.440*	*0.826*	*0.978*
Ash D21	2.2 ± 0.1	2.2 ± 0.2	2.1 ± 0.0	2.0 ± 0.1	2.2 ± 0.0	2.1 ± 0.0	*0.351*	*0.496*	*0.788*
Ash D168	2.2 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	2.0 ± 0.0	*0.824*	*0.078*	*0.806*
Body retention[6]									
Dry matter D21	40 ± 2	39 ± 1	34 ± 5	38 ± 2	38 ± 2	33 ± 1	*0.692*	*0.243*	*0.261*
Dry matter D168	41 ± 1	38 ± 0	39 ± 1	39 ± 0	40 ± 1	41 ± 1	*0.416*	*0.261*	*0.241*
Protein D21	47 ± 2	44 ± 1	42 ± 1	47 ± 1	44 ± 1	45 ± 4	*0.558*	*0.881*	*0.152*
Protein D168	40 ± 1	38 ± 1	37 ± 1	38 ± 0	40 ± 0	40 ± 0	*0.437*	***0.012***	*0.130*
Lipid D21	69 ± 5	65 ± 5	55 ± 16	58 ± 7	62 ± 10	40 ± 7	*0.335*	*0.250*	*0.406*
Lipid D168	98 ± 7	87 ± 0	95 ± 7	97 ± 2	94 ± 6	92 ± 5	*0.398*	*0.789*	*0.511*
Energy D21	45 ± 3	41 ± 4	42 ± 6	41 ± 3	44 ± 4	35 ± 2	*0.158*	*0.678*	*0.473*
Energy D168	49 ± 2	46 ± 0	48 ± 1	48 ± 0	49 ± 2	49 ± 2	*0.431*	*0.633*	*0.449*
Ash D21	48 ± 4	59 ± 14	40 ± 3	38 ± 7	47 ± 2	39 ± 3	*0.962*	*0.148*	*0.405*
Ash D168	33 ± 2	40 ± 2	29 ± 1	29 ± 2	28 ± 3	29 ± 1	*0.078*	***<0.001***	*0.134*

TC, not supplemented; TH, supplemented with MeHg; TI, supplemented with Se(IV); THI, supplemented with both MeHg and Se(IV); TO, supplemented with SeMet; THO, supplemented with both MeHg and SeMet.

Values represent means ± SEM (n = 3). Means not sharing a common superscript letter are significantly different ($P_{Hg \times Se} < 0.05$) according to two-way ANOVA to test the effect of MeHg supplementation (P_{Hg}), Se supplementation (P_{Se}) and the interaction ($P_{Hg \times Se}$) followed by a Tukey's post hoc test. [1]FI, absolute feed intake expressed as g per fish per day = total dry feed intake per tank / (number of fish in tank × number of days over the experimental period). [2]FCR, feed conversion ratio = dry feed intake / wet weight gain. [3]PER, protein efficiency ratio = fish weight gain / crude protein intake. [4]HSI, hepatosomatic index expressed as % = liver weight / fish weight. [5]SR, survival rate expressed as %. [6]Body composition expressed as % for dry matter, crude protein, total lipid and ash and kJ/g for gross energy. [7]Body nutrient retention expressed as % = [(final weight × final body nutrient content) - (initial weight × initial body nutrient content)] / (total feed intake × feed nutrient content) × 100.

Table 4

Hg and Se whole body retention, faecal content and apparent digestibility coefficient in fish fed plant-based diets for 21 (D21) and 168 (D168) days.

Plant based-diets	**PC**	**PH**	**PI**	**PHI**	**PO**	**PHO**	p_{Hg}	p_{Se}	$p_{Hg\times Se}$
Hg retention[1] D21	-	74 ± 6	-	74 ± 2	-	77 ± 2	-	-	-
Hg retention[1] D168	-	56 ± 2	-	66 ± 1	-	69 ± 0	-	-	-
Faecal Hg content[2] D168	1 ± 0	96 ± 4	3 ± 1	115 ± 7	3 ± 0	98 ± 5	***<0.001***	***0.041***	*0.053*
ADC_{Hg}[3] D168	-	87 ± 0	-	86 ± 0	-	87 ± 0	-	-	-
Se retention[4] D21	16 ± 7	24 ± 7	34 ± 0	38 ± 7	66 ± 9	67 ± 4	*0.352*	***<0.001***	*0.812*
Se retention[4] D168	49 ± 2[b]	51 ± 3[b]	21 ± 1[d]	32 ± 1[c]	47 ± 1[b]	57 ± 1[a]	***<0.001***	***<0.001***	***0.041***
Faecal Se content[5] D168	42 ± 2[e]	39 ± 0[e]	371 ± 4[a]	172 ± 14[b]	140 ± 10[c]	104 ± 5[d]	***<0.001***	***<0.001***	***<0.001***
ADC_{Se}[6] D168	58 ± 2	67 ± 0	70 ± 1	82 ± 1	81 ± 1	87 ± 0	***<0.001***	***<0.001***	*0.247*

PC, not supplemented; PH, supplemented with MeHg; PI, supplemented with Se(IV); PHI, supplemented with both MeHg and Se(IV); PO, supplemented with SeMet; PHO, supplemented with both MeHg and SeMet.

Values represent means ± SEM (n = 3). Means not sharing a common superscript letter are significantly different ($P_{Hg*Se} < 0.05$) according to two-way ANOVA performed in each dietary basis at each time to test the effect of methylmercury supplementation (P_{Hg}), selenium supplementation (P_{Se}) and the interaction ($P_{Hg\times Se}$) followed by a Tukey's post hoc test. [1]Hg retention = 100 × [(final body weight × final body Hg content) - (initial body weight × initial body Hg content)) / (total feed intake × dietary Hg content). [2]Hg faeces content (pg/kg wet weight). [3]ADC_{Hg}, apparent digestibility coefficient for Hg = 100 – [100 × (faecal Hg content / dietary Hg content) × (dietary vanadium content / faecal vanadium content)]. [4]Se retention = 100 × [(final body weight × final body Se content) - (initial body weight × initial body Se content)] / (total feed intake × dietary Se content). [5]Se faecal content (µg/kg wet weight). [6]ADC_{Se}, apparent digestibility coefficient for Se = 100 – [100 × (faecal Se content / dietary Se content) × (dietary vanadium content / faecal vanadium content)].

Table 5

Hg and Se whole body retention, faecal content and apparent digestibility coefficient of in fish fed tuna-based diets for 21 (D21) and 168 (D168) days.

Tuna based-diets	**TC**	**TH**	**TI**	**THI**	**TO**	**THO**	p_{Hg}	p_{Se}	$p_{Hg\times Se}$
Hg retention[1] D21	55 ± 3[b]	57 ± 1[b]	75 ± 2[a]	40 ± 2[c]	67 ± 8[ab]	60 ± 3[b]	***<0.001***	*0.156*	***<0.001***
Hg retention[1] D168	57 ± 3	55 ± 3	94 ± 21	54 ± 1	78 ± 2	52 ± 5	***0.010***	*0.180*	*0.141*
Faecal Hg content[2] D168	62 ± 9	366 ± 34	81 ± 12	402 ± 35	63 ± 4	366 ± 49	***<0.001***	*0.536*	*0.943*
ADC_{Hg}[3] D168	49 ± 4	58 ± 1	38 ± 8	61 ± 2	48 ± 2	51 ± 7	***0.007***	*0.667*	*0.129*
Se retention[4] D21	17 ± 1	16 ± 1	18 ± 1	16 ± 1	25 ± 2	22 ± 2	***0.026***	***<0.001***	*0.647*
Se retention[4] D168	9 ± 0	10 ± 1	10 ± 1	12 ± 1	16 ± 1	18 ± 1	***0.036***	***<0.001***	*0.880*
Faecal Se content[5] D168	2264 ± 159	2247 ± 137	2571 ± 286	3026 ± 224	2464 ± 150	2388 ± 128	*0.453*	***0.038***	*0.339*
ADC_{Se}[6] D168	23 ± 1	33 ± 6	40 ± 4	38 ± 2	37 ± 2	38 ± 1	*0.352*	***0.009***	*0.191*

TC, not supplemented; TH, supplemented with MeHg; TI, supplemented with Se(IV); THI, supplemented with both MeHg and Se(IV); TO, supplemented with SeMet; THO, supplemented with both MeHg and SeMet.

Values represent means ± SEM (n = 3). Means not sharing a common superscript letter are significantly different ($P_{Hg*Se} < 0.05$) according to two-way ANOVA performed in each dietary basis at each time to test the effect of methylmercury supplementation (P_{Hg}), selenium supplementation (P_{Se}) and the interaction ($P_{Hg\times Se}$) followed by a Tukey's post hoc test. [1]Hg retention = 100 × [(final body weight × final body Hg content) - (initial body weight × initial body Hg content)) / (total feed intake × dietary Hg content). [2]Hg faeces content (pg/kg wet weight). [3]ADC_{Hg}, apparent digestibility coefficient for Hg = 100 – [100 × (faecal Hg content / dietary Hg content) × (dietary vanadium content / faecal vanadium content)]. [4]Se retention = 100 × [(final body weight × final body Se content) - (initial body weight × initial body Se content)] / (total feed intake × dietary Se content). [5]Se faecal content (µg/kg wet weight). [6]ADC_{Se}, apparent digestibility coefficient for Se = 100 – [100 × (faecal Se content / dietary Se content) × (dietary vanadium content / faecal vanadium content)].

Table 6

Plasma and blood health parameters in fish fed plant-based diets for 21 (D21) and 168 (D168) days.

Plant based-diets	**PC**	**PH**	**PI**	**PHI**	**PO**	**PHO**	p_{Hg}	p_{Se}	$p_{Hg\times Se}$
Lysozyme[1] D21	23 ± 6	15 ± 6	20 ± 5	19 ± 6	17 ± 2	9 ± 4	*0.120*	*0.349*	*0.742*
Lysozyme[1] D168	16 ± 7	9 ± 4	11 ± 3	5 ± 1	11 ± 2	7 ± 2	*0.111*	*0.467*	*0.885*
Total protein[2] D21	23 ± 1	24 ± 1	24 ± 0	25 ± 1	24 ± 1	23 ± 2	*0.399*	*0.702*	*0.666*
Total protein[2] D168	34 ± 1[b]	31 ± 1[b]	31 ± 2[b]	37 ± 1[a]	33 ± 1[b]	31 ± 1[b]	*0.686*	*0.478*	***0.002***
Immunoglobulin[3] D21	17 ± 2	13 ± 1	14 ± 1	16 ± 1	16 ± 1	13 ± 2	*0.149*	*0.931*	*0.134*
Immunoglobulin[3] D168	14 ± 0[ab]	17 ± 1[ab]	18 ± 1[a]	14 ± 1[b]	17 ± 2[ab]	17 ± 1[ab]	*0.764*	*0.495*	***0.022***
Albumin[4] D21	66 ± 3	73 ± 3	68 ± 3	65 ± 2	70 ± 6	64 ± 3	*0.833*	*0.703*	*0.147*
Albumin[4] D168	65 ± 2	73 ± 3	67 ± 2	65 ± 3	67 ± 2	70 ± 2	*0.094*	*0.362*	*0.104*
Glucose[5] D21	1.2 ± 0.0	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	*0.498*	*0.683*	*0.860*
Glucose[5] D168	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.0	0.9 ± 0.1	*0.205*	*0.402*	*0.949*
ASAT[6] D21	233 ± 24	231 ± 11	208 ± 9	194 ± 32	225 ± 15	216 ± 16	*0.563*	*0.271*	*0.942*
ASAT[6] D168	176 ± 21	155 ± 13	161 ± 4	209 ± 67	204 ± 11	151 ± 20	*0.641*	*0.701*	*0.132*
ALAT[7] D21	370 ± 41	279 ± 167	316 ± 57	470 ± 52	198 ± 11	339 ± 166	*0.467*	*0.559*	*0.466*
ALAT[7] D168	278 ± 83	295 ± 33	299 ± 93	136 ± 92	274 ± 68	215 ± 78	*0.299*	*0.678*	*0.533*
Haemoglobin[8] D21	96 ± 4	101 ± 2	100 ± 6	99 ± 11	102 ± 1	93 ± 4	*0.765*	*0.917*	*0.462*
Haemoglobin[8] D168	33 ± 16[b]	69 ± 4[ab]	78 ± 21[ab]	110 ± 9[a]	110 ± 10[a]	82 ± 7[ab]	*0.216*	***0.006***	***0.046***

PC, not supplemented; PH, supplemented with MeHg; PI, supplemented with Se(IV); PHI, supplemented with both MeHg and Se(IV); PO, supplemented with SeMet; PHO, supplemented with both MeHg and SeMet.

Values represent means ± SEM (n = 3). Means not sharing a common superscript letter are significantly different ($P_{Hg*Se} < 0.05$) according to two-way ANOVA performed in each dietary basis at each time to test the effect of methylmercury supplementation (P_{Hg}), selenium supplementation (P_{Se}) and the interaction ($P_{Hg\times Se}$) followed by a Tukey's post hoc test. [1]Lysozyme (mg/L), [2]total protein (g/L), [3]immunoglobulin (% total protein), [4]albumin (% total protein), [5]glucose (g/L), [6]ASAT, aspartate amino-transferase (UI/L), [7]ALAT, alanine aminotransferase (UI/L). [8]Haemoglobin (g/L).

Table 7

Plasma and blood health parameters in fish fed tuna-based diets for 21 (D21) and 168 (D168) days.

Tuna based-diets	**TC**	**TH**	**TI**	**THI**	**TO**	**THO**	p_{Hg}	p_{Se}	$p_{Hg\times Se}$
Lysozyme[1] D21	15 ± 3[ab]	13 ± 3[ab]	21 ± 1[a]	12 ± 3[ab]	10 ± 4[b]	19 ± 1[a]	*0.848*	*0.637*	***0.032***
Lysozyme[1] D168	16 ± 7	15 ± 2	13 ± 2	14 ± 13	23 ± 16	11 ± 2	*0.523*	*0.878*	*0.727*
Total protein[2] D21	26 ± 1	26 ± 1	24 ± 2	26 ± 2	28 ± 2	25 ± 0	*0.786*	*0.558*	*0.217*
Total protein[2] D168	37 ± 2	33 ± 2	35 ± 2	35 ± 3	32 ± 1	36 ± 2	*0.827*	*0.901*	*0.228*
Immunoglobulin[3] D21	15 ± 1	10 ± 2	14 ± 3	15 ± 0	13 ± 1	16 ± 1	*0.714*	*0.428*	*0.128*
Immunoglobulin[3] D168	14 ± 1	16 ± 1	14 ± 1	16 ± 2	16 ± 1	14 ± 1	*0.515*	*0.865*	*0.232*
Albumin[4] D21	64 ± 5	68 ± 3	61 ± 1	66 ± 1	59 ± 3	67 ± 3	***0.039***	*0.597*	*0.711*
Albumin[4] D168	69 ± 4	70 ± 4	77 ± 2	71 ± 3	76 ± 2	71 ± 3	*0.145*	*0.412*	*0.865*
Glucose[5] D21	1.2 ± 0.1	1.6 ± 0.1	1.1 ± 0.1	1.4 ± 0.0	1.4 ± 0.0	1.5 ± 0.1	***0.007***	*0.110*	*0.335*
Glucose[5] D168	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.0	0.7 ± 0.1	0.9 ± 0.1	*0.604*	*0.981*	*0.433*
ASAT[6] D21	184 ± 2	212 ± 24	195 ± 27	195 ± 2	207 ± 13	210 ± 18	*0.563*	*0.764*	*0.763*
ASAT[6] D168	263 ± 66	250 ± 13	372 ± 57	263 ± 63	303 ± 65	254 ± 62	*0.253*	*0.600*	*0.725*
ALAT[7] D21	445 ± 129	304 ± 72	312 ± 76	470 ± 39	342 ± 15	355 ± 81	*0.875*	*0.841*	*0.203*
ALAT[7] D168	372 ± 56	242 ± 101	312 ± 89	278 ± 88	341 ± 19	312 ± 56	*0.308*	*0.911*	*0.739*
Haemoglobin[8] D21	105 ± 4	110 ± 6	103 ± 7	104 ± 14	90 ± 2	101 ± 5	*0.385*	*0.286*	*0.811*
Haemoglobin[8] D168	14 ± 6	41 ± 19	45 ± 14	21 ± 11	33 ± 8	32 ± 9	*0.948*	*0.894*	*0.221*

TC, not supplemented; TH, supplemented with MeHg; TI, supplemented with Se(IV); THI, supplemented with both MeHg and Se(IV); TO, supplemented with SeMet; THO, supplemented with both MeHg and SeMet.

Values represent means ± SEM (n = 3). Means not sharing a common superscript letter are significantly different ($P_{Hg*Se} < 0.05$) according to two-way ANOVA performed in each dietary basis at each time to test the effect of methylmercury supplementation (P_{Hg}), selenium supplementation (P_{Se}) and the interaction ($P_{Hg\times Se}$) followed by a Tukey's post hoc test. [1]Lysozyme (mg/L), [2]total protein (g/L), [3]immunoglobulin (% total protein), [4]albumin (% total protein), [5]glucose (g/L), [6]ASAT, aspartate amino-transferase (UI/L), [7]ALAT, alanine aminotransferase (UI/L). [8]Haemoglobin (g/L).

Figure captions

Fig. 1. Mean body weight of rainbow trout juveniles fed plant-based diets (P, □) or tuna-based diets (T, □) not supplemented (PC and TC), supplemented with MeHg (PH and TH), Se(IV) (PI and TI), both MeHg and Se(IV) (PHI and THI), SeMet (PO and TO) or both MeHg and SeMet (PHO and THO) for 21 (A) or 168 days (B). Bars represent means ± SEM (n = 3). Means not sharing common superscript letter are significantly different ($P_{Hg\times Se} < 0.05$) according to two-way ANOVA performed in each dietary basis at each time to test the effect of methylmercury supplementation (P_{Hg}), selenium supplementation (P_{Se}) and the interaction ($P_{Hg\times Se}$) followed by a Tukey's post hoc test. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Fig. 2. Hg (A and B) and Se (C and D) body contents in rainbow trout juveniles fed plant-based diets (P, □) or tuna-based diets (T, □) not supplemented (PC and TC), supplemented with MeHg (PH and TH), Se(IV) (PI and TI), both MeHg and Se(IV) (PHI and THI), SeMet (PO and TO) or both MeHg and SeMet (PHO and THO) for 21 (A and C) or 168 days (B and D). Bars represent means ± SEM (n = 3). Means not sharing common superscript letter are significantly different ($P_{Hg\times Se} < 0.05$) according to two-way ANOVA performed in each dietary basis at each time to test the effect of methylmercury supplementation (P_{Hg}), selenium supplementation (P_{Se}) and the interaction ($P_{Hg\times Se}$) followed by a Tukey's post hoc test. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Fig. 3. Plasma cytokine concentrations, TNF-α (A and B), IL-1β (C and D) and IL-6 (E and F), in rainbow trout juveniles fed plant-based diets (P, □) or tuna-based diets (T, □) not supplemented (PC and TC), supplemented with MeHg (PH and TH), Se(IV) (PI and TI), both MeHg and Se(IV) (PHI and THI), SeMet (PO and TO) or both MeHg and SeMet (PHO and

THO) for 21 (A, C and E) or 168 days (B, D and F). Bars represent means ± SEM (n = 3 pooled samples). Means not sharing common superscript letter are significantly different ($P_{Hg \times Se} < 0.05$) according to two-way ANOVA performed in each dietary basis at each time to test the effect of methylmercury supplementation (P_{Hg}), selenium supplementation (P_{Se}) and the interaction ($P_{Hg \times Se}$) followed by a Tukey's post hoc test. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

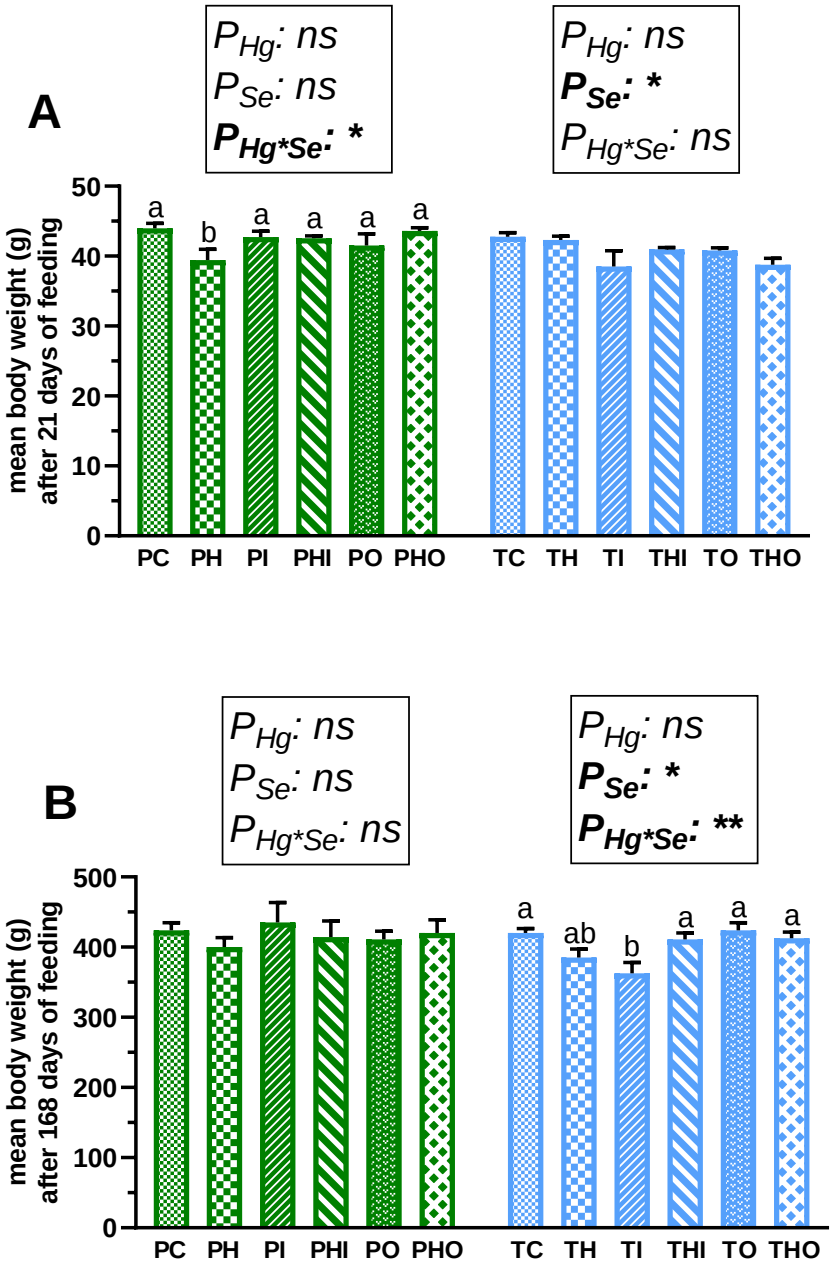
A
P_{Hg}: ns
P_{Se}: ns
P_{Hg*Se}: *
P_{Hg}: ns
P_{Se}: *
P_{Hg*Se}: ns
mean body weight (g) after 21 days of feeding
0
10
20
30
40
50
a
b
a
a
a
a
PC PH PI PHI PO PHO TC TH TI THI TO THO
B
P_{Hg}: ns
P_{Se}: ns
P_{Hg*Se}: ns
P_{Hg}: ns
P_{Se}: *
P_{Hg*Se}: **
mean body weight (g) after 168 days of feeding
0
100
200
300
400
500
a
ab
b
a
a
a
PC PH PI PHI PO PHO TC TH TI THI TO THO

Fig. 1.

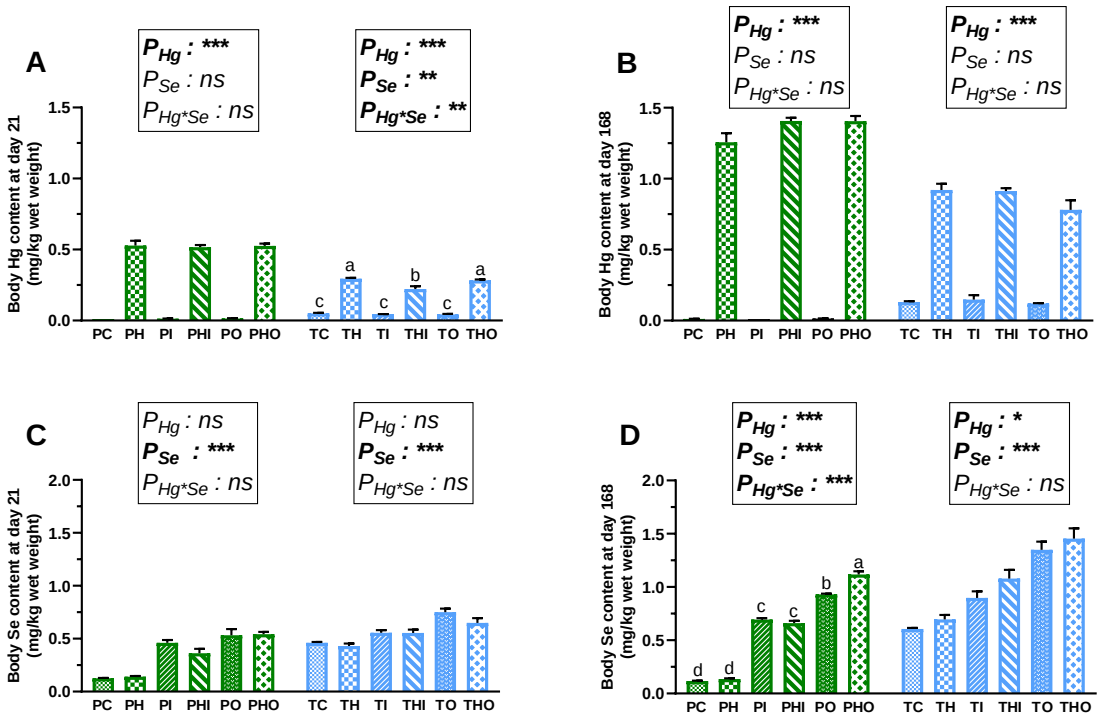
A
P_{Hg} : ***
P_{Se} : ns
P_{Hg*Se} : ns
P_{Hg} : ***
P_{Se} : **
P_{Hg*Se} : **
Body Hg content at day 21 (mg/kg wet weight)
B
P_{Hg} : ***
P_{Se} : ns
P_{Hg*Se} : ns
P_{Hg} : ***
P_{Se} : ns
P_{Hg*Se} : ns
Body Hg content at day 168 (mg/kg wet weight)
C
P_{Hg} : ns
P_{Se} : ***
P_{Hg*Se} : ns
P_{Hg} : ns
P_{Se} : ***
P_{Hg*Se} : ns
Body Se content at day 21 (mg/kg wet weight)
D
P_{Hg} : ***
P_{Se} : ***
P_{Hg*Se} : ***
P_{Hg} : *
P_{Se} : ***
P_{Hg*Se} : ns
Body Se content at day 168 (mg/kg wet weight)
PC PH PI PHI PO PHO TC TH TI THI TO THO

Fig. 2.

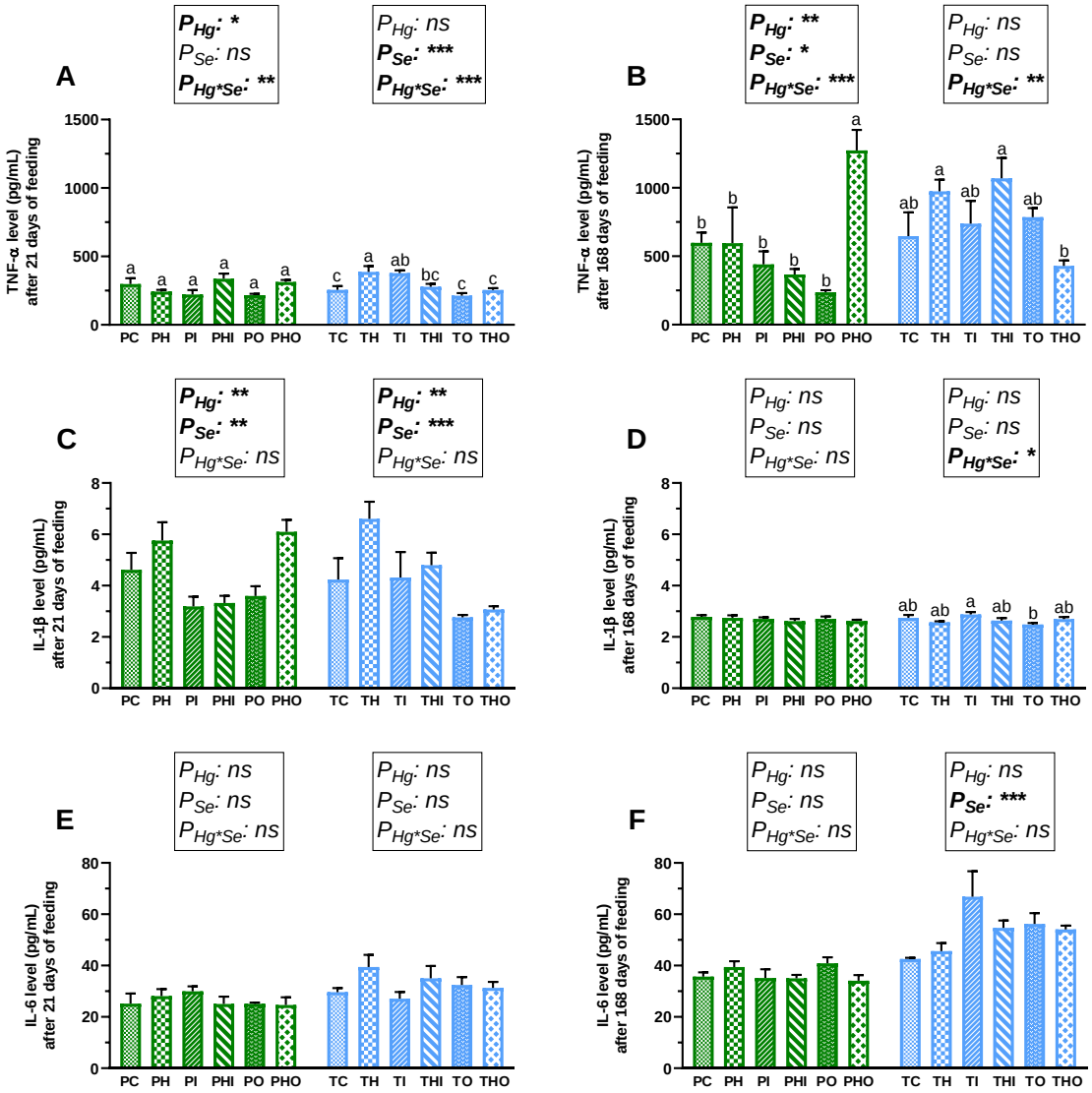
A
PHg: *
PSe: ns
PHg*Se: **
PHg: ns
PSe: ***
PHg*Se: ***
TNF-α level (pg/mL) after 21 days of feeding
B
PHg: **
PSe: *
PHg*Se: ***
PHg: ns
PSe: ns
PHg*Se: **
TNF-α level (pg/mL) after 168 days of feeding
C
PHg: **
PSe: **
PHg*Se: ns
PHg: **
PSe: ***
PHg*Se: ns
IL-1β level (pg/mL) after 21 days of feeding
D
PHg: ns
PSe: ns
PHg*Se: ns
PHg: ns
PSe: ns
PHg*Se: *
IL-1β level (pg/mL) after 168 days of feeding
E
PHg: ns
PSe: ns
PHg*Se: ns
PHg: ns
PSe: ns
PHg*Se: ns
IL-6 level (pg/mL) after 21 days of feeding
F
PHg: ns
PSe: ns
PHg*Se: ns
PHg: ns
PSe: ***
PHg*Se: ns
IL-6 level (pg/mL) after 168 days of feeding
PC PH PI PHI PO PHO TC TH TI THI TO THO

Fig. 3.