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

4

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19 **Highlights**

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

25 **Abstract**

26 This study evaluated the effect of dietary mercury (Hg) and selenium (Se) sources and levels  
27 on growth performance, Hg and Se accumulation, pro-inflammatory cytokine levels and global  
28 health parameters in rainbow trout juveniles. A 6-month feeding trial was carried out with a  
29 control plant-based diet (containing analyzed Se and Hg levels: 0.3 and 0 mg/kg diet,  
30 respectively) or a control tuna-based diet (containing analyzed Se and Hg levels: 7.5 and 0.3  
31 mg/kg diet, respectively) supplemented with 0 or 1.5 mg Se/kg diet supplied either as sodium  
32 selenite (Se(IV)) or selenomethionine (SeMet) combined with 0, 1.6 or 2 mg Hg/kg diet  
33 supplied as methylmercury (MeHg). Fish sampling was carried out at 2 times: after a short (21  
34 days) and a long term dietary exposure (168 days). In the short term, a temporarily reduced  
35 growth was noticed in fish fed MeHg-supplemented plant-based diet in absence of dietary Se  
36 supplementation. MeHg supplementation increased pro-inflammatory cytokine TNF- $\alpha$  and IL-  
37 1 $\beta$  levels in plant and tuna-based diets. MeHg supplementation also affected short-term fish  
38 global health parameters with an increase of glucose and albumin levels in tuna-based diets.  
39 The addition of SeMet in MeHg-supplemented tuna-based diets protected against the increase  
40 of TNF- $\alpha$  level in the long term whereas the addition of Se(IV) in MeHg-supplemented tuna-  
41 based diets decreased whole-body Hg level and retention in the short term. In the long term, a  
42 reduced growth, a higher feed conversion ratio and a lower protein efficiency ratio were  
43 recorded in fish fed Se(IV)-supplemented tuna-based diets in absence of MeHg  
44 supplementation. The addition of Se(IV) or SeMet in tuna-based diets increased plasma IL-6  
45 level. The addition of MeHg in plant and tuna-based diets increased Se accumulation and  
46 retention. This work underlines that different forms of Se supplementation, in two dietary  
47 background context (low basal Se level in plant-based diets and high basal Se level in tuna-  
48 based diets), have specific effects on metabolism and biological consequences of dietary MeHg.  
49 Dietary inorganic Se (but not organic Se) affected MeHg metabolism by reducing Hg

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

52

53 **Keywords (between 4 and 6 words maximum):**

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

## 56 **1. Introduction**

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

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

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

85 In addition to Hg, tunas accumulate high amount of selenium (Se) (Yamashita et al.,  
86 2011). Thus, tuna by-products are expected to contain high Se concentration, probably above  
87 rainbow trout and salmonid Se requirement, defined between 0.15 and 0.65 mg/kg diet (NRC,  
88 2011; Antony Jesu Prabhu et al., 2020). On the contrary, plant-based aquafeeds are known to  
89 contain low Se level, below salmonid requirements (Antony Jesu Prabhu et al., 2020). This  
90 essential element plays also a great role in the protection against Hg toxicity as demonstrated  
91 in goldfish and zebrafish (Bjerregaard et al., 2011; Amlund et al., 2015). These studies  
92 underlined also the importance of dietary Se concentration and chemical forms on Hg  
93 accumulation and detoxification.

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

99

## 100 **2. Material and methods**

### 101 *2.1. Ethical statement*

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

106

## 107 2.2. Experimental diets

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

124

## 125 2.3. Experimental design

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



131 concentration above 90% saturation. Tanks were supplied with flow-through (0.1 L/s, renewal  
132 4.5 times/h at the beginning, then 0.25 L/s, renewal 7 times/h) spring water at  $17 \pm 1$  °C. Fish  
133 were hand-fed twice a day to visual satiation under natural light regimen. Each diet was  
134 distributed to three replicate groups of fish over a 6-month growth trial. Mortality was recorded  
135 daily. Fish from each tank were bulk weighed every three weeks.

136

#### 137 *2.4. Sampling and data collection*

138 Before every weighing and sampling (after 21 and 168 days), fish were overnight  
139 starved during 16 h. At the start, 21 fish in common, then 8 fish from each replicate tank ( $n =$   
140 24 fish per diet) at day 21 and day 168 were anaesthetized with benzocaine (30 mg/L) then  
141 killed with an overdose of benzocaine (60 mg/L). Fish were weighed and blood samples were  
142 collected from the caudal vein. Plasma was recovered from half of the centrifuged ( $5500 \times g$   
143 for 10 min) blood samples, immediately frozen and stored at  $-80$  °C prior to analysis ( $n = 12$   
144 per diet). Three fish collected at the start and two fish per tank at day 21 and day 168 ( $n = 6$  per  
145 diet) were frozen and stored at  $-20$ °C prior to proximate analysis. The liver of other collected  
146 fish ( $n = 18$  at the start and  $n = 18$  per diet at day 21 and day 168) were weighed for calculating  
147 hepatosomatic index (HSI). At the end of the feeding trial, remaining fish (from 3 to 10 per  
148 tank) were fed for another week, anaesthetized and killed 16 h after the last meal for faeces  
149 collection in distal gut for the determination of apparent digestibility coefficient (ADC) of Se  
150 and Hg. The samples of faeces were collected over ice, pooled per tank, frozen immediately  
151 and stored at  $-20$  °C prior to Hg and Se analysis.

152

#### 153 *2.5. Proximate and mineral analyses in diets, whole fish and faeces*

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

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

162

### 163 *2.6. Determination of apparent digestibility coefficient (ADC) of minerals*

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

166

### 167 *2.7. Determination of plasma and blood metabolites*

168 Plasma metabolites were determined in plasma of fish,  $n = 3$  pooled samples per dietary  
169 treatment representing 4 fish each. Fish pro-inflammatory cytokines: tumor necrosis factor  $\alpha$   
170 (TNF- $\alpha$ ), interleukine 1 $\beta$  (IL-1 $\beta$ ) and interleukine 6 (IL-6) were determined with ELISA kit  
171 tests (Cusabio Biotech Co., Wuhan, Hubei, China) according to manufacturer's instructions  
172 using zebrafish TNF- $\alpha$  (cat. No. CSB-E13254Fh), zebrafish IL-1 $\beta$  (cat. No. CSB-E13254Fh)  
173 and Atlantic salmon (*Salmo salar*) IL-6 (cat. No. CSB-E13258Fh). TNF- $\alpha$  and IL-6 ELISA kits  
174 used a competitive inhibition enzyme immunoassay technique whereas IL-1 $\beta$  ELISA kit used  
175 a quantitative sandwich enzyme immunoassay technique. Kits provided cytokines biotin-  
176 conjugated, avidin conjugated horseradish peroxidase and a substrate solution. Horseradish  
177 peroxidase transformed the substrate into a coloured product. In TNF- $\alpha$  and IL-6 ELISA kits,  
178 colour developed in opposite to the amount of pro-inflammatory cytokines in samples. In IL-  
179 1 $\beta$  ELISA kit, the colour developed in proportion to the amount of IL-1 $\beta$  in samples. The  
180 absorbance was measured at 450 nm with a microplate reader and converted into concentrations

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

205

## 206 2.8. Statistical analyses

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

214

## 215 3. Results

### 216 3.1. Dietary MeHg impact on growth performance, proximate body composition of rainbow 217 trout juveniles and interactive effects of dietary Se

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

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

238

### 239 *3.2. Dietary Se impact on growth performance, proximate body composition of rainbow trout* 240 *juveniles and interactive effect of dietary MeHg*

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

248 After 21 days of feeding, fish fed Se-supplemented tuna-based diets TI, THI, TO and  
249 THO displayed decreased body weight compared to fish fed diets TC and TH (Fig 1A). After  
250 168 days of feeding, reduced body weight was recorded in fish fed Se(IV)-supplemented diet  
251 TI compared to fish fed diets TC and TO (Fig. 1B). Fish fed SeMet-supplemented diets TO and  
252 THO displayed higher PER, protein retention and decreased FCR compared to Se(IV)-  
253 supplemented diets TI and THI (Table 3). Fish fed Se-supplemented diets TI, THI, TO and  
254 THO exhibited reduced ash retention compared to fish fed control diets TC and TH (Table 3).

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

257

258 *3.3. Dietary MeHg impact on Hg and Se body composition, retention and digestibility in*  
259 *rainbow trout juveniles and interactive effect of dietary Se*

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

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

277

278 *3.4. Dietary Se impact on Hg and Se body composition, retention and digestibility in rainbow*  
279 *trout juveniles and interactive effect of dietary MeHg*

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

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

299

### 300 *3.5. Dietary MeHg impact on plasma pro-inflammatory cytokines and global health parameters* 301 *in rainbow trout juveniles and interactive effect of dietary Se*

302 Short-term feeding with MeHg-supplemented plant-based diets PH, PHI and PHO  
303 induced higher plasma TNF- $\alpha$  and IL-1 $\beta$  levels compared to control diets PC, PI and PO (Fig.  
304 3A and Fig. 3C). Long-term feeding with MeHg-supplemented plant-based diets also resulted

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

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

319

### 320 *3.6. Dietary Se impact on plasma pro-inflammatory cytokines and global health parameters in* 321 *rainbow trout juveniles and interactive effect of dietary MeHg*

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



329 Short-term feeding with SeMet-supplemented diets TO and THO reduced plasma TNF-  
330  $\alpha$  and IL-1 $\beta$  levels compared to other tuna-based diets TC, TH, TI and THI (Fig. 3A and Fig.  
331 3C). In absence of dietary MeHg supplementation, Se(IV)-supplemented diet TI led to higher  
332 TNF- $\alpha$  levels compared to control diet TC (Fig. 3A) and higher lysozyme levels compared to  
333 SeMet-supplemented diet TO (Table 7). Long-term SeMet supplementation decreased TNF- $\alpha$   
334 level only when supplemented with MeHg in diet THO compared to diets TH and THI (Fig.  
335 3B) whereas both Se(IV) and SeMet supplementation in diets TI, THI, TO and THO increased  
336 IL-6 levels compared to non Se-supplemented diets TC and TH (Fig. 3F).

337

#### 338 **4. Discussion**

339 *4.1. Short-term effect of dietary MeHg on growth, pro-inflammatory cytokines, overall health*  
340 *status and Hg deposition in rainbow trout juveniles and interactive effect of dietary Se*

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

354 gross energy content, those fish displayed no growth alteration. So the transient growth  
355 reduction observed in fish fed MeHg in low Se plant-based diets might not only be related to  
356 the induction of inflammatory response but also to a toxic effect of Hg that would be  
357 emphasized in absence of Se such as the induction of oxidative stress (Baldissera et al., 2020).  
358 Indeed we noticed increased transcript levels of antioxidant enzymes such as glutathione-S-  
359 transferase  $\pi$  and methionine sulfoxide reductase B3 in liver of trout fed the MeHg-  
360 supplemented plant-based diet PH for 21 days compared to the control diet PC (Bidon et al.,  
361 2021). An increased transcript level of the selenoprotein P and glutathione peroxidase 1 in Se-  
362 supplemented diets PI, PHI, PO and PHO was also noticed, suggesting a better antioxidant  
363 protection with dietary Se supplementation that deserves further investigation for a better  
364 characterization and understanding.

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

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

379 MeHg-supplemented plant-based diets (11.6 vs. 2.0, respectively) and might explain why fish  
380 fed MeHg-supplemented tuna-based diets displayed lower whole-body Hg level than fish fed  
381 MeHg-supplemented plant-based diets. However, despite the fact that dietary Se/Hg molar ratio  
382 was negatively correlated to whole-body Hg level in fish, it must be addressed that this  
383 parameter did not take into consideration chemical form of Se, another parameter that can  
384 influence Hg deposition. Indeed, despite a similar or even higher dietary Se/Hg molar ratio in  
385 THO diet compared to THI diet (13.0 vs. 11.9, respectively), fish fed THO displayed higher  
386 whole-body Hg level than fish fed THI in the short term (0.28 vs. 0.22 mg Hg/kg wet weight,  
387 respectively). However, this effect was no more significant after 168 days of feeding (0.78 vs.  
388 0.91 mg Hg/kg wet weight). The reduced Hg digestibility (57% vs. 87% for MeHg-  
389 supplemented tuna and plant-based diets) associated with the reduced Hg retention after short  
390 (52% vs. 75% for MeHg-supplemented tuna and plant-based diets, respectively) and long term  
391 (54% vs. 64% for MeHg-supplemented tuna and plant-based diets, respectively) supports the  
392 reduced body Hg content for fish fed high Se tuna-based diets. It is noteworthy that, in fish fed  
393 MeHg-supplemented plant-based diets, Hg retention was higher after a short-term than a long-  
394 term exposure (75% after 21 days vs. 64% after 168 days of feeding). Similar trends were  
395 observed in muscle of zebrafish fed 5 and 10 mg MeHg/kg diet after 2 weeks by Amlund et al.  
396 (2015) and attributed to a variation in the uptake or in the elimination kinetic. It would have  
397 been interesting to also determine Hg digestibility after a short term exposure to know if there  
398 is variation in Hg uptake through time and describe better the Hg kinetic deposition in rainbow  
399 trout.

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

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

405

#### 406 *4.2. Effect of dietary MeHg on Se deposition in rainbow trout juveniles*

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

424

#### 425 *4.3. Effect of dietary Se on Hg deposition, pro-inflammatory cytokines, growth in rainbow trout* 426 *juveniles and interactive effect of dietary MeHg*

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

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

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

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

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

479

#### 480 *4.4. Tuna by-products an interesting source of selenium for aquafeeds?*

481           Tuna-based diets used in the present study displayed high Se levels, far above salmonid  
482 requirement (Antony Jesu Prabhu et al., 2020). Despite Se concentration in the TC diet equal  
483 to 7.5 mg Se/kg diet and thus below levels reported to reduce fish growth performance: 13 mg  
484 Se/kg diet supplied as Se(IV) for rainbow trout (Hilton et al., 1982) and 11 mg Se/kg diet for  
485 Atlantic salmon (Berntssen et al., 2018), it might be safer to reduce dietary Se level. Tuna, like  
486 other fish, accumulate Se mainly in liver and kidney (Ruelas-Inzunza et al., 2018; Belmonte et  
487 al., 2021). These organs are parts of tuna by-products, thus adjusting their level in by-products  
488 might be interesting to control Se level. Another possibility to reduce dietary Se level is to  
489 decrease the level of tuna by-products inclusion, in our study, we used an inclusion level of  
490 60%, lower level might be suitable.

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

500

## 501 **5. Conclusion**

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

510

## 511 **Abbreviations**

512 ALAT, alanine amino transferase; ASAT, aspartate amino transferase; Hg, mercury; IL-1 $\beta$ ,  
513 interleukine 1 $\beta$ ; IL-6, interleukine 6; MeHg, methylmercury; PC, control plant-based diet; PH,  
514 methylmercury-supplemented plant-based diet; PHI, methylmercury and selenite-  
515 supplemented plant-based diet; PHO, methylmercury and selenomethionine supplemented  
516 plant-based diet; PI, selenite-supplemented plant-based diet; PO, selenomethionine-  
517 supplemented plant-based diet; Se, selenium; Se(IV), selenite; SeMet, selenomethionine; TNF-  
518  $\alpha$ , tumor necrosis factor  $\alpha$ ; TC, control tuna-based diet; TH, methylmercury-supplemented tuna-  
519 based diet; THI, methylmercury and selenite-supplemented tuna-based diet; THO,  
520 methylmercury and selenomethionine-supplemented tuna-based diet; TI, selenite-  
521 supplemented tuna-based diet; TO, selenomethionine-supplemented tuna-based diet.

522

## 523 **Author's contributions**



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

530

### 531 **Declaration of Competing Interest**

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

534

### 535 **Data availability**

536 Data will be made available on request

537

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734 **Table 1a**

735 Experimental diet formulation and composition.

| Diet                                       | Plant-based diets | Tuna-based diets |
|--|-------------------|------------------|
| <i>Ingredients (%)</i>                     |                   |                  |
| Plant-derived proteins <sup>a</sup>        | 73.8              | 25.8             |
| Tuna by-products <sup>b</sup>              | -                 | 60               |
| Crystalline amino acid premix <sup>c</sup> | 3.2               | -                |
| Rapeseed lecithin <sup>d</sup>             | 2                 | -                |
| Vegetable oils <sup>e</sup>                | 8                 | 8                |
| Fish oil <sup>f</sup>                      | 8                 | 4                |
| Vitamin and mineral premixes <sup>g</sup>  | 5                 | 2.2              |
| Sodium selenite (mg Se/kg diet)            | -                 | -                |
| L-selenomethionine (mg Se/kg diet)         | -                 | -                |
| Methylmercury chloride (mg Hg/kg diet)     | -                 | -                |
| <i>Analytical composition</i>              |                   |                  |
| 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              |

736 <sup>a</sup> Plant-derived proteins (% diet in plant-based or tuna meal-based diets respectively): wheat  
737 gluten, 18 or 0 (Roquette, France); corn gluten, 17 or 0 (Inzo, France); soybean protein  
738 concentrate, 15 or 0 (Estril®75 Sopropêche, France); soybean meal, 5 or 0 (Sud-Ouest Aliment,  
739 France); white lupin meal, 5 or 0 (Farilup500 Terrena, France); rapeseed meal, 5 or 0 (Primor  
740 00 Sud-Ouest Aliment, France); dehulled pea mea, 3.8 or 10.8 (Primatex Sotexpro, France),  
741 whole wheat, 5 or 15 (Sud-Ouest Aliment, France).

742 <sup>b</sup> Tuna meal HighPro68 (Port-Louis, Mauritius).

743 <sup>c</sup> Crystalline amino acid premix (% diet): L-lysine, 1.4; DL-methionine, 0.3; glucosamine, 0.5;  
744 taurine, 0.3; betaine, 0.3; glycine, 0.2; alanine, 0.2.

745 <sup>d</sup> Adivec (France).

746 <sup>e</sup> Vegetable oils (% diet): rapeseed oil, 4; linseed oil, 2.4; palm oil 1.6.

747 <sup>f</sup> Sopropêche (France).

748 <sup>g</sup> Vitamin and mineral premixes Se-free (per kg diet): retinyl acetate, 5000 IU; cholecalciferol,  
749 2500 IU; DL- $\alpha$ -tocopheryl acetate, 50 IU; sodium menadione bisulfate, 10 mg, thiamin-HCl, 1

750 mg; riboflavin, 4 mg; niacin, 10 mg; D-calcium pantothenate, 20 mg; pyridoxine-HCl, 3 mg;  
751 myo-inositol, 0.3 g; D-biotin, 0.2 mg; folic acid, 1 mg; cyanocobalamin, 0.01 mg; L-ascorbyl-  
752 2-polyphosphate, 50 mg; choline-HCl, 1 g; CaHPO<sub>4</sub>·2H<sub>2</sub>O (18% P; 22% Ca), 33 or 5 g in plant-  
753 based or tuna meal-based diets respectively; CaCO<sub>3</sub> (40% Ca), 2.15 g; MgOH<sub>2</sub> (42% Mg), 1.24  
754 g; KCl (52% K), 0.9 g; NaCl (39% Na), 0.4 g; FeSO<sub>4</sub>·H<sub>2</sub>O (33% Fe), 20 mg; ZnSO<sub>4</sub>·H<sub>2</sub>O (36%  
755 Zn), 35 mg; MnO (77%); 10 mg; CuSO<sub>4</sub>·5H<sub>2</sub>O (25% Cu), 5 mg; NaF (45% F), 10 mg; CaI<sub>2</sub>  
756 (86% I), 3 mg; CoCO<sub>3</sub> (50% Co), 0.05 mg; BHA, 0.75 mg; BHT, 0.75 mg; propyl gallate, 0.15  
757 mg; sepiolite, 200 mg. All ingredients were diluted with α-cellulose.  
758

759 **Table 1b**

760 Supplementation level for sodium selenite, L-selenomethionine and methylmercury chloride

761 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  |
| <i>Ingredients</i>                        |                   |     |     |     |     |     |                  |     |      |      |      |      |
| 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  |
| <i>Elemental composition (mg/kg diet)</i> |                   |     |     |     |     |     |                  |     |      |      |      |      |
| 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 |

762

763 **Table 2**

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

| <b>Plant based-diets</b>                   | <b>PC</b>                | <b>PH</b>                | <b>PI</b>                | <b>PHI</b>               | <b>PO</b>                | <b>PHO</b>               | <i>p</i> <sub>Hg</sub> | <i>p</i> <sub>Se</sub> | <i>p</i> <sub>Hg×Se</sub> |
|--|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|------------------------|------------------------|---------------------------|
| <b><i>Growth performance</i></b>           |                          |                          |                          |                          |                          |                          |                        |                        |                           |
| FI <sup>1</sup> 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 <sup>1</sup> 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 <sup>2</sup> D21                       | 0.79 ± 0.01 <sup>a</sup> | 0.88 ± 0.02 <sup>a</sup> | 0.85 ± 0.03 <sup>a</sup> | 0.79 ± 0.01 <sup>a</sup> | 0.81 ± 0.03 <sup>a</sup> | 0.82 ± 0.01 <sup>a</sup> | 0.400                  | 0.630                  | <b>0.023</b>              |
| FCR <sup>2</sup> 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 <sup>3</sup> D21                       | 2.6 ± 0.0 <sup>a</sup>   | 2.3 ± 0.1 <sup>a</sup>   | 2.4 ± 0.1 <sup>a</sup>   | 2.6 ± 0.0 <sup>a</sup>   | 2.5 ± 0.0 <sup>a</sup>   | 2.5 ± 0.0 <sup>a</sup>   | 0.309                  | 0.768                  | <b>0.018</b>              |
| PER <sup>3</sup> 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 <sup>4</sup> 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 <sup>4</sup> 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 <sup>5</sup> D21                        | 99 ± 2                   | 98 ± 2                   | 97 ± 3                   | 99 ± 1                   | 100 ± 0                  | 99 ± 1                   | 0.826                  | 0.990                  | 0.499                     |
| SR <sup>5</sup> D168                       | 95 ± 4                   | 98 ± 2                   | 97 ± 3                   | 97 ± 3                   | 96 ± 4                   | 98 ± 2                   | 0.278                  | 0.934                  | 0.743                     |
| <b><i>Body composition<sup>6</sup></i></b> |                          |                          |                          |                          |                          |                          |                        |                        |                           |
| 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 <sup>b</sup>      | 35 ± 0 <sup>ab</sup>     | 37 ± 0 <sup>a</sup>      | 35 ± 0 <sup>ab</sup>     | 36 ± 0 <sup>ab</sup>     | 37 ± 0 <sup>a</sup>      | 0.693                  | <b>0.021</b>           | <b>0.036</b>              |
| 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 <sup>b</sup>      | 16 ± 0 <sup>ab</sup>     | 18 ± 0 <sup>a</sup>      | 17 ± 1 <sup>ab</sup>     | 17 ± 1 <sup>a</sup>      | 18 ± 0 <sup>a</sup>      | 0.577                  | <b>0.004</b>           | <b>0.029</b>              |
| 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 <sup>b</sup>   | 10.5 ± 0.0 <sup>ab</sup> | 11.2 ± 0.1 <sup>a</sup>  | 10.5 ± 0.2 <sup>ab</sup> | 10.7 ± 0.2 <sup>a</sup>  | 10.9 ± 0.0 <sup>a</sup>  | 0.780                  | <b>0.010</b>           | <b>0.017</b>              |
| 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                     |
| <b><i>Body retention<sup>7</sup></i></b>   |                          |                          |                          |                          |                          |                          |                        |                        |                           |
| 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                  | <b>0.036</b>           | 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 <sup>a</sup>      | 28 ± 0 <sup>c</sup>      | 34 ± 1 <sup>b</sup>      | 34 ± 0 <sup>b</sup>      | 34 ± 1 <sup>b</sup>      | 35 ± 1 <sup>b</sup>      | <b>&lt;0.001</b>       | 0.183                  | <b>&lt;0.001</b>          |

766 PC, not supplemented; PH, supplemented with MeHg; PI, supplemented with Se(IV); PHI,

767 supplemented with both MeHg and Se(IV); PO, supplemented with SeMet; PHO, supplemented

768 with both MeHg and SeMet.

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

780 **Table 3**

781 Growth performance, proximate body composition and retention of rainbow trout juveniles fed

782 tuna-based diets for 21 (D21) or 168 days (D168).

| <b>Tuna based-diets</b>                    | <b>TC</b>                 | <b>TH</b>                | <b>TI</b>                | <b>THI</b>                | <b>TO</b>                 | <b>THO</b>               | <i>p</i> <sub>Hg</sub> | <i>p</i> <sub>Se</sub> | <i>p</i> <sub>Hg×Se</sub> |
|--|---------------------------|--------------------------|--------------------------|---------------------------|---------------------------|--------------------------|------------------------|------------------------|---------------------------|
| <b><i>Growth performance</i></b>           |                           |                          |                          |                           |                           |                          |                        |                        |                           |
| FI <sup>1</sup> 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 <sup>1</sup> 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                | <b>0.047</b>           | 0.427                  | 0.062                     |
| FCR <sup>2</sup> D21                       | 0.73 ± 0.01 <sup>a</sup>  | 0.76 ± 0.01 <sup>a</sup> | 0.88 ± 0.09 <sup>a</sup> | 0.74 ± 0.02 <sup>a</sup>  | 0.75 ± 0.01 <sup>a</sup>  | 0.78 ± 0.02 <sup>a</sup> | 0.878                  | 0.427                  | <b>0.028</b>              |
| FCR <sup>2</sup> D168                      | 0.84 ± 0.01 <sup>ab</sup> | 0.88 ± 0.01 <sup>a</sup> | 0.88 ± 0.01 <sup>a</sup> | 0.86 ± 0.01 <sup>ab</sup> | 0.85 ± 0.01 <sup>ab</sup> | 0.83 ± 0.00 <sup>b</sup> | 0.960                  | <b>0.012</b>           | <b>0.010</b>              |
| PER <sup>3</sup> D21                       | 2.7 ± 0.0 <sup>a</sup>    | 2.6 ± 0.0 <sup>a</sup>   | 2.3 ± 0.2 <sup>a</sup>   | 2.7 ± 0.1 <sup>a</sup>    | 2.6 ± 0.0 <sup>a</sup>    | 2.5 ± 0.1 <sup>a</sup>   | 0.460                  | 0.255                  | <b>0.046</b>              |
| PER <sup>3</sup> D168                      | 2.3 ± 0.0 <sup>ab</sup>   | 2.2 ± 0.0 <sup>b</sup>   | 2.2 ± 0.0 <sup>b</sup>   | 2.3 ± 0.0 <sup>ab</sup>   | 2.3 ± 0.0 <sup>ab</sup>   | 2.4 ± 0.0 <sup>a</sup>   | 0.922                  | <b>0.006</b>           | <b>0.017</b>              |
| HSI <sup>4</sup> 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 <sup>4</sup> 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                | <b>&lt;0.001</b>       | 0.406                  | 0.287                     |
| SR <sup>5</sup> D21                        | 99 ± 1                    | 99 ± 1                   | 100 ± 0                  | 100 ± 0                   | 100 ± 0                   | 99 ± 1                   | 0.990                  | 0.138                  | 0.398                     |
| SR <sup>5</sup> D168                       | 96 ± 2                    | 92 ± 7                   | 90 ± 1                   | 90 ± 6                    | 94 ± 0                    | 84 ± 9                   | 0.073                  | 0.298                  | 0.266                     |
| <b><i>Body composition<sup>5</sup></i></b> |                           |                          |                          |                           |                           |                          |                        |                        |                           |
| 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                | <b>0.048</b>           | 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                     |
| <b><i>Body retention<sup>6</sup></i></b>   |                           |                          |                          |                           |                           |                          |                        |                        |                           |
| 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                  | <b>0.012</b>           | 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                  | <b>&lt;0.001</b>       | 0.134                     |

783 TC, not supplemented; TH, supplemented with MeHg; TI, supplemented with Se(IV); THI,

784 supplemented with both MeHg and Se(IV); TO, supplemented with SeMet; THO,

785 supplemented with both MeHg and SeMet.



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

797

798 **Table 4**

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

| <b>Plant based-diets</b>            | <b>PC</b>           | <b>PH</b>           | <b>PI</b>            | <b>PHI</b>            | <b>PO</b>             | <b>PHO</b>           | <b><i>P<sub>Hg</sub></i></b> | <b><i>P<sub>Se</sub></i></b> | <b><i>P<sub>Hg×Se</sub></i></b> |
|-------------------------------------|---------------------|---------------------|----------------------|-----------------------|-----------------------|----------------------|------------------------------|------------------------------|---------------------------------|
| Hg retention <sup>1</sup> D21       | -                   | 74 ± 6              | -                    | 74 ± 2                | -                     | 77 ± 2               | -                            | -                            | -                               |
| Hg retention <sup>1</sup> D168      | -                   | 56 ± 2              | -                    | 66 ± 1                | -                     | 69 ± 0               | -                            | -                            | -                               |
| Faecal Hg content <sup>2</sup> D168 | 1 ± 0               | 96 ± 4              | 3 ± 1                | 115 ± 7               | 3 ± 0                 | 98 ± 5               | <b>&lt;0.001</b>             | <b>0.041</b>                 | <b>0.053</b>                    |
| ADC <sub>Hg</sub> <sup>3</sup> D168 | -                   | 87 ± 0              | -                    | 86 ± 0                | -                     | 87 ± 0               | -                            | -                            | -                               |
| Se retention <sup>4</sup> D21       | 16 ± 7              | 24 ± 7              | 34 ± 0               | 38 ± 7                | 66 ± 9                | 67 ± 4               | 0.352                        | <b>&lt;0.001</b>             | <b>0.812</b>                    |
| Se retention <sup>4</sup> D168      | 49 ± 2 <sup>b</sup> | 51 ± 3 <sup>b</sup> | 21 ± 1 <sup>d</sup>  | 32 ± 1 <sup>c</sup>   | 47 ± 1 <sup>b</sup>   | 57 ± 1 <sup>a</sup>  | <b>&lt;0.001</b>             | <b>&lt;0.001</b>             | <b>0.041</b>                    |
| Faecal Se content <sup>5</sup> D168 | 42 ± 2 <sup>e</sup> | 39 ± 0 <sup>e</sup> | 371 ± 4 <sup>a</sup> | 172 ± 14 <sup>b</sup> | 140 ± 10 <sup>c</sup> | 104 ± 5 <sup>d</sup> | <b>&lt;0.001</b>             | <b>&lt;0.001</b>             | <b>&lt;0.001</b>                |
| ADC <sub>Se</sub> <sup>6</sup> D168 | 58 ± 2              | 67 ± 0              | 70 ± 1               | 82 ± 1                | 81 ± 1                | 87 ± 0               | <b>&lt;0.001</b>             | <b>&lt;0.001</b>             | <b>0.247</b>                    |

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

804 Values represent means ± SEM (n = 3). Means not sharing a common superscript letter are  
 805 significantly different ( $P_{Hg*Se} < 0.05$ ) according to two-way ANOVA performed in each dietary  
 806 basis at each time to test the effect of methylmercury supplementation ( $P_{Hg}$ ), selenium  
 807 supplementation ( $P_{Se}$ ) and the interaction ( $P_{Hg×Se}$ ) followed by a Tukey's post hoc test. <sup>1</sup>Hg  
 808 retention =  $100 \times [(final\ body\ weight \times final\ body\ Hg\ content) - (initial\ body\ weight \times initial$   
 809  $body\ Hg\ content)] / (total\ feed\ intake \times dietary\ Hg\ content)$ . <sup>2</sup>Hg faeces content (pg/kg wet  
 810 weight). <sup>3</sup>ADC<sub>Hg</sub>, apparent digestibility coefficient for Hg =  $100 - [100 \times (faecal\ Hg\ content /$   
 811  $dietary\ Hg\ content) \times (dietary\ vanadium\ content / faecal\ vanadium\ content)]$ . <sup>4</sup>Se retention =  
 812  $100 \times [(final\ body\ weight \times final\ body\ Se\ content) - (initial\ body\ weight \times initial\ body\ Se$   
 813  $content)] / (total\ feed\ intake \times dietary\ Se\ content)$ . <sup>5</sup>Se faecal content (µg/kg wet weight).  
 814 <sup>6</sup>ADC<sub>Se</sub>, apparent digestibility coefficient for Se =  $100 - [100 \times (faecal\ Se\ content / dietary\ Se$   
 815  $content) \times (dietary\ vanadium\ content / faecal\ vanadium\ content)]$ .

816

817 **Table 5**

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

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

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

823 Values represent means ± SEM (n = 3). Means not sharing a common superscript letter are  
 824 significantly different ( $P_{Hg*Se} < 0.05$ ) according to two-way ANOVA performed in each dietary  
 825 basis at each time to test the effect of methylmercury supplementation ( $P_{Hg}$ ), selenium  
 826 supplementation ( $P_{Se}$ ) and the interaction ( $P_{Hg×Se}$ ) followed by a Tukey's post hoc test. <sup>1</sup>Hg  
 827 retention =  $100 \times [(final\ body\ weight \times final\ body\ Hg\ content) - (initial\ body\ weight \times initial$   
 828  $body\ Hg\ content)] / (total\ feed\ intake \times dietary\ Hg\ content)$ . <sup>2</sup>Hg faeces content (pg/kg wet  
 829 weight). <sup>3</sup>ADC<sub>Hg</sub>, apparent digestibility coefficient for Hg =  $100 - [100 \times (faecal\ Hg\ content /$   
 830  $dietary\ Hg\ content) \times (dietary\ vanadium\ content / faecal\ vanadium\ content)]$ . <sup>4</sup>Se retention =  
 831  $100 \times [(final\ body\ weight \times final\ body\ Se\ content) - (initial\ body\ weight \times initial\ body\ Se$   
 832  $content)] / (total\ feed\ intake \times dietary\ Se\ content)$ . <sup>5</sup>Se faecal content (µg/kg wet weight).  
 833 <sup>6</sup>ADC<sub>Se</sub>, apparent digestibility coefficient for Se =  $100 - [100 \times (faecal\ Se\ content / dietary\ Se$   
 834  $content) \times (dietary\ vanadium\ content / faecal\ vanadium\ content)]$ .

835

836 **Table 6**

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

838 days.

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

839 PC, not supplemented; PH, supplemented with MeHg; PI, supplemented with Se(IV); PHI,

840 supplemented with both MeHg and Se(IV); PO, supplemented with SeMet; PHO, supplemented

841 with both MeHg and SeMet.

842 Values represent means ± SEM (n = 3). Means not sharing a common superscript letter are

843 significantly different ( $P_{Hg \times Se} < 0.05$ ) according to two-way ANOVA performed in each dietary844 basis at each time to test the effect of methylmercury supplementation ( $P_{Hg}$ ), selenium845 supplementation ( $P_{Se}$ ) and the interaction ( $P_{Hg \times Se}$ ) followed by a Tukey's post hoc test.846 <sup>1</sup>Lysozyme (mg/L), <sup>2</sup>total protein (g/L), <sup>3</sup>immunoglobulin (% total protein), <sup>4</sup>albumin (% total847 protein), <sup>5</sup>glucose (g/L), <sup>6</sup>ASAT, aspartate amino-transferase (UI/L), <sup>7</sup>ALAT, alanine848 aminotransferase (UI/L). <sup>8</sup>Haemoglobin (g/L).

849

850 **Table 7**

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

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

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

856 Values represent means ± SEM (n = 3). Means not sharing a common superscript letter are  
857 significantly different ( $P_{Hg*Se} < 0.05$ ) according to two-way ANOVA performed in each dietary  
858 basis at each time to test the effect of methylmercury supplementation ( $P_{Hg}$ ), selenium  
859 supplementation ( $P_{Se}$ ) and the interaction ( $P_{Hg×Se}$ ) followed by a Tukey's post hoc test.

860 <sup>1</sup>Lysozyme (mg/L), <sup>2</sup>total protein (g/L), <sup>3</sup>immunoglobulin (% total protein), <sup>4</sup>albumin (% total  
861 protein), <sup>5</sup>glucose (g/L), <sup>6</sup>ASAT, aspartate amino-transferase (UI/L), <sup>7</sup>ALAT, alanine  
862 aminotransferase (UI/L). <sup>8</sup>Haemoglobin (g/L).

863

864 **Figure captions**

865

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

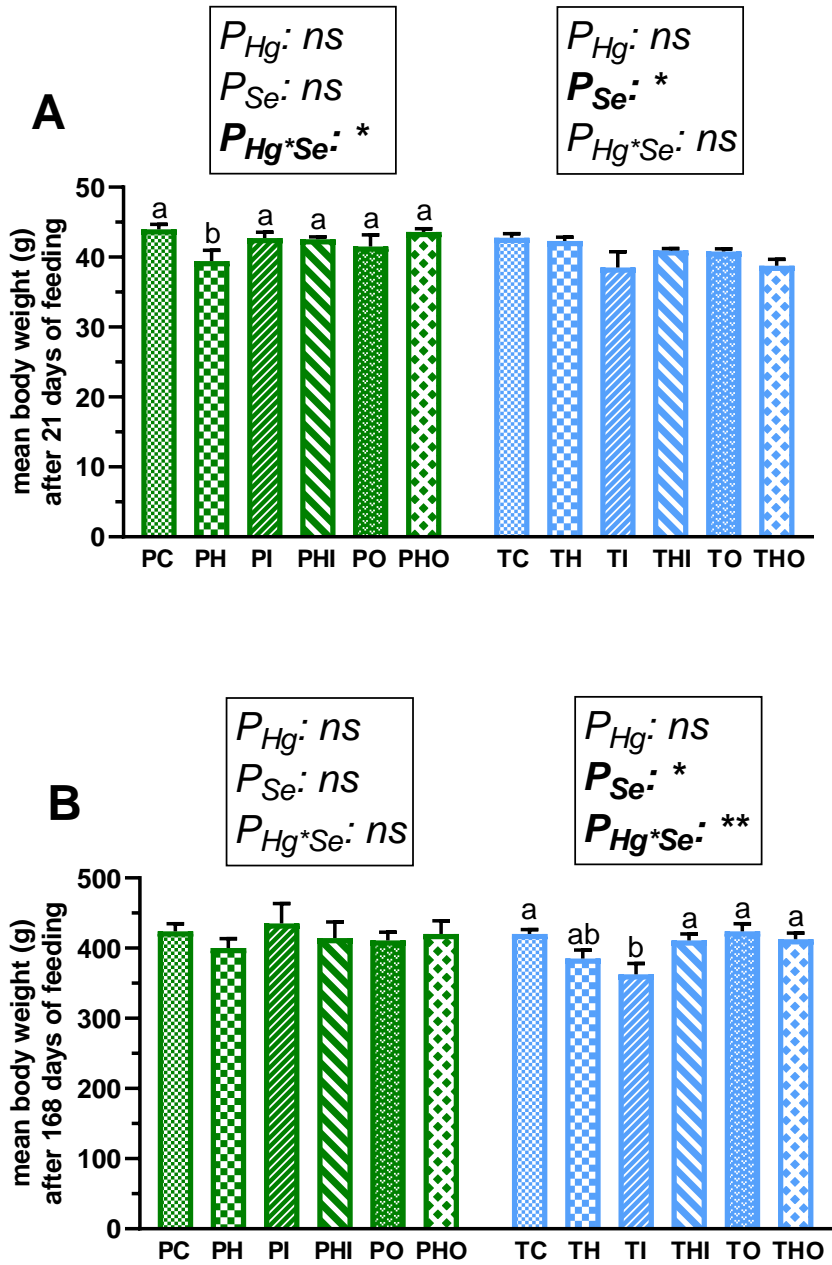
874

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

884

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

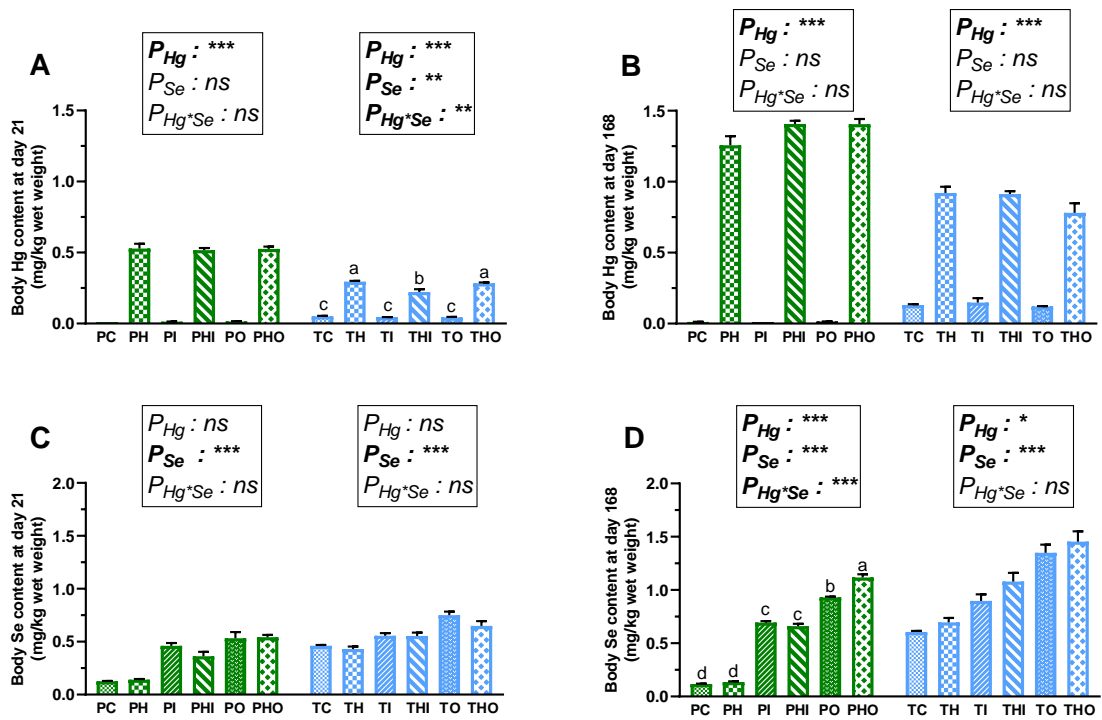
889 THO) for 21 (A, C and E) or 168 days (B, D and F). Bars represent means  $\pm$  SEM (n = 3 pooled  
890 samples). Means not sharing common superscript letter are significantly different ( $P_{\text{Hg} \times \text{Se}} <$   
891 0.05) according to two-way ANOVA performed in each dietary basis at each time to test the  
892 effect of methylmercury supplementation ( $P_{\text{Hg}}$ ), selenium supplementation ( $P_{\text{Se}}$ ) and the  
893 interaction ( $P_{\text{Hg} \times \text{Se}}$ ) followed by a Tukey's post hoc test. ns, not significant; \*,  $P < 0.05$ ; \*\*,  $P$   
894  $< 0.01$ ; \*\*\*,  $P < 0.001$ .



895

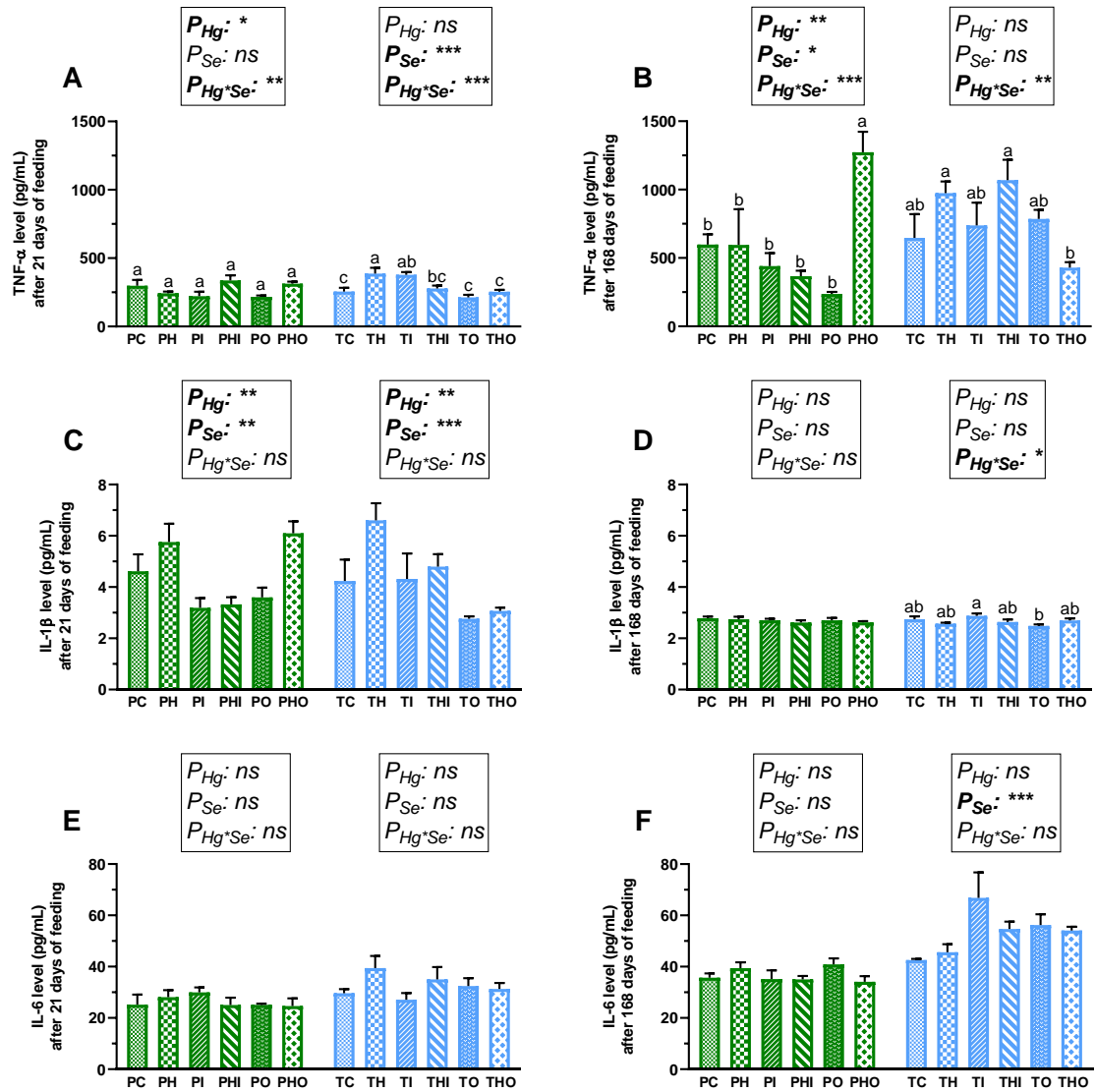
896 Fig. 1.





897

898 **Fig. 2.**



899

900 **Fig. 3.**