Effect of dietary Se in rainbow trout (*Oncorhynchus mykiss*) broodstock on antioxidant status, its parental transfer and oxidative status in the progeny

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Abstract

We studied the effects of supplementing a plant-ingredient based rainbow trout broodstock diet with different forms of selenium (Se) on reproductive performance, parental Se transfer and antioxidant metabolism in their progeny before first-feeding. Three groups of rainbow trout were fed a diet either without selenium supplementation (NC, basal Se level: 0.3 ppm) or supplemented with 0.3 ppm Se either in the form of sodium selenite (SS) or as hydroxy-selenomethionine (SO, dietary Se level: 0.7 ppm) over a 6-month period prior to spawning. In Se-supplemented groups, the total number of females spawning was significantly higher compared to the negative control group and the females fed SO began to spawn earlier compared to females fed SS or NC. Total Se concentrations were significantly higher in female muscle of SO group. Higher Se concentrations in the oocytes of both Se-supplemented groups confirmed a maternal transfer of Se, while total Se concentrations in

milt samples were not significantly different between dietary groups. There was no effect on glutathione peroxidase (GPX) or other antioxidant enzyme activity in the liver of female or male broodstock, whereas in the swim-up fry, GPX activity was significantly higher in both Se-supplemented treatments with the highest activity in the SO group. Se-supplementation enhanced the expression of hepatic SelPa in broodstock males and females along with MsrB1, GPx1a, GPx4a2, CAT, Gclc, Keap1 and MsrB2 in male liver. In swim-up fry, only organic Se-supplementation led to a higher gene expression for SelPa, GPX1a, GPX1b2, CAT and MsrB2. An improvement of the oxidative status in whole-body of swim-up fry could not be confirmed in this study as the GSH/GSSG was even lower in swim-up fry of Se-supplemented groups, whereas the 8-isoprostane levels were lower in the SS group and no effect on protein carbonyl levels could be detected. Organic Se-supplementation led to a significant increase in the α-tocopherol and vitamin C levels in the progeny. These results show that dietary Se in broodstock nutrition influences spawning occurrence and along with the parental transfer, several traits in the progeny are affected.

Keywords:

Rainbow trout; Broodstock; Progeny; Selenium; Antioxidant

1. Introduction

While fishmeal based diets naturally contain high amounts of selenium (Se) covering the nutritional requirements (Hilton et al., 1980), plant based diets are generally lower and vary more widely in their Se content (Combs, 2001). With the ongoing replacement of fish meal and fish oil in aquafeeds, there is a need to better understand the metabolic role of Se, which is complex and not yet fully explored. The Se requirements in fish are reported to vary between 0.15 and 0.70 mg/kg diet (Antony Jesu Prabhu et al., 2016), depending on species (Watanabe et al., 1997), life stage (Bell et al., 1985) and bioavailability of the Se form (Wang and Lovell, 1997; Penglase et al., 2014a). Thereby, organic Se forms have been described to have a higher bioavailability compared to inorganic forms, leading to increased tissue Se content (Le and Fotedar, 2014; Fontagné-Dicharry et al., 2015; Antony Jesu Prabhu et al., 2016). Dietary Se, both in surplus and deficiency, can have severe consequences for the fish. Already a dietary Se level of 3 mg/kg, slightly above the recommended level, can in the long term induce adverse effects (Berntssen et al., 2018). On the other hand, Se deficiency can reduce the expression and activity of selenoproteins (Hesketh, 2008; Elia et al., 2011; Penglase et al., 2014a), of which many are involved in the antioxidant protection of the cell (Papp et al., 2007). In a previous study, working with low Se levels in rainbow trout (*Oncorhynchus mykiss*) fry, the positive impact of Se supplementation on Se-dependent GPX activity and gene expression was demonstrated, especially when an organic Se supplement was used (Fontagné-Dicharry et al., 2015). Se supplementation was found to increase the expression of selenoprotein P not only in rainbow trout, but also in zebrafish (*Danio rerio*) (Penglase et al., 2014a; Fontagné-Dicharry et al., 2015; Pacitti et al., 2015; Wang et al., 2018). Under a dietary Se deficiency, besides the effects at the cellular level, a reduced growth and an increased mortality in fish are also reported (Wang et al., 2013). In rainbow trout, the negative impact of Se deficiency on body weight could not be detected (Bell et al., 1986), but liver vitamin E levels were significantly reduced and the combined effect of vitamin E and Se deficiency led to a reduced weight gain (Bell et al., 1985). Most of the studies investigating the effect of dietary Se on the metabolism in fish have been

undertaken with juveniles or early stages and there is little information on the effects of Se in broodstock nutrition, except studies investigating the impact of Se-supplementation at high levels (Hardy et al., 2010; Penglase et al., 2014a, 2014b). However, the reproduction period is substantially different to other life stages as energy is not used for growth, but mobilized to the reproductive organs; especially in females where nutrients are accumulated in the ovarian oocytes during vitellogenesis. Therefore, Se deficiency effects observed at early stages might be different in broodstock fish and might affect spawn quality. Male fertility and reproduction have been shown to be reduced in several animal species fed Se-deficient diets (Hosnedlova et al., 2017) and could be linked to the presence of the selenoprotein GPx4 in male sperm (Imai et al., 2009). A reduced antioxidant capacity of offspring by maternal Se deficiency have already been described in broilers (Pappas et al., 2006; Zhang et al., 2014; Xiao et al., 2016). Furthermore, the dietary supplementation of Se in poultry breeders led to an increased fertility of males and maternal transfer of Se, improving the oxidative status in the progeny (Słowińska et al., 2011, Wang et al., 2011; Zhang et al., 2014). Se toxicity trials undertaken with zebrafish (Thomas and Janz, 2015) and brown trout (*Salmo trutta*) (Covington et al., 2018) suggest that a parental transfer of Se in fish exists at toxic levels. The current study was undertaken to address whether similar effects are detected in broodstock fish fed nutritionally relevant dietary levels of Se in different forms.

2. Material and Methods

2.1. Experimental set up

Three-year-old rainbow trout (*Oncorhynchus mykiss*) females and males of the same genetic origin from the INRA experimental fish farm of Lées-Athas (Pyrénées-Atlantiques, France) were used as broodstock. Rainbow trout broodstock were randomly allocated to three circular 2-m diameter tanks supplied with flow-through (1L per sec) spring water at 8 ± 1 °C with 25 females (initial mean weight: 1.1 ± 0.2 kg, second reproduction season) and 15 males (0.9 ± 0.3 kg) per tank (stocking density: 40 kg/m^3). The fish were hand fed once a day to visual satiation for a period of 6 months prior to spawning. Each fish was tagged in

the dorsal muscle with a passive integrated transponder (PIT, 12 x 2 mm, ISO 11784/11785, IER, Suresnes, France) and the individual growth was followed until spawning. During the spawning period, fish were regularly checked for ovulation and at spawning, oocytes of each female were collected through stripping. At the first spawning date, one female from the SS-group and two females from the SO-group were over-mature (with oocytes exhibiting polar condensation of vitellus and lipid goblets) and they were excluded from the trial as well as one NC female during the second spawning date due to low spawning quantity and quality. Otherwise, two pools of 50 oocytes from each female were lined up to measure total length and afterwards weighed to calculate the average diameter and weight of oocytes, respectively. All other eggs were fertilized with sperm collected on the same day from a pool of males of the same dietary group. Eggs from each female were reared separately in small trays supplied with flow-through spring water at 8 ± 1 °C to follow individual hatching data. The embryonic development was followed until swim-up fry stage. The survival was monitored and dead eggs were removed every two days. Survival rates were calculated at eyed stage (33 dpf), hatching (46 dpf) and swim-up stage (65 dpf).

2.2. Sample collection

Broodstock fish were sampled at spawning. First, fish were anaesthetized with benzocaine (30 mg/L) for stripping and afterwards killed by a sharp blow on the head for liver and muscle sampling. Muscle tissue was sampled in duplicate at the dorsal fin as approximately 15g of fillet. The weights of whole fish, spawn (total eggs collected from each female after stripping and removal of coelomic fluid) and liver were recorded to calculate absolute and relative fecundity (number of eggs/female and number of eggs/kg female, respectively), gonado-somatic index (GSI, percentage of spawn weight to weight of female) and hepatosomatic index (HSI, percentage of liver weight to weight of fish). Tissue samples and oocytes (2 x 50 units per female) were immediately frozen in liquid nitrogen and stored at -80 °C until further analysis. The progeny was sampled at swim-up fry stage. Pooled fry, for every female separately, were euthanized with an overdose of benzocaine (60 mg/L), weighed and

afterwards subdivided in different sample size according to planned analysis, before freezing as whole-body fry in liquid nitrogen and stored at -80°C until further analysis.

All procedures were performed in compliance with the European Directive 2010/63/EU for the protection of animals used for scientific purposes and the French Decree no. 2013-118 for animal experimentation.

2.3. Experimental diets

Diets were manufactured using a twin-screw extruder (BC 45, Clextral, Firminy, France) at the INRA experimental facilities in Donzacq (Landes, France). The three diets (NC, SS and SO) were based on plant-derived proteins and had similar levels of crude protein (50%) and total lipid (22%) and differed only in Se content (Table 1). The NC-diet containing 0.3 mg Se/kg feed was not supplemented with Se and served as negative control. The SS and SO diets were supplemented with either sodium selenite (SS-diet) or hydroxy-selenomethionine (Selisseo®, SO-diet). In both supplemented diets, the targeted Se concentration was 0.6 mg Se/kg, which remained lower to concentrations usually found in fish meal based diets (Fontagné-Dicharry et al., 2015), but feed analyses gave a final dietary Se content of 0.8 mg Se/kg in SS diet and 0.7 mg Se/kg in SO diet.

2.4. Proximate, mineral and fatty acid analysis

For proximate composition of diets, dry matter (DM) was determined after drying at 105 °C for 24h, protein (N × 6.25) by Kjeldahl method after acid digestion (ISO, 1984), ash after 10h incineration at 550 °C and gross energy in an adiabatic bomb calorimeter. Total lipid in diets and broodstock muscle was extracted and quantified gravimetrically following Folch et al. (1957), using dichloromethane instead of chloroform. Fatty acid methyl esters were prepared and analyzed as previously described (Fontagné et al., 2008). Total phosphorus was determined by the molybdate blue/ascorbic acid method at 820 nm after mineralization and acid digestion (AFNOR, 1992). Total Se was measured using inductively coupled plasma mass spectrometry (ICP-MS, Agilent series 7500cx) by Ultra Trace Analyses Aquitaine

(UT2A, Pau, France) for diets, broodstock tissues and oocytes according to Vacchina and Dumont (2018) and by the Institut des Sciences Analytiques et de Physico-Chimie pour l'Environnement et les Matériaux (IPREM, Pau, France) for swim-up fry as described by Godin et al. (2015). Selenomethionine (SeMet) and selenocysteine (SeCys) were analyzed by liquid chromatography (HPLC, Agilent series 1200) coupled to ICP-MS by UT2A for oocytes according to Vacchina et al. (2018) and by IPREM for swim-up fry according to Godin et al. (2015).

2.5. Antioxidant enzyme activity and mRNA levels

Antioxidant enzyme activity was measured as previously described by Fontagné-Dicharry et al. (2014) in 0.3 g samples of broodstock liver and pooled whole-body fry. Briefly, all samples were first homogenized with an Ultra-turrax in an eight-time dilution of 20 mM-phosphate buffer (pH 7.4) containing 1mM EDTA, afterwards centrifuged at 2000g for 10 min, the supernatant diluted in 0.5% triton solution and incubated for one hour before using the different enzyme assays. The glutathione peroxidase (GPX, EC 1.11.1.9) activity was measured in a solution of 50 mM phosphate buffer (pH 7.4), 1 mM EDTA, 2 mM sodium azide, 2 mM GSH, 0.1 mM NADPH and 0.2 mM glutathione reductase (GR) following the reduction of cumene hydroperoxide (0.2 mM) for Se-dependent GPX (SeGPX) and H_2O_2 (50 µM) for total GPX (totGPX) at 30 °C and 340 nm. Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by monitoring the inhibition of nitrotetrazolium reduction at 550 nm, and was expressed as the amount of enzyme required to inhibit the rate of nitrotetrazolium reduction by 50% per mg protein at 37 °C. The catalase (CAT, EC 1.11.1.6) activity was measured by following the reduction of H_2O_2 in a solution of 67 mM phosphate buffer, 1 mM EDTA and 20 mM H_2O_2 (pH 7.4) at 30 °C and 240 nm. GR (EC 1.8.1.7) activity was determined measuring the reduction of NADPH in a solution containing 100 mM phosphate buffer (pH 7.5), 1 mM GSSG and 50 µM NADPH at 30 °C and 340 nm. Glutathione-S-transferase (GST, EC 2.5.1.18) activity was measured in a solution of 100 mM phosphate buffer (pH 6.5), 1 mM EDTA, 1 mM GSH and 1 mM CDNB at 30°C and 340nm, using the

extinction coefficient of 9.61 mM^{-1}/cm. One unit of enzyme is defined as the amount needed to catalyze the reaction of 1 µM substrate per minute. The protein concentration was determined according to Lowry et al. (1951), using bovine serum albumin (BSA) as a standard.

Total RNA was isolated from 100 mg samples of broodstock liver and a pool of three whole-body swim-up fry by using Trizol reagent (Invitrogen, Cergy-Pontoise, France). For quantitative RT-PCR, complementary DNA was generated from 1 mg total RNA using SuperScript III RT (Invitrogen) and a mix of oligo (dT)15 and random primers (Promega, Charbonnières, France). For each sample, RT was performed in duplicate and quantitative PCR analyses were performed in LightCycler® 480 Instrument II (Roche Diagnostics, Meylan, France) using LightCycler® 480 SYBR Green I Master mix (Roche Diagnostics). Total reaction volume was 6 µL, with 2 µL of the diluted RT reaction mixture (dilution 40) and 4 µL of master mix added with 0.4 mM of each primer (Table 2). The PCR protocol was initiated at 95 °C for 10 min for initial denaturation of the cDNA and hot-start Taq-polymerase activation, followed by 45 cycles of a three-step amplification program (15 s at 95 °C, 10 s at the melting temperature of 60 °C, 4.8 s at 72 °C). Melting curves were systematically monitored (5 s at 95 °C, 1 min at 65 °C, temperature slope at 0.11 °C/s from 65 to 97 °C) at the end of the last amplification cycle to confirm the specificity of the amplification reaction. Relative quantification of target gene transcripts was performed using β-actin as the reference gene and NC as the reference group using the ΔΔCt method (Pfaffl, 2001).

2.6. Glutathione and oxidative status in the progeny

Total (GSHt), oxidized (GSSG) and reduced glutathione (GSH) were measured in 0.3 g homogenized liver tissues and 1 g of pooled oocyte and fry samples using Cayman glutathione assay kit (Bertin Pharma, Montigny-le-Bretonneux, France) according to the manufacturer's instructions with protein concentration assessed by the method of Lowry et al. (1951) using BSA as a standard. Lipid peroxidation was assessed by measuring concentration of 8-isoprostanes (8-isoPT), the product of non-enzymatic peroxidation of

arachidonic acid by reactive oxygen species in 0.5 g of pooled fry as described by Fontagné et al. (2006) using Cayman Chemical 8-isoprostane enzyme immunoassay kit (Spi-Bio, Massy, France) according to the manufacturer's instructions. Protein oxidation was assessed using the spectrophotometric determination of protein carbonyls by derivatization with 2,4-dinitrophenylhydrazine (DNPH) at 370 nm according to Armenteros et al. (2009) with protein concentration calculated from absorption at 280 nm in the blank sample using bovine serum albumin (BSA) as standard. HPLC was used for determination of vitamin C according to the method described by Mæland and Waagbø (1998). Tocopherols were extracted from a pool of six oocytes and three whole-body fry according to Folch et al. (1957) using dichloromethane instead of chloroform. They were analyzed by HPLC on a Phenomenex Luna® PFP(2) column (100 Å, 3 µm, 100 x 2 mm), with an isocratic mobile phase of methanol/water (93:7 v/v) at a flow rate of 0.5 mL/min and column oven temperature of 40 °C using a Waters Alliance 2695 separation module with a Waters 2475 multi wavelength fluorescence detector set at 295/330 nm (excitation/emission) according to Górnaś et al. (2014). The concentration of tocopherol per individual was calculated using the average weight of oocyte or whole-body fry.

2.7. Statistical analysis

Results are given as mean ± standard error (SEM). Differences between dietary groups were analyzed in statistical software R (R Development Core Team, 2008) or SigmaStat 3 (SPSS, Chicago, IL, USA) using one-way ANOVA. Tocopherol data were analyzed using two-way ANOVA to compare data from oocytes with data at swim-up fry stage. All data were tested for normal distribution and homogeneity and only in case the assumptions were not respected, the ANOVA was performed instead on ranks. The binary spawning data were analyzed pairwise using χ^2 test. Percentages were arc-sin transformed before analysis. A Newman-Keuls post-hoc test was used to compare means, when a significant difference ($P < 0.05$) was found.

3. Results

3.1. Growth and reproductive performance in broodstock

All three diets were readily accepted by rainbow trout broodstock. In males and females, no significant difference in initial or final body weight was detected between the three dietary groups (Table 3). In females, no significant difference in daily growth between the control group and the supplemented groups was found. However, the daily growth index was higher in females fed SO compared to SS. In the groups NC and SS, six males and in SO, one female and four males died during the feeding trial before spawning. HSI, muscle lipid content and muscle fatty acid profile (data not shown) were not significantly different between groups for both males and females. At the end of the spawning period in January, the total number of fish spawning was significantly higher in the two Se-supplemented groups SS and SO compared to the control group NC. Also, females fed the organic Se-supplemented diet SO began to spawn significantly earlier compared to females fed the inorganic Se-supplemented diet SS (Fig. 1). No significant difference in reproductive performance, including GSI, fecundity or egg size could be detected (Table 3). During embryonic development, there was no significant difference in survival between NC and the supplemented groups. However, the survival rate was significantly lower in the SS-group than in SO from hatching onwards.

3.2. Se and its transfer from broodstock to progeny

Total Se content was significantly higher in muscle of females fed SO, compared to the ones fed NC or SS (Fig. 2A). The same tendency was noticed in female liver, but no significant difference between the three treatments was detected (Fig. 2B). Total Se content in milt was not significantly different between groups (Fig. 2C). On the other hand, total Se content in oocytes was significantly higher for females fed SO and lower in NC group compared to the SS treatment (Fig. 2D).

The majority of Se in oocytes was present in the form of SeMet, which was significantly higher in Se-supplemented groups compared to the control group NC with the highest

concentration in the organic Se treatment, while the SeCys content was not significantly different between the groups (Table 4). As for oocytes, the total Se content in swim-up fry before first-feeding was the lowest in NC, followed by SS and highest in SO (Fig. 2E). However, with smaller sample size for Se speciation analyses (n = 3), no significant difference between the groups SS and SO were found (Table 4). SeCys content was not significantly different between the three dietary groups of swim-up fry. Only swim-up fry from the organic Se-supplemented group SO showed an increase in SeMet concentration compared to the two other groups (Table 4). The total Se content per individual decreased significantly from oocyte to swim-up fry stage in SS (18 ± 2 to 15 ± 1 ng Se per individual) and SO (29 ± 3 to 23 ± 1 ng Se per individual) groups whereas no significant change in Se content was noticed for NC (11 ± 1 ng Se per individual).

3.3. Changes in selenoproteins and other antioxidant enzyme activity and mRNA levels in broodstock and progeny

There was no effect of broodstock nutrition on GPX (Fig. 3A and 3B) or other antioxidant enzyme activity in liver of female or male (Table 5). In whole-body swim-up fry, totGPX activity was significantly higher in both Se-supplemented treatments with the highest activity in the SO group (Fig. 3C). This was associated with higher SeGPX activity in SO-group compared to the two other groups. The activity of other antioxidant enzymes was not significantly affected by Se supplementation (Table 5).

The mRNA expression of selenoprotein genes including the different GPX genes was not significantly affected by dietary Se supplementation in broodstock female liver except for SelPa and SelPa2 that were higher expressed in liver of broodstock females fed the inorganic Se-supplemented diet SS compared to the two other groups (Fig. 4A). The other antioxidant genes not coding for selenoproteins were not significantly affected by dietary Se supplementation in female liver (Fig. 4B). In male liver, all the three selenoproteins P were found to be higher expressed in the Se-supplemented treatments SS and SO while GPx1a was significantly higher expressed only in the inorganic Se treatment SS and MsrB1 only in

the organic Se treatment SO (Fig. 5A). GPX4a2 mRNA levels were significantly higher in liver of broodstock male fed the organic Se-supplemented diet SO than in the inorganic Se-supplemented group SS. Regarding non-selenoprotein genes, CAT, Gclc and MsrB2 mRNA levels were significantly higher in liver of broodstock males fed the two Se-supplemented groups while Keap1 mRNA levels were only higher in male livers from the inorganic Se supplemented treatment SS compared to the control group NC (Fig. 5B). In whole-body swim-up fry, gene expression of the SO group with organic Se supplementation was enhanced not only for the two selenoproteins Pa and Pa2, but also for the glutathione peroxidases GPx1a and GPx1b2 (Fig. 6A). For other non-selenoproteins involved in antioxidant protection, no significant difference in mRNA levels could be detected between the swim-up fry groups except for CAT and MsrB2 that were higher expressed in the organic Se-supplemented group SO (Fig. 6B).

3.4. Glutathione levels in rainbow trout broodstock and offspring

GSHt, GSSG and GSH contents in male or female liver were not significantly different between the three dietary groups (Fig. 7A and 7B). In whole-body swim-up fry, GSH was significantly lower in the organic Se-supplemented group SO, while GSSG was higher in both Se-supplemented groups compared to the control group NC (Fig. 7C). Glutathione content was also measured in oocytes, but GSSG was not detectable, while no significant difference in total glutathione was detected between the three dietary groups of oocytes (0.68 ± 0.04 µmol/mg protein).

3.5. Oxidative status in rainbow trout offspring

At whole-body swim-up fry stage, the GSH/GSSG ratio was significantly lower in the offspring from broodstock fed the Se-supplemented diets SS and SO compared to the control group NC (Table 6). The concentration of 8-isoprostanes was significantly lower in whole-body swim-up fry in the inorganic Se treatment SS compared to the control group NC and organic Se-supplemented group SO. Parental Se-supplementation had no significant

effect on the concentration of protein carbonyls in whole-body swim-up fry. However, there was a tendency for lower protein carbonyl content in the organic Se treatment SO, indicative of an improved antioxidant status. This was confirmed with vitamin C that displayed significantly higher amounts in whole-body swim-up fry from the organic Se-supplemented SO treatment compared to the two other groups.

3.6. Tocopherol content in rainbow trout offspring

Organic Se supplementation in rainbow trout broodstock diet significantly increased the α-tocopherol concentration per individual in the offspring. The concentrations of γ- and δ-tocopherols were not significantly affected by dietary broodstock Se supplementation. A significant decrease between oocyte and swim-up fry stage was noticed for δ-tocopherol (Table 7).

4. Discussion

The recent edition of NRC (2011) stated that micronutrient requirements for farmed fish and shrimp are another area where knowledge is lacking; whether dietary micronutrient requirements should be based on minimal levels required to prevent clinical deficiency signs or impaired growth or based on other criteria is an open question. Studies on the determination of nutrient requirements should rely on more than one response criterion to test the effect of increasing levels of a nutrient and eventually estimate minimal dietary inclusion levels for each criterion tested, whenever possible.

4.1. Organic and inorganic Se supplementation affects spawning rate and timing

We did not find any positive effect of Se-supplementation to a plant-ingredient based diet on the growth performance of rainbow trout broodstock. It could indicate that the Se levels used in the control diet did not provoke a severe deficiency situation, as was also shown, in an earlier study with rainbow trout, that a Se level of 0.025 mg Se/kg did not significantly affect growth rates (Bell et al., 1986). On the other hand, the growth performance in all treatments

of this experiment was low, which can be attributed in part to low water temperatures and the use of plant-based diets or it might be related to the increased demand of energy in reproductive organs during spawning period. These factors might have masked the impact of Se-supplementation on broodstock growth. We did not also find any significant effect of the form of Se-supplementation on reproductive performance parameters, including absolute and relative fecundity, GSI and egg size and weight. These results are in line with observations in zebrafish, where no significant difference in number of eggs and mating success could be found between groups fed deficient (0.09 mg Se/kg) vs. replete (0.65 mg Se /kg) diets (Penglase et al., 2014a). Also, similar to the mentioned zebrafish trial, the survival of the progeny was not significantly different between the control group and fish fed Se supplemented diets, however we found a slightly lower survival in the inorganic supplemented group compared to the control group. But even without a detectable effect on growth, the number of females spawning was significantly higher in both Se-supplemented groups and females fed organic Se in form of hydroxy-selenomethionine spawned significantly earlier compared to females receiving the sodium selenite diet. The low survival in progeny of selenite group can be traced back to given ascending females, spawning at the early spawning dates, while progeny of the majority of females in this group did not show any elevated mortality. The differences in spawning pattern and survival between the groups receiving different forms of dietary Se supplementation could be connected to modified vitellogenesis and blood steroid concentrations as these parameters were described to be affected in rainbow trout females fed dietary SeMet at a high level of 4.54 mg/kg (Wiseman et al., 2011). It might have been interesting to also analyze plasma vitellogenin levels in the present study.

The results indicate that a plant-protein based diet with a basal Se level of 0.3 ppm was not sufficiently deficient to affect growth of rainbow trout broodstock, but might negatively affect reproduction by decreasing the number of females spawning, which can be improved by supplementing Se, especially as organic form.

4.2. Effective Se transfer from broodstock to progeny

Tissue analysis revealed elevated female liver and muscle tissue Se concentrations in supplemented groups, compared to the control group, and the concentration in fish fed diets with organic Se supplementation were even higher compared to fish fed inorganic Se diets. The increase of Se in muscle tissue of fish fed Se-supplemented diets was also recently reported in Atlantic salmon (*Salmo salar*), finding higher Se retention for organic Se supplementation in the form of SeMet compared to inorganic supplementation (Sele et al., 2018). The higher retention for organic Se (SeMet), compared to inorganic Se (selenite) was previously described also in rainbow trout fry (Rider et al., 2009; Godin et al., 2015). However, this effect is not systematic, as in some other studies, no effect on tissue Se accumulation was described, when working with similar amounts of Se as studied here (Han et al., 2011). We found elevated Se concentrations in the progeny at oocyte and swim-up fry stage before first feeding in Se-supplemented groups with higher transfer in the group supplemented with hydroxy-selenomethionine. These results are in line with results obtained in hens that describe the parental transfer of Se to the progeny (Pappas et al., 2006; Wang et al., 2011). The Se speciation in oocytes and swim-up fry could show that the SeMet can be found in tissues in higher concentrations compared to SeCys. Thereby, Se supplementation increased the SeMet levels, while SeCys was not affected. The elevated levels of SeMet in oocytes of the selenite treatment are surprising, especially as they are not found any more at swim-up fry stage. Indeed selenite in the Se metabolism is known to be metabolized to selenide and not transformed to SeMet (Burk and Hill, 2015). The elevated status of SeMet in the oocytes of the inorganic Se supplemented group found in this study might be linked to a transfer of SeMet from broodstock to the progeny, a process which could have been limited in the control group compared to the Se-supplemented groups. In a study with rainbow trout fry fed a diet supplemented with sodium selenite or SeMet, SeMet was found to be higher in the SeMet treatment compared to control group and selenite treatment, whereas in comparison to SeMet, the SeCys concentration was not affected by the supplementation at all (Godin et al., 2015). However, there were huge differences in the

amount of Se identified as SeMet and SeCys between the two tissues analyzed in this study, which could be also connected to different extraction methods used. In this case, the absolute concentration values should be considered carefully and preferably trends at each stage regarded. However, it cannot be excluded that other seleno-components are present in fry as other Se-containing peaks were observed in chromatograms.

These results showed that Se-supplementation to diets with low basal levels could increase the tissue Se concentration and that there was a transfer of Se from the broodstock to the progeny. The bioavailability and transfer of Se was stronger in broodstock fed hydroxy-selenomethionine compared to selenite.

4.3. An altered antioxidant metabolism in deficient Se broodstock and offspring compensated by Se supplementation especially in organic form

In animals, selenoprotein P is a selenoprotein often found to be regulated by Se level (Yuan et al., 2013; Penglase et al., 2014a; Fontagné-Dicharry et al., 2015) and this was confirmed in the present study where the SelPa expression was significantly lower in the control group. In hepatic tissue of both males and females, the strongest effect was found in the group fed inorganic Se supplemented diets, while in the whole-body swim-up fry, the effect is detectable only in the organic Se supplemented groups. In humans, Steinbrenner et al. (2006) found that hepatocyte-derived selenoprotein P concentrations can stimulate the GPX1 expression and activity in astrocytes. Similar to selenoprotein P, GPX1 mRNA levels and activity are often described to decrease in mammals under Se deficiency (Chow and Tappel, 1974; Sunde et al., 2009) and have been found to be a good biomarker of Se status in fish (Bell et al., 1985; Penglase et al., 2014a; Fontagné-Dicharry et al., 2015; Pacitti et al., 2015). Likewise, the present results showed a lower GPX1a expression in male liver of the control group, compared to the inorganic Se treatment, but there was no effect in female liver or GPX activity in broodstock male or female liver. In whole-body swim-up fry, GPX1a1 and GPX1b2 expression, in correlation with GPX activity was increased in fry coming from broodstock fed the organic Se supplemented diet. In male liver, also GPX4a2 was influenced

by dietary Se supplementation with significantly higher levels in the organic Se supplemented treatment, compared to the group fed inorganic Se, while in a study with rainbow trout fry GPX4a1 was found to be the most sensitive isoform in terms of low Se levels (Fontagné-Dicharry et al., 2015). Se deficiency was described to lead to decreased levels of MsrB protein activity and mRNA expression in various tissues (Moskovitz and Stadtman, 2003). In the present study, the selenoprotein MrsB1 was higher expressed only in male liver of the group fed organic Se-supplemented diets, while the non-selenoprotein MsrB2 was regulated by dietary Se not only in male liver of both supplemented groups, but also in the progeny of the group fed organic selenium. In comparison to mice, where the selenoprotein MsrB1 is the most dominant Msr in liver and affected by dietary Se levels (Novoselov et al., 2010), these results in rainbow trout could indicate a different MsrB hierarchy in fish as MsrB2 was more sensitive to low dietary Se levels than MsrB1.

These results showed that parental Se nutrition, especially supplementation with hydroxy-selenomethionine as an organic source of Se, can enhance not only the expression of proteins involved in antioxidant defense, such as SelP, GPX and MsrB2, but can also increase the activity of GPX in the progeny, while the expression of some hepatic proteins in the broodstock was more strongly affected even by a supplementation with selenite.

4.4. Contrasted oxidative status in progeny related to Se broodstock nutrition

As an integral part of several glutathione peroxidases, Se plays an important role in metabolizing hydrogen peroxides and lipid hydroperoxides into water and lipid alcohols (Brigelius-Flohé, 1999). This function is complementary to that of vitamin E as a lipid-soluble antioxidant and both are functioning in close link (Hamre, 2011). It has been shown that a combined deficiency of vitamin E and Se can lead to severe consequences, including suppressed growth, muscular dystrophy, exudative diathesis and death in fish (Poston et al., 1976; Gatlin et al., 1986; Watanabe et al., 1997), while Se-deficiency symptoms appear less severe, if adequate levels of vitamin E are provided (Bell et al., 1985, 1986). In the present study, vitamin E requirements were assumed to be fulfilled as per the NRC (2011) with more

than 50 mg tocopherol per kg diet. As organic Se supplementation led to an increase in α-tocopherol levels in the progeny, this possibly indicates a Se deficiency or reduced Se availability in the control group and in the group receiving sodium selenite. Indeed, Se-deficiency has been reported to cause a reduction of vitamin E levels (Hamre, 2011). These results are in line with an earlier study in hens, describing significant increase in yolk vitamin E concentration by the supplementation of hydroxy-selenomethionine (Tufarelli et al., 2016). Elevated vitamin E and Se levels in the progeny of supplemented groups thus should provide them a better protection against oxidative damage. This was supported also by an elevated vitamin C level in organic Se-supplemented group in this study, as was also reported by Kumar et al. (2018) in striped catfish. However, protein carbonyls as an indicator of protein oxidation were not significantly affected by Se-supplementation in the present study, even though there was a tendency for lower protein carbonyl levels in the group with organic Se-supplementation. An effect might have been found at the single tissue level as in mice, where fewer protein carbonyls were found in isolated liver, kidney, cerebrum and cerebellum, when fed higher Se levels (Moskovitz and Stadtman, 2003). A closer look on 8-isoprostane levels showed a decrease only in the group with sodium selenite supplementation. This result is surprising as Küçükbay et al. (2009) found that, both inorganic and organic Se supplementation led to a linear decrease of both serum 8-isoprostane and malondialdehyde (MDA) levels. Similarly, in common carp, selenite supplementation did not even affect MDA levels, which were only decreased by feeding SeMet or nano-Se (Saffari et al., 2017). As 8-Isoprostanes are products of fatty acid (arachidonic acid) peroxidation, a more indirect effect of Se could be suspected in the present study as dietary Se has been shown to affect fatty acid composition in a previous study (Fontagné-Dicharry et al., 2015). Also, the GSH/GSSG ratio in the whole-body swim-up fry was found to be lower in supplemented groups compared to the control group, which is accompanied by an absence of effect on the mRNA levels of both glutamate-cysteine ligase catalytic subunit (Gclc), needed for the synthesis of glutathione, and glutathione reductase (GR), needed for the reduction of glutathione, while the GPX activity was higher in

supplemented groups. In more developed fry fed with different Se level, not only the GPX activity, but also the GSH/GSSG ratio was higher in groups fed Se-supplemented diets (Fontagné-Dicharry et al., 2015). The different results might be connected to differences in the glutathione metabolism, which seems to be boosting at swim-up fry stage as levels of total glutathione increased about ten times compared to oocytes and oxidized glutathione levels only became detectable at swim-up fry stage in this study. The indirect effect of parental feeding on glutathione status in the progeny will need further investigation.

The absence of significant effects on some markers of oxidative status, especially linked to glutathione metabolism, may indicate that even with the clear difference in Se transfer from broodstock to the progeny, the Se deficiency in the non-supplemented group was not critical enough to provoke a strong oxidative stress. Otherwise, the Se deficiency in broodstock might induce other metabolic effects leading to changes in oxidative status such as modification of one-carbon metabolism as suggested by Speckmann et al. (2017) in mice.

5. Conclusion

The present results demonstrate that dietary Se-supplementation in plant-ingredient based diets with low basal selenium levels can enhance the total number of females spawning, while hydroxy-selenomethionine supplementation even led to an earlier spawning. It was demonstrated for the first time a parental transfer of Se in the progeny with the higher effect observed with the better bioavailability of organic Se. In the progeny before first feeding, the high Se levels led to an increase in the activity and mRNA expression of GPX and other proteins involved in antioxidant protection, along with elevated vitamin E and C levels. The impact of Se broodstock nutrition on glutathione metabolism in the progeny is intriguing and requires further investigation. Further work with juvenile fish at later developmental stages is warranted to show, if the parental effects persist after exogenous feeding of the progeny.

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Table 1. Formulation and composition of experimental diets (g/100g dry weight).

Diet	**NC**	**SS**	**SO**
Ingredients			
Plant meals[1]	74	74	74
Crystalline amino acids and attractant mixture[2]	3.14	3.14	3.14
Soybean lecithin[3]	2	2	2
Fish oil[3]	8	8	8
Vegetable oils[4]	8	8	8
Carophyll® pink[5]	0.04	0.04	0.04
Vitamin and mineral mixture without Se[6]	4.82	4.82	4.82
Sodium selenite (µg/g diet)[7]	-	0.7	-
Selisseo® (µg/g diet)[7]	-	-	15
Analytical composition			
Dry matter (DM, %)	96	98	97
Crude protein (% DM)	49	50	50
Total lipid (% DM)	23	22	23
Gross energy (kJ/g DM)	25	25	25
Ash (% DM)	6	6	6
Phosphorus (% DM)	1.2	1.1	1.2
Se (mg/kg)	0.3	0.8	0.7
Fatty acid profile (% total fatty acids)			
14:0	5.1	4.6	5.0
16:0	25.5	24.4	25.6
18:0	3.1	3.2	3.3
Total saturates[8]	35.1	33.6	35.3
16:1	3.8	3.5	3.7
18:1	28.0	28.8	27.5
Total monoenes[8]	32.5	33.0	31.8
18:2 n-6	16.7	17.3	17.0
20:4 n-6	0.5	0.4	0.5
Total n-6	17.2	17.7	17.4
18:3 n-3	9.9	10.1	9.7
18:4 n-3	0.6	0.5	0.5
20:5 n-3	2.3	2.1	2.2
22:6 n-3	1.5	1.8	2.1
Total n-3	14.3	14.6	14.6

[1]Plant meals (% diet): 20% wheat gluten (Roquette), 18% corn gluten meal (Inzo), 15% soybean protein concentrate Estril®75 (Sopropêche), 6% soybean meal (Sud-Ouest Aliment), 5% rapeseed meal 00 (Sud-Ouest Aliment), 5% white lupin meal Farilup 500 (Terrena), 3% dehulled pea meal Primatex (Sotexpro), 2% whole wheat (Sud-Ouest Aliment).

[2]Crystalline amino acids and attractant mixture (% diet): 1.34% L-lysine, 0.3% DL-methionine, 0.5 % glucosamine, 0.3% taurine, 0.3% betaine, 0.2% glycine, 0.2% alanine.

[3]Soybean lecithin from Louis François and fish oil from Sopropêche.

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

[5]Contained 10% astaxanthin (DSM).

[6]Vitamin and mineral mixture without Se (per kg diet): retinol acetate, 55,000 IU; cholecalciferol, 2,500 IU; DL-α-tocopherol acetate, 50 IU; sodium menadione bisulfate, 10 mg; thiamin-HCl, 1 mg; riboflavin, 4 mg; niacin, 10 mg; D-calcium pantothenate, 20 mg; pyridoxine-HCl, 3 mg; D-biotin, 0.2 mg; folic acid, 1 mg; cyanocobalamin, 10 µg; L-ascorbyl-2-polyphosphate, 50 mg; myo-inositol, 0.3 g; choline, 1 g; $CaHPO_4 \cdot 2H_2O$, 33 g; $CaCo_3$, 2.15 g; $Mg(OH)_2$, 1.24 g; KCl, 0.9 g; NaCl, 0.4 g; $FeSO_4 \cdot 7H_2O$, 0.2 g; $ZnSO_4 \cdot 7H_2O$, 40 mg;

$MnSO_4 \cdot H_2O$, 30 mg; $CuSO_4 \cdot 5H_2O$, 30 mg; NaF, 10 mg; KI, 0.4 mg; $CoCl_2 \cdot 6H_2O$, 0.2 mg. All ingredients were diluted with α-cellulose.

[7]Sodium selenite contained 42% Se (Sigma-Aldrich) and Selisseo contained 2% Se (Adisseo).

[8]Total saturates include 12:0, 15:0, 17:0 and 20:0 and total monoenes include 14:1 and 20:1.

Table 2. Oligonucleotide primers used to assay mRNA levels by real-time quantitative RT-PCR.

Gene	Accession no.	Forward primer sequence	Reverse primer sequence	Amplicon size
SelPa	HF969249.1	cagccacctggttggagtat	cctggagtagggccacca	82
SelPa2	MH085054.1	accttgctgagccagaaact	cagacgaccacacctgtcat	129
SelPb	HF969250.2	gtgtcgttcctcatcgtgaa	ggaacgtcagtctcccacat	187
GPX1a	HE687021	aatgtggcgtcactctgagg	caattctcctgatggccaaa	131
GPX1b1	CA357669.1	cgagctccatgaacggtacg	tgcttcccgttcacatccac	183
GPX1b2	HE687023	tcggacatcaggagaactgc	tccttcccattcacatccac	121
GPX4a1	HE687024	gaaaggcttcctgggaaatg	ctccaccacactgggatcat	112
GPX4a2	HE687025	agaaatacaggggcgacgtt	gcatctccgcaaactgagag	90
GPX4b	CA344428.1	ttggaggtcaggagccaggt	acccttttcccttgggctgtt	152
TR	HF969247.1	acaaaatcaaggcgaccaac	ggcagagagaacaggtcgtc	148
MsrB1	BX313019.3	ctggcctgccttcactgaga	ttcccacatcggaccttgaa	87
MsrB2	BX311214.3	aggggacagagatgcccttc	cccatgagcctctttgaacg	149
MsrB3	CX041708.1	tcaggttggccttccttcta	cgaggtgagaaccacactga	118
MsrA	BT073595.1	agatggctatgcttgggatg	acctgtgcaggtctcttcgt	137
SOD1	AF469663.1	tggtcctgtgaagctgattg	ttgtcagctcctgcagtcac	201
SOD2	CA352127.1	tccctgacctgacctacgac	ggcctcctccattaaacctc	201
CAT	BX087110.3	tgatgtcacacaggtgcgta	gtgggctcagtgttgttgag	195
GR	HF969248.1	ctaagcgcagcgtcatagtg	acacccctgtctgacgacat	108
Gclc	GSONMT00065033001	caaccaactggcagacaatg	cctttgacaaggggatgaga	189
GSTπ	BX302932.3	tcgctgactggacgaaagga	cgaaggtcctcaacgccatc	196
Nrf2	CA360709.1	tgagctgcagcaatgtctga	gttgggcaatgggtagaagc	124
Keap1	GSONMT00034445001	gctacgtgatgtctgcccct	ggtacctcatagcggccagt	116
Keap1X	GSONMT00019673001	actgggaggttatgacggga	cctccgtcctctcatgcttc	179
NF-κB	BX880658.3	cagcgtcctaccaggctaaagagat	gctgttcgatccatccgcactat	181
IκBα	BT074199.1	agagacagactgcgctccac	cggccttcagtagcctctct	72
β-Actin	AJ438158.1	gatgggccagaaagacagcta	tcgtcccagttggtgacgat	105

SelP, selenoprotein P; GPX1, glutathione peroxidase 1 ; GPX4, glutathione peroxidase 4; TR, thioredoxin reductase; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2; CAT, catalase; GR, glutathione reductase; Gclc, glutamate-cysteine ligase catalytic subunit; GSTπ, glutathione S-transferase π; Nrf2, nuclear factor erythroid-2 related factor 2; Keap1, Kelch-like ECH-associated protein 1; NF-κB, nuclear factor kappa-light chain-enhancer of activated B cells; IκBα, NF-κB inhibitor α.

Table 3. Growth, spawning parameters and offspring performance of rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of 8 ± 1°C.

Diet	NC	SS	SO
Male performance			
Initial body weight (kg, n=15)	0.88 ± 0.06	0.90 ± 0.09	0.82 ± 0.07
Body weight after 6 month (kg, n=9-11)	1.20 ± 0.12	1.04 ± 0.09	1.03 ± 0.13
DGI[1] (%, n=9-11)	0.34 ± 0.08	0.28 ± 0.14	0.29 ± 0.12
HSI[1] (%, n=5)	1.1 ± 0.1	1.1 ± 1.1	1.5 ± 0.2
Muscle lipid content (%, n=5)	6.8 ± 0.8	6.7 ± 1.1	6.3 ± 0.6
Female performance			
Initial mean body weight (kg, n=25)	1.13 ± 0.05	1.11 ± 0.03	1.10 ± 0.04
Final mean body weight (kg, n=24-25)	1.30 ± 0.07	1.21 ± 0.04	1.32 ± 0.05
DGI[1] (%, n=10-21)	0.27 ± 0.05^{ab}	0.17 ± 0.04^{b}	0.32 ± 0.05^{a}
HSI[1] (%, n=10-21)	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.2
Muscle lipid content (%, n=8-18)	7.9 ± 0.6	6.2 ± 0.5	6.9 ± 0.4
GSI[1] (%, n=13-24)	15.6 ± 1.1	14.3 ± 1.0	15.6 ± 0.7
Spawn weight (g, n=13-24)	220 ± 16	168 ± 12	205 ± 13
Absolute fecundity (eggs/female, n=13-24)	3378 ± 209	2687 ± 266	3119 ± 136
Relative fecundity (eggs/kg female, n=13-24)	2381 ± 148	2269 ± 213	2418 ± 114
Offspring performance			
Egg diameter (mm, n=13-24)	4.1 ± 0.1	3.9 ± 0.1	4.1 ± 0.0
Egg mean weight (mg, n=13-24)	54 ± 3	50 ± 2	54 ± 1
Survival at eyed stage (%, n=12-21)	91 ± 2	79 ± 7	94 ± 1
Hatching rate (%, n=12-21)	89 ± 2^{ab}	76 ± 6^{b}	92 ± 1^{a}
Survival at swim-up fry stage (%, n=12-21)	87 ± 2^{ab}	75 ± 6^{b}	91 ± 1^{a}
Mean weight of swim-up fry (mg, n=12-21)	85 ± 3	82 ± 3	85 ± 2

Values are means ± SEM. Within rows, means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by a Newman-Keuls test.

[1]DGI, daily growth index = 100 x (final body weight$^{1/3}$ – initial body weight$^{1/3}$)/duration (182 days); HSI, hepato-somatic index = 100 x (wet liver weight/fish weight); GSI, gonado-somatic index = 100 x (wet spawn weight/female weight); Spawn weight = total egg weight per female after stripping and removal of cœlomic fluid; absolute fecundity = egg number per female; relative fecundity = egg number/kg female.

Table 4. Total Se, SeMet and SeCys concentrations (µg Se/kg wet weight), ratio of SeMet and SeCys over total Se, SeMet to SeCys ratio and percentage of total Se in oocytes and whole-body swim-up fry

	Setot	SeMet	SeCys	SeMet/Setot	SeCys/Setot	SeMet/SeCys	%Setot
Oocytes							
NC	214 ± 12^{c}	118 ± 18^{c}	89 ± 8	0.55 ± 0.06	0.42 ± 0.04^{a}	1.35 ± 0.25^{b}	97 ± 3
SS	321 ± 14^{b}	220 ± 24^{b}	102 ± 14	0.69 ± 0.06	0.32 ± 0.03^{ab}	2.24 ± 0.38^{ab}	100 ± 3
SO	521 ± 19^{a}	373 ± 5^{a}	116 ± 19	0.72 ± 0.02	0.22 ± 0.03^{b}	3.40 ± 0.56^{a}	94 ± 1
Swim-up fry							
NC	197 ± 17^{b}	47 ± 9^{b}	13 ± 2	0.24 ± 0.06	0.07 ± 0.01	3.68 ± 0.77^{b}	31 ± 6
SS	276 ± 11^{a}	40 ± 5^{b}	13 ± 1	0.15 ± 0.03	0.05 ± 0.01	2.95 ± 0.03^{b}	19 ± 4
SO	312 ± 10^{a}	84 ± 6^{a}	12 ± 2	0.27 ± 0.01	0.04 ± 0.00	6.89 ± 0.78^{a}	31 ± 1

Values are mean ± SEM (n = 3). Within tissue and column values not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by Newman-Keuls post-hoc test.

Table 5. Antioxidant enzyme activity in liver and whole-body swim-up fry from rainbow trout fed different Se sources over 6 months at a constant water temperature of 8 ± 1°C.

	Female liver			Male liver			Swim-up fry		
	NC	SS	SO	NC	SS	SO	NC	SS	SO
Enzyme activity									
SOD[1]	14 ± 1	14 ± 1	14 ± 1	19 ± 2	24 ± 2	18 ± 2	5.0 ± 0.2	4.9 ± 0.2	5.1 ± 0.3
CAT[1]	409 ± 32	373 ± 45	391 ± 50	502 ± 60	574 ± 52	424 ± 49	11 ± 1	11 ± 1	11 ± 1
GR[2]	8 ± 1	9 ± 1	9 ± 1	12 ± 2	7 ± 1	11 ± 1	8 ± 1	8 ± 1	8 ± 1
GST[2]	145 ± 10	154 ± 22	145 ± 17	232 ± 19	229 ± 35	220 ± 33	54 ± 4	57 ± 3	55 ± 4

Mean values ± SEM (n = 8, except for male liver: n = 5).. Within rows and for each tissue, means not sharing a common superscript letter are significant different ($P < 0.05$) according to one-way ANOVA followed by Newman-Keuls post-hoc test.

[1]Expressed in U/mg protein

[2]Expressed in mU/mg protein

Table 6. Oxidative status assessed by GSH/GSSG ratio, 8-Isoprostanes, protein carbonyls content and vitamin C in whole-body swim-up fry from rainbow trout broodstock fed different Se sources over 6 months at a constant temperature of 8 ± 1°C.

	NC	SS	SO
GSH:GSSG	3.7 ± 0.3[a]	2.7 ± 0.2[b]	2.4 ± 0.1[b]
8-Isoprostanes (pg/g)	44 ± 2[a]	35 ± 3[b]	50 ± 5[a]
Protein carbonyls (nmol/mg protein)	19 ± 1	19 ± 1	16 ± 1
Vitamin C (mg/kg)	29 ± 1[b]	30 ± 2[b]	37 ± 2[a]

Within rows means ± SEM (n = 8) not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by Newman-Kauls post-hoc test.

Table 7. Tocopherol content in offspring (oocyte and whole-body swim-up fry) from rainbow trout broodstock fed different Se sources over 6 months at a constant temperature of 8 ± 1°C.

	Dietary group			Stage		p-value		
	NC	SS	SO	oocyte	swim-up fry	Diet	Stage	DxS
α-tocopherol	1815 ± 146[b]	2056 ± 176[ab]	2472 ± 168[a]	2157 ± 154	2071 ± 132	0.043	0.773	0.794
γ-tocopherol	254 ± 23	249 ± 26	254 ± 15	263 ± 17	241 ± 18	0.348	0.426	0.400
δ-tocopherol	5.15 ± 0.68	5.67 ± 0.69	4.68 ± 0.66	6.31 ± 0.36[a]	4.02 ± 0.60[b]	0.967	<0.001	0.613

Values expressed in ng tocopherol per individual are means ± SEM calculated from samples of offspring (n = 8 per dietary group and stage). Within rows and within dietary groups (n = 16) and developmental stages (n = 24), means not sharing a common superscript letter are significantly different ($P < 0.05$) according to two-way ANOVA followed by a Newman-Keuls test.

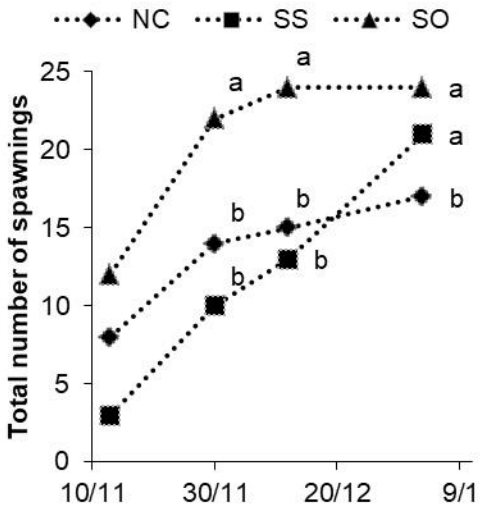

Fig. 1. Total numbers of females spawning in rainbow trout broodstock fed different Se sources over a six months period prior to spawning at a constant water temperature of 8 ± 1°C. For each date, total numbers of females spawning, not sharing the same superscript letter, are significantly different ($P < 0.05$) by pairwise comparison of the χ^2.

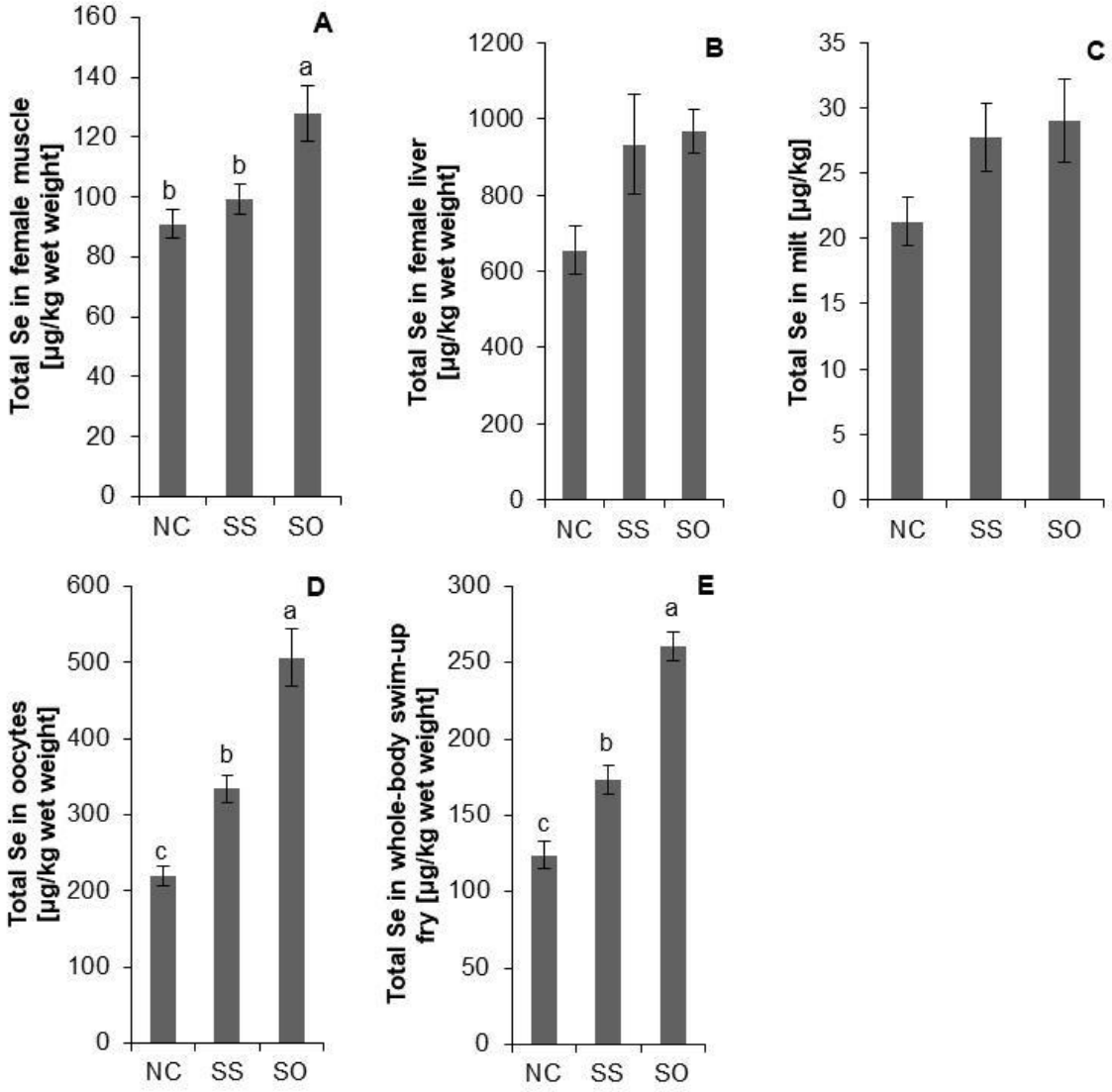

Fig. 2. Total Se content in female muscle (A), female liver (B) and in male milt (C) as well as in oocytes (D) and whole-body swim-up fry (E) from rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of 8 ± 1°C. Given as means ± SEM (n = 8, except Se2 milt: n = 4 and Se3 milt: n = 6). Means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by Newman-Keuls post-hoc test.

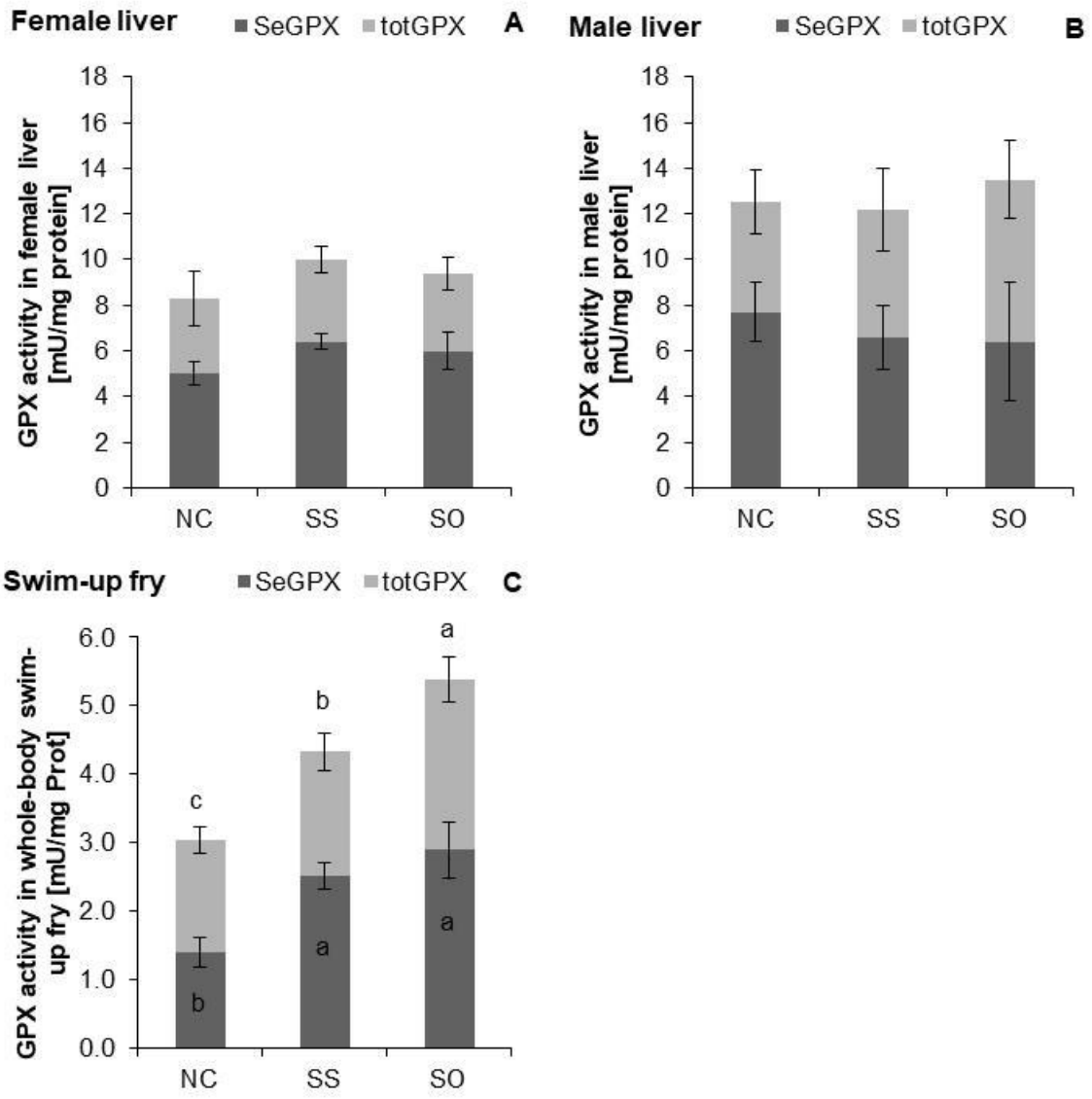

Fig. 3. Total and Se-GPX activity in female liver (A), male liver (B) and whole-body swim-up fry (C) from rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of 8 ± 1°C. Bars represent means ± SEM (n = 8, except for male liver: n = 5). Means not sharing the same superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by Newman-Keuls post-hoc test.

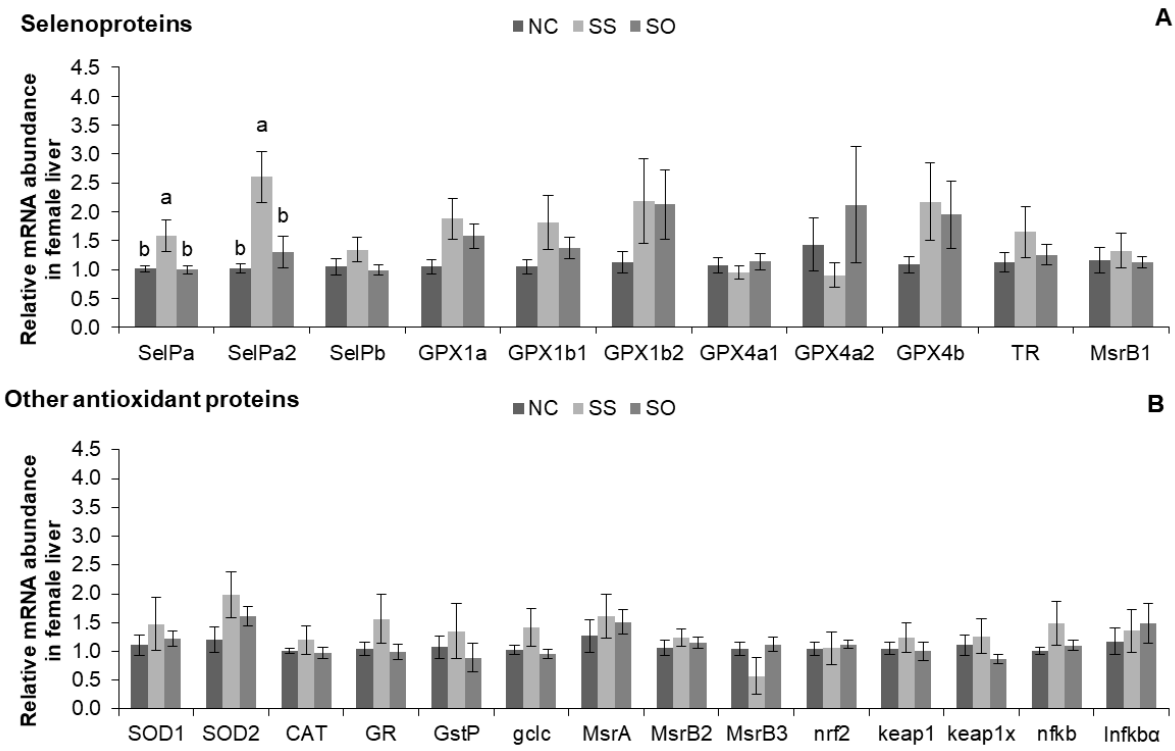

Fig. 4. Relative mRNA abundance of selenoproteins (A) and other proteins involved in antioxidant metabolism (B) in female liver from rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of 8 ± 1°C. Data are normalized to ß-actin and expressed as fold-changes of mRNA abundance compared with the control group Se1. Bars represent means ± SEM (n = 8). Means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by Newman-Keuls test.

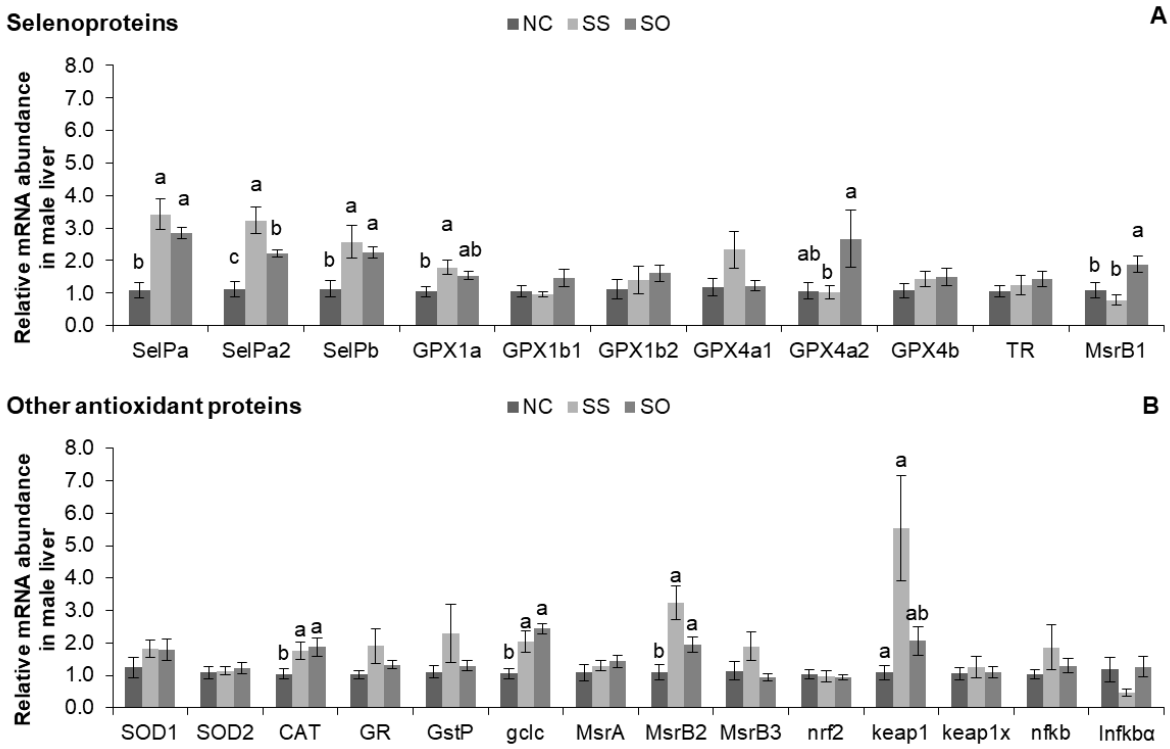

Fig. 5. Relative mRNA abundance of selenoproteins (A) and other proteins involved in antioxidant metabolism (B) in male liver from rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of 8 ± 1°C. Data are normalized to ß-actin and expressed as fold-changes of mRNA abundance compared with the control group Se1. Bars represent means ± SEM (n = 5). Means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by Newman-Keuls test.

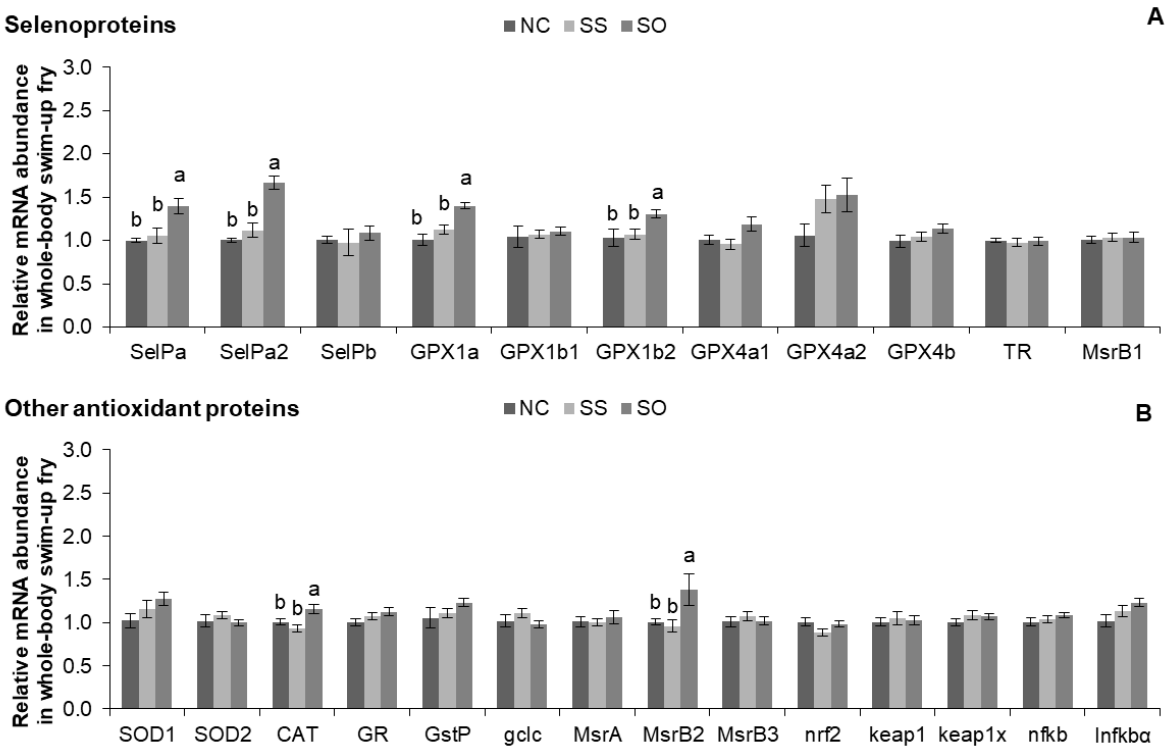

Fig. 6. Relative mRNA abundance of selenoproteins (A) and other proteins involved in antioxidant metabolism (B) in whole-body swim-up fry stage from rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of 8 ± 1°C. Data are normalized to ß-actin and expressed as fold-changes of mRNA abundance compared with the control group Se1. Bars represent means ± SEM (n = 8). Means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by Newman-Keuls test.

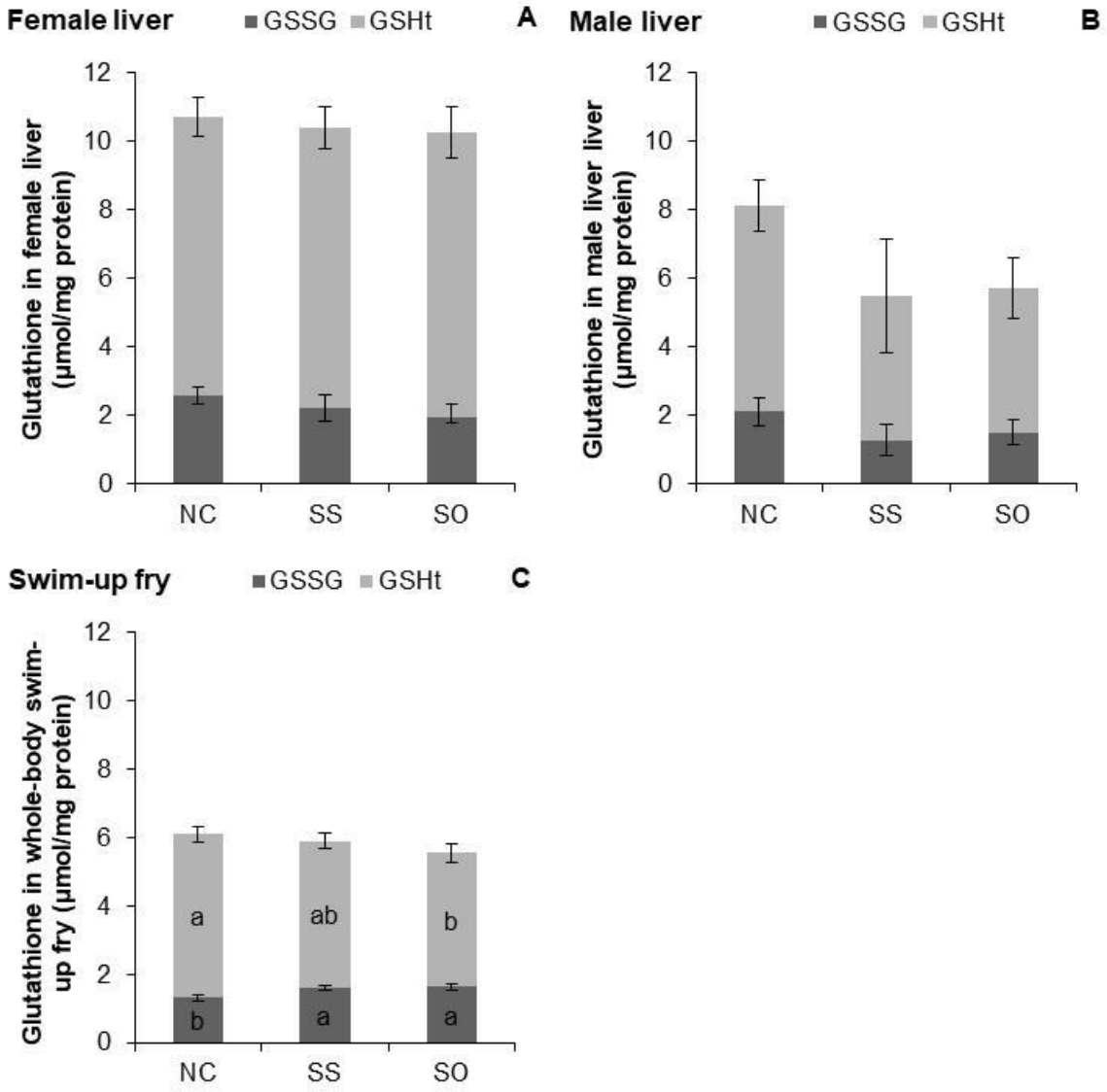

Fig. 7. Glutathione contents in female liver (A), male liver (B), and whole-body swim-up fry (C) from rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of 8 ± 1°C. Bars represent means ± SEM (n = 8, except for male liver: n = 5). Difference between total (GSHt) and oxidized glutathione (GSSG) represents reduced glutathione (GSH). Means not sharing a common superscript letter are significantly different ($P < 0.05$) according to one-way ANOVA followed by Newman-Keuls post-hoc test.

Dietary Se affected the number and timing of spawning in rainbow trout females.

Better bioavailability of Se in broodstock diets increased its transfer to the progeny.

Highest GPX activity and mRNA expression of selenoproteins in offspring of broodstock fed organic Se.

Organic Se supplementation increased vitamin E and C levels in progeny.