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1 **Effect of selenium sources in plant-based diets on antioxidant status and oxidative**  
2 **stress-related parameters in rainbow trout juveniles under chronic stress exposure**

3

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7

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13

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16

17 **Abstract**

18 Selenium (Se) is an essential micronutrient required for normal development and antioxidant  
19 protection. Oxidative stress induced by stressful conditions has been shown to be reduced by  
20 dietary Se supplementation. However, replacing fishmeal, an important source of Se, with  
21 ingredients of plant origin usually results in decreased Se content in aquafeeds. The objective  
22 of the study was hence to assess the impact of dietary Se supplementation by an inorganic or  
23 organic source on the antioxidant defense system of rainbow trout (*Oncorhynchus mykiss*)  
24 juveniles fed practical plant-based feeds under normal or chronic stress conditions. Fish  
25 (initial mean weight:  $42 \pm 2$  g) were maintained under normal (25 fish/tank, dissolved oxygen:  
26 7.9 mg/L) or stressful conditions combining decreased water quality with hypoxia and  
27 increased stocking density (50 fish/tank, dissolved oxygen: 5.9 mg/L) for 12 weeks at 17°C.  
28 Rainbow trout juveniles were fed three plant-derived protein-based diets containing 0.5 mg  
29 Se/kg diet supplemented or not with 0.3 mg Se/kg diet supplied as sodium selenite or Se-  
30 enriched yeast, Selsaf®. The apparent digestibility coefficients (ADC) of dry matter, protein,  
31 ash and Se were improved by Se-enriched yeast supplementation while the ADC of Se was  
32 reduced by sodium selenite supplementation and the ADC of dry matter, starch, energy, ash  
33 and phosphorus were reduced by chronic stress exposure. Dietary Se supplementation had no  
34 significant impact on growth performance contrary to chronic stress exposure which led to  
35 decreased final body weight and feed intake. Total Se content of fish was increased by both  
36 dietary Se supplementations but to a larger extent with Se-enriched yeast. Fish fed Se-  
37 enriched yeast displayed increased hepatic selenomethionine content and decreased muscle  
38 anisidine value. Blood glutathione content was reduced by chronic stress exposure. While  
39 hepatic catalase activity and transcript expression were increased by chronic stress exposure,  
40 both hepatic and renal glutathione peroxidase (GPX) activity and hepatic transcript expression  
41 of *gpx1b2* and *gpx4a2* were decreased in non-Se supplemented fish. These results suggest the

42 necessity to supplement plant-based diets with Se for rainbow trout juveniles, independently  
43 of chronic stress exposure and highlight the superiority of organic form of Se to fulfil the  
44 dietary Se requirement and sustain the antioxidant status of fish.

45

46 **Keywords**

47 Selenium; Chronic stress exposure; Oxidative stress; Glutathione peroxidase; Rainbow trout

48 **Highlights**

49

- 50 • Dietary selenium enhanced glutathione peroxidase activity in trout liver and kidney
- 51 • Se-enriched yeast supplementation reduced anisidine value in trout muscle
- 52 • Chronic stress exposure reduced growth, feed intake and nutrient digestibility
- 53 • Chronic stress exposure altered blood glutathione and hepatic antioxidant transcripts

## 54 **1. Introduction**

55

56 Selenium (Se) is an essential trace element for animals, including fish, required for  
57 normal development and antioxidant protection (Watanabe et al., 1997; Surai et al., 2006;  
58 NRC, 2011; Khan et al., 2017). This role is mainly attributed to the presence of Se within  
59 proteins and enzymes, named selenoproteins, in the form of selenocysteine, considered as the  
60 21<sup>st</sup> amino acid. The selenoproteins involved in the protection of cell from deleterious  
61 damages of oxidized compounds include glutathione peroxidases (GPX) used as a specific  
62 response criterion to estimate dietary Se requirements of fish (Antony Jesu Prabhu et al.,  
63 2016), thioredoxine reductases (TR) and methionine sulfoxide reductase B (MSRB).

64 Se can be added as a supplement to aquafeeds in either inorganic or organic forms.  
65 There is evidence in rainbow trout as in other animals that inorganic Se (sodium selenite or  
66 selenate) is less bioavailable and bioactive than organic Se, such as Se-yeast or  
67 selenomethionine (Küçükbay et al., 2009; Rider et al., 2009; Rider et al., 2010; Fontagné-  
68 Dicharry et al., 2015; Godin et al., 2015; Antony Jesu Prabhu et al., 2016). If levels of 0.15 to  
69 0.38 mg Se/kg diet as sodium selenite were required to provide maximum growth and GPX  
70 activity in rainbow trout according to Hilton et al. (1980), other recent studies recommend  
71 much higher doses of organic Se supplementation, up to 2-4 mg Se/kg diet, to promote growth  
72 and flesh quality in rainbow trout (Wang et al., 2018a,b). Fish meal-based diets generally  
73 provide sufficient Se to satisfy the nutritional requirements of fish (Watanabe et al., 1997), but  
74 can contain levels of Se well above the maximum dietary levels of 0.5 mg/kg permitted by  
75 European Food Safety Authority (European Commission, 2003), even though this upper limit  
76 only applies when Se is added to the feed, not when it appears naturally from the feed  
77 ingredients (Sissener et al., 2013). However, currently, there is significant reduction in  
78 fishmeal levels in aquafeeds, particularly in salmonid feeds, replaced to a large extent by plant

79 protein sources (Tacon and Metian, 2008) that vary widely in their Se content (Watanabe et  
80 al., 1997; Sauvant et al., 2004; Rayman et al., 2008). Accordingly, recent reports suggest that  
81 dietary Se required in plant-based feeds for Atlantic salmon and gilthead seabream may  
82 exceed the present EU legal limit of 0.5 mg/kg (Antony Jesu Prabhu et al., 2020; Domínguez  
83 et al., 2020).

84 Some studies in fish have shown that dietary Se supplementation can reduce oxidative  
85 stress induced by stressful conditions such as handling (Rider et al., 2009), crowding  
86 (Küçükbay et al., 2009), heavy metal contamination (Lin et al., 2007; Talas et al., 2008;  
87 Penglase et al., 2014a) or oxidized oil ingestion (Chen et al., 2013). Hypoxia, another stressful  
88 condition, is recognized as a major concern in aquaculture as this naturally occurring  
89 phenomenon in most aquatic environments, especially in intensive fish farming with high  
90 stocking density, has been reported to have many adverse effects (Van Raaij et al., 1996; Wu et  
91 al., 2016; Li et al., 2018; Del Rio et al., 2019). Hypoxia induces metabolic changes that may  
92 elicit oxidative stress due to the generation of reactive oxygen species (Lushchak and  
93 Bagnyukova, 2006; Bickler and Buck, 2007; Pérez-Jiménez et al., 2012). The antioxidant  
94 status of fish exposed to hypoxia can nonetheless be restored by proper supplementation of  
95 dietary antioxidants (Varghese et al., 2017).

96 However, with the fishmeal replacement by plant ingredients, the dietary Se level  
97 tends to decline in aquafeeds (Sissener et al., 2013). The objective of the study was hence to  
98 assess the impact of dietary Se supplementation by an organic or inorganic source on the  
99 antioxidant defense system of rainbow trout juveniles fed practical plant-based feeds under  
100 normal or stressful conditions combining decreased water quality with hypoxia and increased  
101 stocking density.

102

## 103 **2. Materials and Methods**

104

## 105 *2.1. Experimental diets*

106

107 Three practical diets based on plant-derived proteins were formulated to contain 51%  
108 crude protein and 23% crude lipid with different levels and forms of selenium (Table 1). Diets  
109 were supplemented with mineral mixture to meet all the essential mineral requirements of  
110 rainbow trout (NRC, 2011), except for Se. The Se0 diet containing 0.5 mg Se/kg feed was not  
111 supplemented with Se and served as control. The other two diets were supplemented with  
112 either sodium selenite (SeS diet) or the Se-enriched yeast Selsaf from Phileo by Lesaffre  
113 (France) containing 2.2 g Se/kg with 99% of organic Se (SeY diet) at a concentration of 0.3  
114 mg Se/kg feed. All the diets were prepared with incorporation of an inert marker, (yttrium  
115 oxide at 0.01 %) for apparent digestibility measurements, manufactured using a twin-screw  
116 extruder (BC 45, Clextal), vacuum-coated with oils and stored at 4 °C at the INRAE  
117 experimental facilities in Donzacq (Landes, France).

118

## 119 *2.2. Apparent digestibility trial*

120

121 Rainbow trout juveniles (initial body weight: 84 g) from the same parental stock were  
122 held (12 for normal conditions or 24 fish per unit for stressful conditions) in 18 cylindro-  
123 conical fiberglass tanks (each 60 l) connected to a re-circulation system (one per condition)  
124 with a flow rate of 2 l/min at Saint-Pée-sur-Nivelle, INRAE, France. The fish were fed one of  
125 the three diets (Se0, SeS or SeY) in triplicate groups. The water was well aerated and  
126 thermostatically maintained at  $17.0 \pm 0.5$  °C with dissolved oxygen levels of  $7.5 \pm 0.4$  mg/l  
127 for normal condition and  $5.8 \pm 0.2$  mg/l for stressful conditions representing 78 and 60%  
128 saturation, respectively. This low dissolved oxygen level was selected to be within the



129 minimum range of dissolved oxygen level for farmed trout (5-6 mg/l) to avoid sub-lethal  
130 effects (Ellis et al. 2002). Other water quality parameters were monitored every 3 days to  
131 ensure animal welfare (pH:  $7.4 \pm 0.2$ , ammonia: non detectable, nitrite:  $<0.015$  mg/l). The fish  
132 were allowed to adapt to the rearing conditions for 2 weeks, after which collection of feces  
133 was performed by the method of Choubert et al. (1982). The feces were collected on a daily  
134 basis for a period of 2 weeks and stored at  $-20$  °C. The feces collected over 2 weeks from each  
135 tank were pooled, freeze-dried and used for further chemical analysis. The apparent  
136 digestibility coefficients (ADC, %) of Se and other dietary components were calculated using  
137 yttrium oxide as digestibility marker as follows  $ADC (\%) = 100 - [100 * (\% X \text{ in feces} / \% X$   
138  $\text{in diet}) * (\% Y_2O_3 \text{ in diet} / \% Y_2O_3 \text{ in feces})]$  where X is the dietary component or energy.

139 All experimental procedures complied with the European Directive 010/63/EU for the  
140 protection of animals used for scientific purposes, and the French Decree no. 2013-118 for  
141 animal experimentation.

142

### 143 2.3. *Experimental fish and dietary trial conditions*

144

145 The feeding trial was carried out in the INRAE experimental fish farm at Donzacq  
146 supplied with flow through spring water at  $17.5 \pm 0.5$ °C. Rainbow trout (*Oncorhynchus*  
147 *mykiss*) juveniles from the same parental stock with a mean body weight of  $42 \pm 2$  g were  
148 randomly allocated to 150-l fiberglass tanks receiving a natural photoperiod. Fish were  
149 maintained under normal (N, 25 fish/tank, 20 kg/m<sup>3</sup>) and stressful (S, 50 fish/tank, 42 kg/m<sup>3</sup>)  
150 conditions for 12 weeks after acclimatization to the rearing conditions two weeks prior to the  
151 start of the experiment. These two stocking densities were selected to be within the normal  
152 density range of 15-40 kg/m<sup>3</sup> for rainbow trout reared in tanks (Ellis et al., 2002). For stressful  
153 conditions, the flow rate was reduced from 24 l/min to below 12 l/min to decrease dissolved

154 oxygen levels from  $7.9 \pm 0.4$  mg/l to  $5.9 \pm 1.0$  mg/l, representing 83 and 62% saturation,  
155 respectively. Fish were hand fed twice a day to visual satiation. Each diet was distributed to  
156 three replicate groups of fish over a 12-week growth trial.

157

### 158 *2.3. Sampling*

159

160 At the beginning of the feeding trial, 6 fish were sampled for analysis of whole-body  
161 composition. During the study, fish from each tank were bulk weighed every three weeks.  
162 Feed was withheld for 16 h before every weighing and sampling. At the end of the 12-week  
163 growth trial, a pooled sample of 3 fish from each experimental unit was taken for final body  
164 composition analysis. Further, 3 more fish from each replicate were withdrawn, anaesthetized  
165 (benzocaine, 30 mg/L) and blood samples were collected from the caudal vein into  
166 heparinized syringes. Fish were euthanized subsequently by a sharp blow to the head and  
167 liver, kidney and muscle were dissected. The liver was weighed for calculating hepato-  
168 somatic indice (HSI). Tissues were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$   
169 before analysis.

170

### 171 *2.5. Proximate, mineral and fatty acid analyses*

172

173 Proximate composition of diets was determined according to following procedures:  
174 dry matter after drying at  $105^{\circ}\text{C}$  for 24 h, protein ( $\text{N} \times 6.25$ ) by the Kjeldahl method after  
175 acid digestion, ash by incineration at  $550^{\circ}\text{C}$  for 10 h and gross energy in an adiabatic bomb  
176 calorimeter. Starch content was determined as glucose by a kit, using the  
177 amyloglucosidase/hexokinase/glucose-6-phosphate dehydrogenase method (Invivo Labs,  
178 France). Total lipid was extracted and measured gravimetrically according to Folch et al.

179 (1957) using dichloromethane instead of chloroform. Total P was determined by the  
180 molybdate blue/ascorbic acid method at 802 nm after mineralization and acid digestion  
181 (AFNOR, 1992). Total Se and yttrium concentrations were determined after acid digestion  
182 using concentrated HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>, using inductively coupled plasma-mass spectrometry  
183 (ICP-MS) as previously described (Fontagné-Dicharry et al., 2015). Typical analytical  
184 precision was lower than 5% (relative standard deviation) and detection limit of 3 µg/kg.  
185 Selenomethionine (SeMet) and selenocysteine (SeCys) concentrations were determined in  
186 rainbow trout tissues after enzymatic digestion using HPLC-ICP QMS as previously  
187 described (Godin et al., 2015). Fatty acid methyl esters were prepared and analyzed according  
188 to Fontagné et al. (2008).

189

## 190 *2.6. Oxidative status*

191

192 Conjugated dienes (E232) and trienes (E268) were measured as specific extinctions at  
193 the wavelengths of 232 and 268 nm, respectively (CEN, 2002). Anisidine value (AV) was  
194 determined according to standard procedures (CEN, 2000). Lipid-soluble fluorescent products  
195 (LSFP) and thiobarbituric acid-reactive substances (TBARS) were analyzed as previously  
196 described (Fontagné-Dicharry et al., 2014). Oxidized glutathione (GSSG) and reduced  
197 glutathione (GSH) were measured in blood and whole-body using Cayman glutathione assay  
198 kit (Bertin Pharma, Montigny-le-Bretonneux, France) according to the manufacturer's  
199 instructions with protein concentration assessed by the method of Lowry et al. (1951) using  
200 bovine serum albumin as the standard.

201

## 202 *2.7. Antioxidant enzyme activity*

203

204 Total and Se-dependent GPX (EC 1.11.1.9), glutathione reductase (GR, EC1.6.4.2),  
205 glutathione-S-transferase (GST, EC2.5.1.18), superoxide dismutase (SOD, EC1.15.1.1) and  
206 catalase (CAT, EC1.11.1.6) activities were assayed in liver as previously described (Fontagné  
207 et al., 2008; Fontagné-Dicharry et al., 2014). Total and Se-dependent GPX activities were also  
208 assayed in kidney. Specific enzymatic activities were assessed with protein concentration  
209 determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

210

### 211 *2.8. Antioxidant transcript level*

212

213 Total RNA was isolated from liver using Trizol reagent (Invitrogen, Cergy-Pontoise,  
214 France). For quantitative RT-PCR, complementary DNA was generated from 1 mg total RNA  
215 using SuperScript III RT (Invitrogen) and a mix of oligo(dT)15 and random primers  
216 (Promega, Charbonnières, France). For each sample, RT was performed in duplicate and  
217 quantitative PCR analyses were performed in LightCycler® 480 Instrument II (Roche  
218 Diagnostics, Meylan, France) using LightCycler® 480 SYBR Green I Master mix (Roche  
219 Diagnostics). Total reaction volume was 6 µL, with 2 µL of the diluted RT reaction mixture  
220 (dilution 40) and 4 µL of master mix added with 0.4 mM of each primer (Table 2). The PCR  
221 protocol was initiated at 95 °C for 10 min for initial denaturation of the cDNA and hot-start  
222 Taq-polymerase activation, followed by 45 cycles of a three-step amplification program (15  
223 sec at 95 °C, 10 sec at the melting temperature of 60 °C, 4.8 sec at 72 °C). Melting curves  
224 were systematically monitored (5 sec at 95 °C, 1 min at 65 °C, temperature slope at  
225 0.11 °C/sec from 65 to 97 °C) at the end of the last amplification cycle to confirm the  
226 specificity of the amplification reaction. Relative quantification of target gene transcripts were  
227 performed using Elongation Factor 1α (EF1α) as the reference gene and Se0-N as the  
228 reference group using the  $\Delta\Delta C_t$  method (Pfaffl, 2001).

229

## 230 *2.9. Statistical analyses*

231

232 Data are expressed as mean  $\pm$  standard deviation (SD). Differences between dietary  
233 groups were analyzed using two-way ANOVA to test the effect of dietary Se  
234 supplementation, rearing conditions and their interaction. The Newman-Keuls multiple range  
235 test was used to compare means when a significant difference was found. Gene transcript data  
236 were rank-transformed before analysis. Statistical analyses were performed with the  
237 computing program SigmaStat 3 (SPSS, Chicago, IL, USA) and differences were considered  
238 significant when P values were  $< 0.05$ .

239

## 240 **3. Results**

241

### 242 *3.1. Diet digestibility*

243

244 The ADC of dry matter, protein, ash and Se were significantly affected by diet  
245 composition, being improved by Se-enriched yeast (Selsaf) supplementation (Table 3). The  
246 ADC of Se was significantly reduced by sodium selenite supplementation. A significant  
247 interaction of dietary Se supplementation with rearing conditions was observed for ADC of  
248 starch as a significant decrease was noticed in Se0 group compared to SeS group but only in  
249 N conditions. The ADC of dry matter, starch, energy, ash and phosphorus were significantly  
250 reduced by chronic stressful conditions.

251

### 252 *3.2. Growth performance*

253

254 Rainbow trout juveniles readily accepted the plant-based diets from the beginning of  
255 the study and maintained normal behavior throughout the growth trial. The survival was not  
256 significantly affected by dietary treatments and conditions (Table 4). Rainbow trout from all  
257 dietary groups almost tripled their body weight at the end of the 12-week feeding trial and  
258 attained an individual final body weight ranging from 109 g in S groups to 124 g in N  
259 conditions. Daily growth index, feed intake and hepato-somatic index were also significantly  
260 reduced by stressful conditions and not affected by dietary Se supplementation. On the other  
261 hand, feed conversion ratio was not significantly affected by stressful conditions and no  
262 significant interaction between dietary Se supplementation and rearing conditions was noticed  
263 on the different growth parameters

264

### 265 *3.3. Se contents in rainbow trout*

266

267 Final whole-body dry matter and ash contents were not significantly affected by Se  
268 supplementation and rearing conditions (Table 5). Total Se content of whole-body rainbow  
269 trout was significantly increased by both dietary Se supplementations but to a larger extent  
270 with Se-enriched yeast compared to sodium selenite (Fig. 1). Both dietary Se  
271 supplementations increased also total Se content in liver and rainbow trout fed Se-enriched  
272 yeast displayed increased SeMet content in liver whereas hepatic SeCys content was not  
273 significantly affected. No significant effect of stress exposure or interaction between dietary  
274 Se supplementation and rearing conditions was noticed on the different rainbow trout Se  
275 contents (whether total Se content in whole-body and liver, or SeMet and SeCys content in  
276 liver).

277

### 278 *3.4. Oxidative status in rainbow trout*

279

280           The oxidative status in whole-body rainbow trout assessed by TBARS and glutathione  
281 contents was not significantly affected by dietary Se supplementation and rearing conditions  
282 (Table 5). Total glutathione contents were lower in whole-body than in blood (2.4 vs. 5.2  
283  $\mu\text{mol}/\text{mg}$  protein), with GSH and GSSG mean values of 1.7 and 0.8 for whole-body and 5.0  
284 and 0.2 for blood (Fig. 2), respectively. The GSSG/GSH ratio was thus higher in whole-body  
285 than in blood (0.61 vs. 0.04, respectively). Unlike whole-body glutathione levels, chronic  
286 stress exposure significantly reduced blood glutathione levels, both reduced and oxidized  
287 forms, leading to a reduced GSSG/GSH ratio (Fig. 2).

288           Lipid peroxidation and fatty acid profile were also assessed specifically in the flesh  
289 (Table 6). Levels of conjugated and lipid-soluble fluorescent products in rainbow trout muscle  
290 were not significantly affected by dietary Se supplementation or rearing conditions (Table 6).  
291 On the contrary, anisidine value was decreased by Se-enriched yeast supplementation  
292 compared to control group Se0 (Fig. 3). Levels of eicosapentaenoic acid (EPA) were  
293 increased by both sodium selenite supplementation and chronic stress exposure whereas other  
294 fatty acid levels were not significantly affected (Table 6).

295           No significant interaction between dietary Se supplementation and rearing conditions  
296 was noticed on the different oxidative status parameters (Tables 5 and 6, Fig. 2).

297

### 298 *3.5. Antioxidant enzyme activity and transcript levels in rainbow trout*

299

300           In both liver and kidney, total and Se-dependent GPX activities were increased by  
301 dietary Se supplementation whether as sodium selenite or Se-enriched yeast (Fig. 4) while  
302 hepatic CAT activity was increased by chronic stress exposure (Table 7). Other antioxidant

303 enzyme activities were not significantly affected and no significant interaction between  
304 dietary Se supplementation and rearing conditions was recorded (Table 7).

305 The transcript levels of *gpx1b2* and *gpx4a2* were increased in both Se-supplemented  
306 groups similar to GPX activity (Table 8). Chronic stress exposure modulated antioxidant  
307 transcript expression rather differently to antioxidant enzyme activity: transcript levels of  
308 *gpx1a*, *gpx4a2* and *gpx4b* were reduced by chronic stress exposure whereas *tr3a* and *msrb2*  
309 were increased by chronic stress exposure. Only *cat* mRNA content was increased by chronic  
310 stress exposure similar to CAT activity. No significant interaction between dietary Se  
311 supplementation and rearing conditions was noticed on the transcript level according to two-  
312 way ANOVA.

313

#### 314 **4. Discussion**

315

##### 316 *4.1. Impact of Se supplementation in plant-based diets on antioxidant metabolism in rainbow* 317 *trout juveniles*

318

319 Similar to our previous study in rainbow trout fry (Fontagné-Dicharry et al., 2015),  
320 growth performance of juveniles was not affected by dietary Se sources and levels ranging  
321 from 0.47 to 0.93 mg/kg diet. These dietary Se levels correspond to available Se levels of 0.42  
322 mg/kg diet for the non-Se supplemented diet Se0, 0.81 mg/kg diet for the selenite-  
323 supplemented diet, SeS and 0.83 mg/kg diet for the SeMet-supplemented diet, SeY. This  
324 finding is in accordance with the earlier results of Hilton et al. (1980), who reported a Se  
325 requirement of 0.15 mg/kg diet based on weight gain as response criterion or 0.38 mg/kg diet  
326 based on maximal plasma GPX activity. According to the meta-analysis of Antony Jesu  
327 Prabhu et al. (2016), hepatic GPX activity is a more stringent and robust criterion than weight



328 gain to define Se deficiency in fish. In the present study, both hepatic and kidney GPX  
329 activities were improved by dietary Se supplementation (from 31-40% in liver to 45-56% in  
330 kidney), suggesting that the basal dietary Se level of 0.47 mg/kg diet meaning 0.42 mg/kg diet  
331 of available Se in the non-Se supplemented diet Se0 was not sufficient to sustain proper GPX  
332 activity in rainbow trout juveniles fed plant-based diets. Besides, this suggests that kidney  
333 GPX activity can be a reliable marker to define Se deficiency. These results are consistent  
334 with our previous observations of reduced whole-body GPX activity in rainbow trout fry fed a  
335 similar plant-based diet containing 0.50 mg Se/kg diet (Fontagné-Dicharry et al., 2015). These  
336 plant-based diets contained predominantly SeMet, with some selenite and selenate (Godin et  
337 al., 2015). This is coherent with the present ADC of Se that is intermediate for the non-Se  
338 supplemented diet Se0 compared to the selenite- and SeMet-supplemented diets, SeS and  
339 SeY, respectively. So, the higher dietary need for Se in rainbow trout from the present study  
340 compared to the study of Hilton et al. (1980) using semi-purified diets supplemented with  
341 sodium selenite might not be attributed to a reduced Se availability in the basal diet Se0. On  
342 the other hand, an interacting effect of dietary vitamin E level could not be excluded. The  
343 dietary vitamin E level used in the present study, even meeting the vitamin E requirement of  
344 rainbow trout according to NRC (2011), was eight times lower than the one used by Hilton et  
345 al (1980) and could have increased the dietary need for Se in this study as was suggested in  
346 the meta-analysis by Antony Jesu Prabhu et al. (2016).

347         The reduction of hepatic and kidney Se-GPX activities in fish fed the non-Se  
348 supplemented diet was associated with a reduction of total-GPX activity. There was no  
349 compensatory increase of non-Se-dependent GPX activity in agreement with Bell et al. (1985,  
350 1986) in the liver of rainbow trout but contrary to our previous findings in whole-body of  
351 rainbow trout fry (Fontagné-Dicharry et al., 2015). Besides, the other antioxidant enzymes  
352 were not either affected in agreement with Fontagné-Dicharry et al. (2015) and Bell et al.

353 (1985) but contrary to Bell et al. (1986) and Gatlin et al. (1986) who reported an increased  
354 hepatic GST activity in Se-deficient fish. The reduced activity of Se-GPX in fish fed Se0 was  
355 associated with concomitant decreased expression of the Se-GPX genes *gpx1b2* and *gpx4a2*  
356 confirming the observations on GPX transcript expression in rainbow trout hepatocytes  
357 (Pacitti et al., 2013), fry (Fontagné-Dicharry et al., 2015) and progeny (Wischhusen et al.,  
358 2019). On the other hand, *gpx1a* and *gpx4a* were not affected by increased Se intake in the  
359 present study whereas these transcripts were reported to be the most sensitive ones among  
360 cytosolic and phospholipid hydroperoxide GPX (Pacitti et al., 2013). Except for *gpx1b2* and  
361 *gpx4a2*, antioxidant or selenoprotein transcript expressions were not affected by Se intake, in  
362 conjunction with antioxidant enzyme activities. The selenoprotein P, considered as a major  
363 selenoprotein involved in the transport and delivery of Se to remote tissues (Papp et al. 2007)  
364 and a good marker of Se status in rainbow trout fry and broodstock (Fontagné-Dicharry et al.,  
365 2015; Wischhusen et al., 2019), was not affected by dietary Se level in the present study with  
366 rainbow trout juveniles whereas diet compositions, Se levels and forms were comparable. In  
367 zebrafish, the expression of the selenoprotein P paralogues has been shown to differ, with  
368 only *sepp1a* responding to Se status (Penglase et al., 2014b). Similar results were reported in  
369 rainbow trout broodstock with *selpa* and *selpa2* responding to Se status contrary to *selpb*  
370 (Wischhusen et al., 2019). In the present study, we assessed the expression of *selpa2*, a  
371 putative Se-sensitive paralogue and this difference of regulation might be attributed to a  
372 difference of sensitivity between life stages, with juvenile stages being less sensitive or able to  
373 adapt more quickly to Se deficiency than fry or broodstock as suggested for oxidative stress  
374 (Fontagné-Dicharry et al., 2018). If selenoprotein P level has been proved to be a good  
375 biomarker of Se status in higher vertebrates, its mRNA level is not always decreased by Se  
376 deficiency contrary to GPX1 (Sunde et al., 2011). Such difference between protein and  
377 mRNA levels might exist in fish and it would have been interesting to also analyze tissue

378 selenoprotein P levels in the present study to obtain more information about the hierarchy of  
379 Se regulation of the various selenoproteins and their mRNA.

380         The oxidative status was mildly altered by dietary Se levels and forms with only a  
381 decrease of anisidine value in muscle of rainbow trout juveniles fed Se-enriched yeast  
382 compared to the control group. This result supports the superiority of organic Se form to  
383 sustain the antioxidant status of fish reported by Küçükbay et al. (2009) and Fontagné-  
384 Dicharry et al. (2015) in rainbow trout and by Saffari et al. (2016) and Mechlaoui et al. (2019)  
385 in other fish species. This finding is probably related to the superior bioavailability of the  
386 organic form, resulting in improved body Se contents compared to the inorganic form (171 vs.  
387 156% compared to the non-Se supplemented diet Se0). Nevertheless, this could not be due  
388 only to the higher digestibility of the organic form (91 vs 87%) that results in a small  
389 difference of available Se between yeast and selenite (201 vs. 194% compared to the non-Se  
390 supplemented diet Se0) but probably also to a different metabolism between Se forms. In  
391 Atlantic salmon, organic Se intake has been shown to result in improved body Se especially in  
392 muscle (Lorentzen et al., 1994, Antony Jesu Prabhu et al., 2020) as SeMet (Sele et al, 2018)  
393 compared to inorganic Se intake. However, if organic Se supplementation has been shown to  
394 increase SeMet levels in liver of rainbow trout, hepatic SeCys levels were not significantly  
395 affected similar to observations in the whole-body of rainbow trout fry (Godin et al., 2015).  
396 These results would suggest the non-specific incorporation of dietary Se as SeMet into hepatic  
397 protein rather than its incorporation as SeCys into functional selenoenzymes such as GPX,  
398 even if this assumption is not sustained by the hepatic GPX activity and transcript expression  
399 data. Nevertheless, different metabolism of selenite and SeMet between liver and muscle  
400 probably occur, resulting in differential Se deposition as suggested in salmon (Lorentzen et  
401 al., 1994; Sele et al., 2018; Antony Jesu Prabhu et al., 2020). It would have been interesting to  
402 also analyze muscle Se levels in the present study, coupled with the muscle selenoenzymes to

403 figure out the role of non-specific SeMet incorporation into proteins on the antioxidant  
404 protection in rainbow trout muscle.

405

#### 406 *4.2. Impact of chronic stress exposure on oxidative stress in rainbow trout juveniles*

407

408         Similar to our results on the impact of chronic stress exposure combining stocking  
409 density and hypoxia, reduced growth and feed intake were reported in rainbow trout subjected  
410 to crowding conditions but with reduced feed efficiency (Trenzado et al., 2006; Küçükbay et  
411 al., 2009; Naderi et al., 2017). Moreover, contrary to the present study with chronic stress  
412 exposure, even in different tissues, Küçükbay et al. (2009) also reported a reduction of serum  
413 and muscle Se levels and serum GPX activity, with an increase of serum and muscle TBARS  
414 content with crowding conditions and Naderi et al. (2017) observed a decrease of the serum  
415 total antioxidant capacity and hepatic vitamin E content. In the present study, we only  
416 observed a reduction of some hepatic GPX mRNA contents that could relate to the changes of  
417 oxidative stress markers reported in the former studies. These differences could be linked to  
418 the intensity of the stress that was lower in the present study with a density of 42 kg/m<sup>3</sup>  
419 compared to the three previous ones with a density of 100 kg/m<sup>3</sup>. Moreover, according to Ellis  
420 et al. (2002), the magnitude of the density effect depends upon study-specific conditions,  
421 including behavioral interactions and water quality deterioration with a special attention to  
422 dissolved oxygen level.

423         The reduction of growth, feed intake and nutrient digestibility by chronic stress  
424 exposure combining both decreased water quality with hypoxia and increased stocking  
425 density noticed in the present study is in accordance with results reported in a rainbow trout  
426 isogenic line exposed to hypoxia by Magnoni et al. (2018). However, the effects of chronic  
427 stress exposure on oxidative stress induction were less clear as blood GSH level was reduced

428 and hepatic CAT activity and mRNA content were increased as well as *msrb2* and *tr3a*  
429 transcript levels but along with some GPX mRNA contents that were reduced as well as blood  
430 GSSG level and GSSG/GSH ratio. Such discrepancy between antioxidant enzymes has been  
431 highlighted in different fish species by Lushchak and Bagnyukova (2006) who distinguished a  
432 conventional response to hypoxic stress with antioxidant enzymes remaining unchanged or  
433 being lowered over hypoxia in fish and the preparation to oxidative stress response with an  
434 increase of antioxidant enzymes only in cyprinids exposed to hypoxia. Likewise, Magnoni et  
435 al. (2019) reported no increase of oxidative stress in liver of a rainbow trout isogenic line  
436 contrary to Varghese et al. (2017) who noticed an oxidative stress induction in the mrigal  
437 carp, *Cirrhinus mrigala*. On the other hand, Pérez-Jiménez et al. (2012) recorded dual  
438 response of antioxidant enzymes similar to our results, but with decreased SOD and increased  
439 GPX activity in gilthead sea bream exposed to an acute hypoxic challenge and CAT activity  
440 was increased by hypoxia only if fish were fed a methionine-supplemented diet.

441

#### 442 *4.3. Interaction between dietary Se supplementation and chronic stress exposure on oxidative* 443 *stress in rainbow trout juveniles*

444

445 In the present study, no significant interaction on oxidative stress induction between  
446 chronic stress exposure combining hypoxia and stocking density and dietary Se  
447 supplementation was detected. The beneficial effect of dietary Se supplementation was  
448 observed only for starch digestibility under normal conditions but not under stressful  
449 conditions. A tendency for an increased lipid peroxidation with reduced antioxidant transcript  
450 level in stressful conditions could be noticed only in non-supplemented (for muscle LSFP,  
451 AV and hepatic *gpx4b* data) or selenite-supplemented fish (for whole-body GSH and hepatic  
452 *gpx4b* data) but it was significant only for hepatic *gpx4b* mRNA levels according to one-way

453 ANOVA (Fig. 5). If significant, it could have indicated that Se requirements may not be met  
454 by the non-supplemented or selenite-supplemented diet in stressed fish similar to the results  
455 reported by Rider et al. (2009) in juvenile rainbow trout fed a non-supplemented diet  
456 containing 0.73 mg Se/kg diet subjected to chronic physical stress. However, this interaction  
457 was not significant for almost all parameters. So according to the present results, dietary Se  
458 supplementation did not appear to alleviate the detrimental effects of chronic stress exposure  
459 that were, however, quite limited on oxidative stress induction. Similarly, no effect of dietary  
460 Se supplementation was noticed on rainbow trout under high stocking density conditions with  
461 80 kg/m<sup>3</sup> by Naderi et al. (2017) contrary to dietary vitamin E supplementation. On the other  
462 hand, Rider et al. (2009) and Küçükbay et al. (2009) indicated that dietary Se supplementation  
463 could prevent the negative effects of chronic physical stress (daily handling and confinement  
464 stressors) and crowding conditions with 100 kg/m<sup>3</sup> in rainbow trout juveniles. In the present  
465 study, even if the stocking density was doubled in stressful conditions to reach 42 kg/m<sup>3</sup>, this  
466 could not be considered as crowding conditions for rainbow trout according to the review of  
467 Ellis et al. (2002). So stress conditions were different and could explain the observed  
468 differences between studies. In order to ascertain this, the impact of different stress conditions  
469 on oxidative stress induction and antioxidant metabolism in rainbow trout deserves further  
470 studies to gain a better understanding of the mechanisms involved.

471

## 472 **5. Conclusion**

473

474 In summary, our results suggest the necessity to supplement plant-based diets with Se  
475 for rainbow trout juveniles, independently of chronic stress exposure and highlight the  
476 superiority of organic form to fulfil the dietary Se requirement probably due in part to a better  
477 apparent availability and to sustain the antioxidant status of fish. However, dietary Se

478 supplementation did not allow to alleviate the detrimental effects on growth performance and  
479 antioxidant metabolism induced by chronic stress exposure.

480

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482

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487

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489

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728

729 **Table 1**

730 Formulation and composition of the experimental diets.

Diet	Se0	SeS	SeY
<i>Ingredients (%)</i>			
Plant meals <sup>a</sup>	75.1	75.1	75.1
Vegetable and fish oils <sup>b</sup>	15.6	15.6	15.6
Soybean lecithin <sup>c</sup>	2.0	2.0	2.0
Crystalline amino acids <sup>d</sup>	1.6	1.6	1.6
Attractant premix <sup>e</sup>	1.5	1.5	1.5
Vitamin premix <sup>f</sup>	1	1	1
Mineral premix without Se <sup>g</sup>	3.2	3.2	3.2
Yttrium oxide (g/kg diet)	0.1	0.1	0.1
Sodium selenite (mg/kg diet)	-	0.7	-
Se-enriched yeast, Selsaf (mg/kg diet)	-	-	150
<i>Proximate composition</i>			
Dry matter (DM, %)	97	97	97
Crude protein (% DM)	51	51	52
Total lipid (% DM)	24	23	23
Gross energy (kJ/g DM)	25	25	25
Ash (% DM)	5	5	5
P (% DM)	1.1	1.1	1.1
Se (mg/kg)	0.5	0.9	0.9

731 <sup>a</sup> Plant meals (% diet): wheat gluten (Roquette, France), 20; corn gluten meal (Inzo, France),

732 18; soybean protein concentrate (Estril, Spropêche, France), 17; soybean meal (Sud-Ouest

733 Aliment, France), 5; white lupin meal (Farilup 500, Terrena, France), 5; rapeseed meal  
734 (Primor 00, Sud-Ouest Aliment, France), 4; dehulled pea meal (Primatex, Sotexpro, France),  
735 3; whole wheat (Sud-Ouest Aliment, France), 3.1.  
736 <sup>b</sup> Vegetable and fish oils (% diet): linseed oil (Daudruy, France), 7.8; fish oil (Sopropêche,  
737 France), 7.8.  
738 <sup>c</sup> Supplied by Louis François (France).  
739 <sup>d</sup> Crystalline amino acids (% diet): L-lysine (Ajinomoto-Eurolysine, France), 1.34; DL-  
740 methionine (Evonik, Germany), 0.3.  
741 <sup>e</sup> Attractant premix (g/kg diet): glucosamine, 5; taurine, 3; betaine, 3; glycine, 2; alanine, 2.  
742 <sup>f</sup> Vitamin premix (IU or g/kg diet): retinyl acetate, 5,000 IU; cholecalciferol, 2,500 IU; DL- $\alpha$ -  
743 tocopheryl acetate, 50 IU; sodium menadione bisulfate, 10 mg; thiamin-HCl, 1 mg; riboflavin,  
744 4 mg; niacin, 10 mg; D-calcium pantothenate, 20 mg; pyridoxine-HCl, 3 mg; *myo*-inositol,  
745 0.3 g; D-biotin, 0.2 mg; folic acid, 1 mg; cyanocobalamin, 10  $\mu$ g; L-ascorbyl-2-  
746 polyphosphate, 50 mg; choline, 1 g. All ingredients were diluted with  $\alpha$ -cellulose.  
747 <sup>g</sup> Mineral mixture (g/kg diet): CaHPO<sub>4</sub>·2H<sub>2</sub>O, 27; CaCO<sub>3</sub>, 2.15; NaCl, 0.4; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.2;  
748 MnSO<sub>4</sub>·H<sub>2</sub>O, 0.03; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.04; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.03; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.0002; KI, 0.0004;  
749 NaF, 0.01; MgO, 1.24; KCl, 0.9.

750 **Table 2**

751 Oligonucleotide primers used to assay gene transcript levels by real-time quantitative RT-

752 PCR.

Gene	Accession no.	Forward primer sequence (5' → 3')	Reverse primer sequence (5' → 3')	Amplicon size (bp)
<i>gpx1a</i> <sup>a</sup>	HE687021	AATGTGGCGTCACTCTGAGG	CAATTCTCCTGATGGCCAAA	131
<i>gpx1b1</i> <sup>a</sup>	CA357669.1	CGAGCTCCATGAACGGTACG	TGCTTCCCGTTCACATCCAC	183
<i>gpx1b2</i> <sup>a</sup>	HE687023	TCGGACATCAGGAGAACTGC	TCCTTCCCATTACATCCAC	121
<i>gpx4a1</i> <sup>a</sup>	HE687024	GAAAGGCTTCCTGGGAAATG	CTCCACCACACTGGGATCAT	112
<i>gpx4a2</i> <sup>a</sup>	HE687025	AGAAATACAGGGGCGACGTT	GCATCTCCGCAAACCTGAGAG	90
<i>gpx4b</i> <sup>a</sup>	CA344428.1	TTGGAGGTCAGGAGCCAGGT	ACCCTTTCCCTTGGGCTGTT	152
<i>selpa2</i>	MH085054.1	ACCTTGCTGAGCCAGAAACT	CAGACGACCACACCTGTCAT	129
<i>tr3a</i>	HF969246.1	GAGCCTCCCTCAAGTGTGAC	AGTGAACCTCCAGAGGCCAGA	158
<i>tr3b</i>	HF969247.1	ACAAAATCAAGGCGACCAAC	GGCAGAGAGAACAGGTCGTC	148
<i>msrb2</i>	CA354807.1	AGGGGACAGAGATGCCCTTC	CCCATGAGCCTCTTTGAACG	149
<i>sod1</i>	AF469663.1	TGGTCCTGTGAAGCTGATTG	TTGTCAGCTCCTGCAGTCAC	201
<i>sod2</i>	CA352127.1	TCCCTGACCTGACCTACGAC	GGCCTCCTCCATTAAACCTC	201
<i>cat</i>	BX087110.3	TGATGTACACAGGTGCGTA	GTGGGCTCAGTGTGTTGAG	195
<i>gr</i>	HF969248.1	CTAAGCGCAGCGTCATAGTG	ACACCCCTGTCTGACGACAT	108
<i>gstπ</i>	BX302932.3	TCGCTGACTGGACGAAAGGA	CGAAGGTCCTCAACGCCATC	196
<i>gclc</i>	FR904377.1	AGGCCAGAGTATGGCAGCTA	TTGGGAATGATGTGATGGTG	166
<i>nrf2</i>	CA360709.1	TGAGCTGCAGCAATGTCTGA	GTTGGGCAATGGGTAGAAGC	124
<i>ikba</i>	BT074199.1	AGAGACAGACTGCGCTCCAC	CGGCCTTCAGTAGCCTCTCT	72
<i>ahra</i>	AF065137.3	CTCTCGCCTGGACAAACTCT	GCGTTCATCCCATTTCATGTT	138
<i>ef1α</i>	AF498320.1	TCCTCTTGGTCGTTTCGCTG	ACCCGAGGGACATCCTGTG	159

753 *gpx1*, cytosolic glutathione peroxidase; *gpx4*, phospholipid hydroperoxide glutathione754 peroxidase; *selp*, selenoprotein P; *tr*, thioredoxin reductase; *msrb*, methionine-R-sulfoxide755 reductase B; *sod1*, cytosolic Cu/Zn superoxide dismutase; *sod2*, mitochondrial Mn superoxide756 dismutase; *cat*, catalase; *gr*, glutathione reductase; *gst*, glutathione S-transferase; *gclc*,

757 glutamate-cysteine ligase catalytic subunit; nrf2, nuclear factor erythroid-2-related factor 2;

758 ikba, nuclear factor kappa-light chain-enhancer of activated B cells inhibitor  $\alpha$ ; ahra, aryl

759 hydrocarbon receptor  $\alpha$ ; ef1 $\alpha$ , elongation factor 1 $\alpha$  .

760 <sup>a</sup> As described by Pacitti et al. (2013).

761 **Table 3**

762 Apparent digestibility coefficients (ADC) of Se and other nutrients in rainbow trout juveniles  
 763 fed the experimental diets not supplemented with Se (Se0), supplemented with sodium  
 764 selenite (SeS) or Se-enriched yeast, Selsaf (SeY) and kept under normal (N) or stressful (S)  
 765 conditions for 2 weeks at  $17 \pm 1$  °C.

ADC (%)	Dietary Se supplementation			Rearing conditions		Interaction
	Se0	SeS	SeY	N	S	
Dry matter	77±2 <sup>b</sup>	77±3 <sup>b</sup>	79±1 <sup>a</sup>	79±1 <sup>z</sup>	76±3 <sup>y</sup>	0.091
Protein	91.7±0.4 <sup>b</sup>	91.9±0.4 <sup>b</sup>	92.5±0.3 <sup>a</sup>	92.2±0.4	91.9±0.4	0.588
Starch	88±3	88±5	89±4	92±2 <sup>z</sup>	85±2 <sup>y</sup>	<b>0.041</b>
Energy	84±1	83±1	84±1	85±1 <sup>z</sup>	83±1 <sup>y</sup>	0.240
Ash	21±5 <sup>b</sup>	22±6 <sup>b</sup>	28±4 <sup>a</sup>	28±3 <sup>z</sup>	20±4 <sup>y</sup>	0.236
Phosphorus	37±5	38±6	39±4	42±2 <sup>z</sup>	34±2 <sup>y</sup>	0.161
Selenium	89±1 <sup>b</sup>	87±1 <sup>c</sup>	91±1 <sup>a</sup>	89±2	89±2	0.743

766 Values are means ± SD of six or nine rearing tanks (two conditions per Se level or three diets  
 767 per condition). Within rows and for each effect: dietary Se supplementation or rearing  
 768 condition, means not sharing a common superscript letter are significantly different according  
 769 to two-way ANOVA. Significant interactions are highlighted in bold type.

770 **Table 4**

771 Growth performance of rainbow trout juveniles fed the experimental diets not supplemented  
 772 with Se (Se0), supplemented with sodium selenite (SeS) or Se-enriched yeast, Selsaf (SeY)  
 773 and kept under normal (N) or stressful (S) conditions for 12 weeks at  $17 \pm 1$  °C.

	Dietary Se supplementation			Rearing conditions		Interaction
	Se0	SeS	SeY	N	S	
Survival (%)	78±9	93±9	86±16	80±14	91±9	0.977
Mean body weight (g)	112±13	118±12	118±10	124±11 <sup>a</sup>	109±6 <sup>b</sup>	0.837
Daily growth index <sup>1</sup>	1.6±0.3	1.7±0.2	1.7±0.2	1.8±0.2 <sup>a</sup>	1.5±0.1 <sup>b</sup>	0.848
Absolute feed intake <sup>2</sup>	1.0±0.1	1.1±0.1	1.0±0.1	1.1±0.1 <sup>a</sup>	1.0±0.1 <sup>b</sup>	0.952
Feed intake <sup>3</sup>	8.3±0.6	9.4±0.7	9.0±1.2	9.3±0.9 <sup>a</sup>	8.5±0.8 <sup>b</sup>	0.794
Feed conversion ratio <sup>4</sup>	1.5±0.3	1.3±0.1	1.4±0.1	1.4±0.3	1.4±0.1	0.803
Hepatosomatic index <sup>5</sup>	1.3±0.2	1.4±0.2	1.4±0.1	1.5±0.2 <sup>a</sup>	1.3±0.1 <sup>b</sup>	0.475

774 Values are means ± SD of six or nine rearing tanks (two conditions per Se level or three diets  
 775 per condition). Within rows and for each effect: dietary Se supplementation or rearing  
 776 condition, means not sharing a common superscript letter are significantly different according  
 777 to two-way ANOVA.

778 <sup>1</sup> Daily growth index was calculated as  $100 \times [(\text{final mean body weight})^{1/3} - (\text{initial mean}$   
 779  $\text{body weight})^{1/3}] / \text{duration of experimental period (84 days)}$ .

780 <sup>2</sup> Absolute feed intake, expressed in g DM per fish per day, was calculated on dry matter  
 781 (DM) basis as  $FI_{\text{tot}} / (n \times t)$  where  $FI_{\text{tot}}$  is the total feed intake per tank (in g DM) over  
 782 experimental period, n is the number of fish in tank and t is the duration of experimental  
 783 period.

784 <sup>3</sup> Feed intake per metabolic body weight, expressed in g DM per  $\text{kg}^{0.8}$  per day was calculated  
 785 as  $\text{absolute feed intake} / [\sqrt[3]{(\text{final mean body weight} \times \text{initial mean body weight}) / 1000}]^{0.8}$ .

786 <sup>4</sup> Feed conversion ratio was calculated as dry feed intake (g) / wet weight gain (g).

787 <sup>5</sup> Hepato-somatic index was calculated as wet liver weight (g) / fish weight (g).



788 **Table 5**

789 Whole-body composition and oxidative status of rainbow trout juveniles fed the experimental  
 790 diets not supplemented with Se (Se0), supplemented with sodium selenite (SeS) or Se-  
 791 enriched yeast, Selsaf (SeY) and kept under normal (N) or stressful (S) conditions for 12  
 792 weeks.

	Dietary Se supplementation			Rearing conditions		Interaction
	Se0	SeS	SeY	N	S	
Dry matter (% wet weight)	30.9±0.8	31.2±0.6	30.9±1.3	31.2±1.1	30.7±0.8	0.081
Ash (% wet weight)	2.3±0.2	2.3±0.3	2.5±0.2	2.3±0.3	2.4±0.2	0.386
TBARS (µg/g wet weight)	5.8±3.9	4.9±1.7	3.9±1.5	6.0±3.1	3.7±1.4	0.934
GSH (µmol/mg protein)	1.5±0.5	1.7±0.7	1.9±0.8	1.7±0.7	1.7±0.8	0.059
GSSG (µmol/mg protein)	0.7±0.5	0.8±0.5	0.8±0.4	0.8±0.4	0.8±0.6	0.709
GSSG/GSH ratio	0.7±0.5	0.6±0.3	0.5±0.4	0.5±0.2	0.7±0.5	0.611

793 Values are means ± SD of six or nine rearing tanks (two conditions per Se level or three diets  
 794 per condition). Within rows and for each effect: dietary Se supplementation or rearing  
 795 condition, means not sharing a common superscript letter are significantly different according  
 796 to two-way ANOVA.

797 **Table 6**

798 Lipid peroxidation and fatty acid composition in flesh of rainbow trout juveniles fed the  
 799 experimental diets not supplemented with Se (Se0), supplemented with sodium selenite (SeS)  
 800 or Se-enriched yeast, Selsaf (SeY) and kept under normal (N) or stressful (S) conditions for  
 801 12 weeks.

	Dietary Se supplementation			Rearing conditions		Interaction
	Se0	SeS	SeY	N	S	
Total lipid (% wet weight)	3.8±0.7	3.5±0.5	3.7±0.4	3.7±0.5	3.6±0.6	0.288
<i>Lipid peroxidation level</i>						
E <sub>232</sub>	12±2	13±2	12±2	13±2	12±2	0.594
E <sub>268</sub>	4.3±1.7	4.7±1.4	3.9±1.2	4.4±1.3	4.2±1.6	0.615
LSFP	3.9±0.4	3.8±0.2	3.8±0.2	3.8±0.3	3.9±0.3	0.051
<i>FA composition (% total FA)</i>						
Saturated FA	23.0±1.0	22.8±1.1	22.6±0.7	23.0±1.0	22.7±0.8	0.572
Monounsaturated FA	23.0±1.7	22.2±1.2	22.3±1.4	22.4±1.2	22.6±1.7	0.294
n-6 PUFA	18.0±0.7	18.0±0.6	18.4±0.4	18.2±0.6	18.0±0.6	0.516
n-3 PUFA	32.4±1.6	33.3±1.0	33.3±1.3	32.8±1.1	33.2±1.6	0.812
Long-chain n-3 PUFA	15.6±1.7	16.3±1.3	15.9±1.1	15.8±1.3	16.1±1.4	0.540
EPA	4.8±0.4 <sup>b</sup>	5.1±0.3 <sup>a</sup>	4.9±0.4 <sup>ab</sup>	4.8±0.4 <sup>y</sup>	5.1±0.5 <sup>z</sup>	0.978
DHA	8.3±1.2	8.7±1.1	8.4±0.7	8.4±1.0	8.5±1.1	0.230
Unsaturation index	203±7	207±5	206±5	204±5	206±6	0.852

802 E<sub>232</sub>, conjugated dienes; E<sub>268</sub>, conjugated trienes; LSFP, lipid-soluble fluorescent products;  
 803 FA, fatty acid; PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA,  
 804 docosaheptaenoic acid. Unsaturation index=sum (fatty acid percent)×(number of double  
 805 bonds).

806 Values are means  $\pm$  SD of 18 or 27 fish (two conditions per Se level or three diets per  
807 condition). Within rows and for each effect: dietary Se supplementation or rearing condition,  
808 means not sharing a common superscript letter are significantly different according to two-  
809 way ANOVA.

810 **Table 7**

811 Antioxidant enzyme activity in liver of rainbow trout juveniles fed the experimental diets not  
 812 supplemented with Se (Se0), supplemented with sodium selenite (SeS) or Se-enriched yeast,  
 813 Selsaf (SeY) and kept under normal (N) or stressful (S) conditions for 12 weeks.

	Dietary Se supplementation			Rearing conditions		Interaction
	Se0	SeS	SeY	N	S	
SOD (U/mg protein)	31±9	25±5	28±4	27±6	31±7	0.686
CAT (U/mg protein)	542±94	511±111	531±97	489±86 <sup>b</sup>	568±98 <sup>a</sup>	0.545
GR (mU/mg protein)	24±15	26±9	25±13	28±12	23±13	0.540
GST (mU/mg protein)	334±66	319±46	310±54	318±54	324±59	0.292

814 Values are means ± SD of 18 or 27 fish (two conditions per Se level or three diets per  
 815 condition). Within rows and for each effect: dietary Se supplementation or rearing condition,  
 816 means not sharing a common superscript letter are significantly different according to two-  
 817 way ANOVA.

818 **Table 8**

819 Antioxidant transcript levels in liver of rainbow trout juveniles fed the experimental diets not  
 820 supplemented with Se (Se0), supplemented with sodium selenite (SeS) or Se-enriched yeast,  
 821 Selsaf (SeY) and kept under normal (N) or stressful (S) conditions for 12 weeks.

	Dietary Se supplementation			Rearing conditions		Interaction
	Se0	SeS	SeY	N	S	
<i>gpx1a</i>	0.9±0.4	0.9±0.3	1.0±0.2	1.0±0.3 <sup>z</sup>	0.8±0.2 <sup>y</sup>	0.959
<i>gpx1b1</i>	1.0±0.3	0.9±0.3	1.1±0.3	1.0±0.3	1.0±0.3	0.795
<i>gpx1b2</i>	1.1±0.3 <sup>b</sup>	1.2±0.3 <sup>a</sup>	1.5±0.3 <sup>a</sup>	1.2±0.4	1.3±0.3	0.876
<i>gpx4a1</i>	0.9±0.2	0.8±0.2	0.9±0.2	0.9±0.2	0.9±0.2	0.965
<i>gpx4a2</i>	1.0±0.3 <sup>b</sup>	1.4±0.3 <sup>a</sup>	1.4±0.3 <sup>a</sup>	1.4±0.4 <sup>z</sup>	1.1±0.3 <sup>y</sup>	0.123
<i>gpx4b</i>	0.8±0.2	0.9±0.3	0.8±0.2	0.9±0.2 <sup>z</sup>	0.7±0.2 <sup>y</sup>	0.089
<i>selpa2</i>	1.1±0.3	1.0±0.4	1.1±0.2	1.0±0.3	1.1±0.3	0.605
<i>tr3a</i>	1.3±0.6	1.1±0.4	1.2±0.5	1.0±0.4 <sup>y</sup>	1.3±0.5 <sup>z</sup>	0.837
<i>tr3b</i>	1.0±0.5	0.9±0.4	1.0±0.4	1.0±0.3	0.9±0.5	0.928
<i>msrb2</i>	1.1±0.3	1.1±0.4	1.2±0.3	1.0±0.3 <sup>y</sup>	1.3±0.3 <sup>z</sup>	0.169
<i>sod1</i>	1.1±0.4	1.1±0.5	1.4±0.7	1.2±0.6	1.2±0.5	0.631
<i>sod2</i>	0.9±0.2	0.8±0.2	0.9±0.3	0.9±0.2	0.9±0.3	0.213
<i>cat</i>	1.0±0.3	0.8±0.3	1.0±0.3	0.9±0.3 <sup>y</sup>	1.1±0.3 <sup>z</sup>	0.506
<i>gr</i>	1.1±0.3	1.0±0.3	1.2±0.5	1.1±0.4	1.1±0.4	0.356
<i>gst</i>	1.2±0.4	1.1±0.4	1.1±0.4	1.1±0.3	1.2±0.4	0.736
<i>gclc</i>	1.2±0.3	1.3±0.5	1.2±0.4	1.1±0.3	1.3±0.5	0.762
<i>nrf2</i>	1.0±0.5	0.9±0.2	0.9±0.3	0.9±0.2	1.0±0.4	0.520

822 Values are means ± SD of 18 or 27 fish (two conditions per Se level or three diets per

823 condition), normalized to EF1α RNA and expressed as fold-changes of mRNA abundance

824 compared with the control group Se0-N. Within rows and for each effect: dietary Se  
825 supplementation or rearing condition, means not sharing a common superscript letter are  
826 significantly different according to two-way ANOVA.

827 **Figure captions**

828

829 Fig. 1. Se contents of rainbow trout juveniles fed the experimental diets not supplemented  
830 with Se (Se0), supplemented with sodium selenite (SeS) or Se-enriched yeast, Selsaf (SeY)  
831 and kept under normal (N) or stressful (S) conditions for 12 weeks. Bars represent means  $\pm$   
832 SD of six or nine rearing tanks (two conditions per Se level or three diets per condition).  
833 Means not sharing a common superscript letter are significantly different ( $P < 0.05$ ) according  
834 to two-way ANOVA followed by a Newman-Keuls test. The interaction between dietary Se  
835 supplementation and rearing conditions was not significant.

836

837 Fig. 2. Glutathione contents in blood of rainbow trout juveniles fed the experimental diets not  
838 supplemented with Se (Se0), supplemented with sodium selenite (SeS) or Se-enriched yeast,  
839 Selsaf (SeY) and kept under normal (N) or stressful (S) conditions for 12 weeks. Bars  
840 represent means  $\pm$  SD of 18 or 27 fish (two conditions per Se level or three diets per  
841 condition). Means not sharing a common superscript letter are significantly different ( $P <$   
842  $0.05$ ) according to two-way ANOVA followed by a Newman-Keuls test. The interaction  
843 between dietary Se supplementation and rearing conditions was not significant.

844

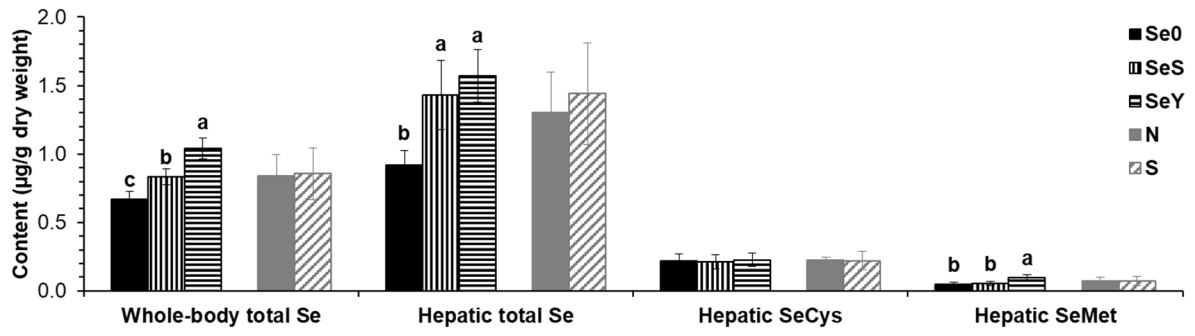
845 Fig. 3. Anisidine value (AV) in muscle of rainbow trout juveniles fed the experimental diets  
846 not supplemented with Se (Se0), supplemented with sodium selenite (SeS) or Se-enriched  
847 yeast, Selsaf (SeY) and kept under normal (N) or stressful (S) conditions for 12 weeks. Bars  
848 represent means  $\pm$  SD of 18 or 27 fish (two conditions per Se level or three diets per  
849 condition). Means not sharing a common superscript letter are significantly different ( $P <$   
850  $0.05$ ) according to two-way ANOVA followed by a Newman-Keuls test. The interaction  
851 between dietary Se supplementation and rearing conditions was not significant.

852 Fig. 4. Total, Se-dependent and non-Se dependent glutathione peroxidase activity in liver (A)  
853 and kidney (B) of rainbow trout juveniles fed the experimental diets not supplemented with  
854 Se (Se0), supplemented with sodium selenite (SeS) or Se-enriched yeast, Selsaf (SeY) and  
855 kept under normal (N) or stressful (S) conditions for 12 weeks. Bars represent means  $\pm$  SD of  
856 18 or 27 fish (two conditions per Se level or three diets per condition). Means not sharing a  
857 common superscript letter are significantly different ( $P < 0.05$ ) according to two-way  
858 ANOVA followed by a Newman-Keuls test. The interaction between dietary Se  
859 supplementation and rearing conditions was not significant.

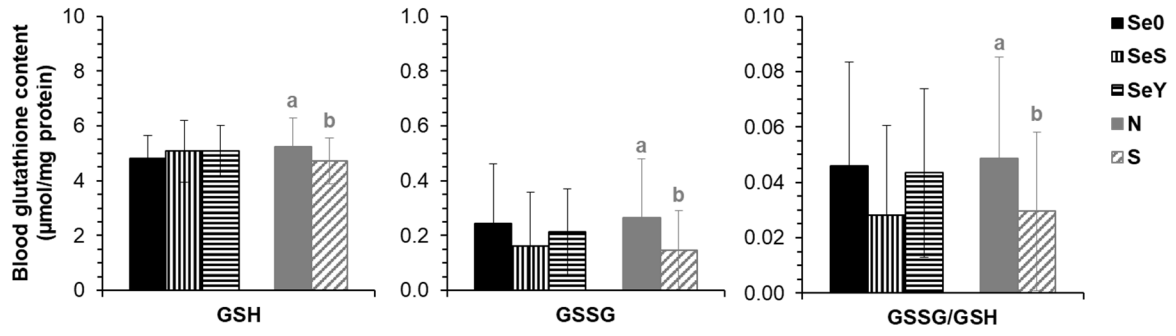
860

861 Fig. 5. Oxidative stress parameters displaying a tendency towards an interaction between  
862 dietary Se supplementation and rearing conditions in rainbow trout juveniles fed the  
863 experimental diets not supplemented with Se (Se0), supplemented with sodium selenite (SeS)  
864 or Se-enriched yeast, Selsaf (SeY) and kept under normal (N) or stressful (S) conditions for  
865 12 weeks. Means not sharing a common superscript letter are significantly different ( $P < 0.05$ )  
866 according to one-way ANOVA followed by a Newman-Keuls test.

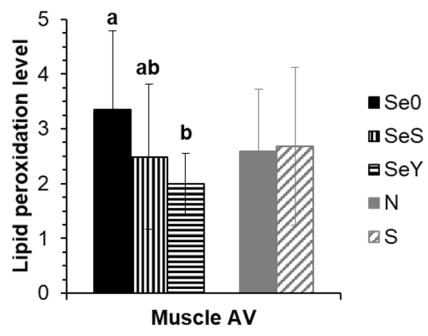




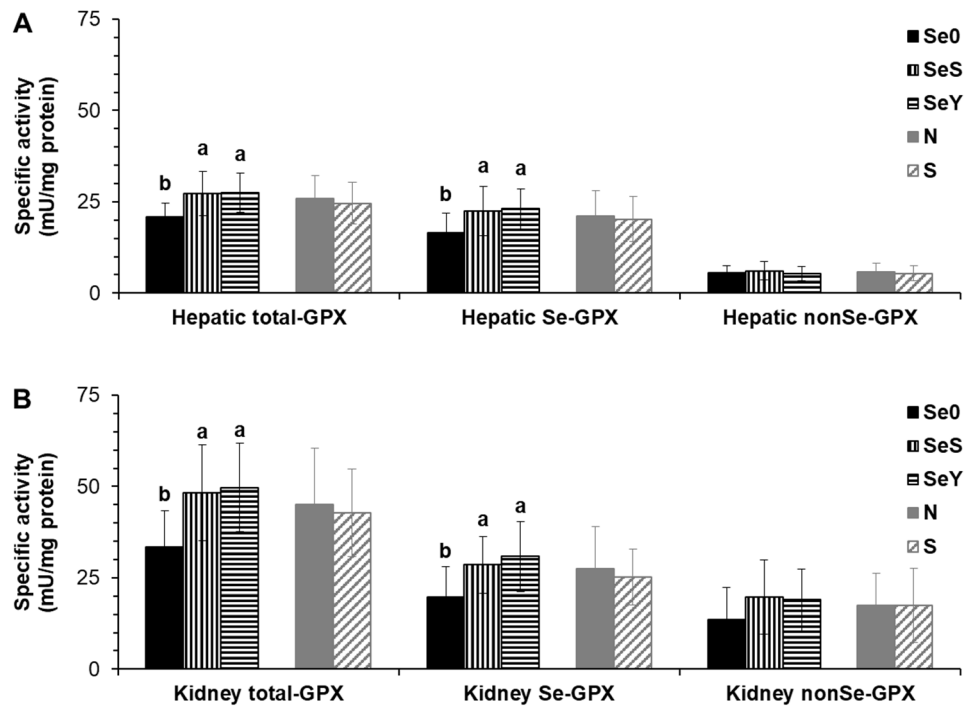
867  
868 Fig. 1.



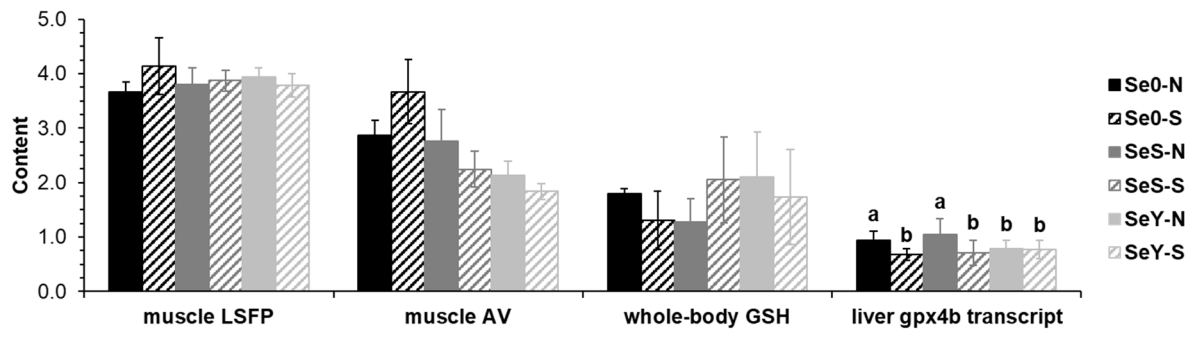
869  
870 Fig. 2.



871 Fig. 3.



872 Fig. 4.



873  
874 Fig. 5.