

Roles of selenoprotein antioxidant protection in zebrafish, *Danio rerio*, subjected to dietary oxidative stress

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Abstract In vertebrates, selenium (Se) is an essential micronutrient for vertebrates that is involved in antioxidant protection and thyroid hormone regulation among other roles and functions through its incorporation into proteins, the selenoproteins. Long-chain polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), are essential nutrients for fish although high dietary levels may lead to increased oxidative stress due to the high degree of unsaturation. The present study investigated the effects of Se supplementation on zebrafish, *Danio rerio*, oxidative status together with selenoprotein expression profiles when subjected to a high-DHA diet. Fish were fed for 8 weeks with one of the four experimental diets, containing high or low DHA in combination with or without organic Se (7 mg/kg). Fish performance, Se content, fatty acid composition and TBARS of zebrafish were determined, as well as gene expression of selected selenoproteins in liver and muscle. The Se levels in whole fish reflected dietary content. High dietary DHA increased oxidative stress as indicated by reduced growth and high TBARS content, although Se supplementation reduced oxidation. The expression patterns of selenoproteins varied between liver and muscle with only *deiodinase type II* displaying a transcriptional response when high dietary Se was

supplied. High dietary DHA decreased *selenoprotein W* expression in muscle and *sps2* expression in liver regardless of the dietary Se content. These data suggest that oxidative stress protection associated with a high dietary intake of Se may not be solely mediated by transcriptional changes in teleost selenoprotein expression.

Keywords Zebrafish · Selenium · Docosahexaenoic acid · Oxidative stress · Selenoproteins

Abbreviations

BACT	Beta-actin
B2M	Beta-2-microglobulin
DIO2	Deiodinase type II
DHA	Docosahexaenoic acid
FAME	Fatty acid methyl esters
GPX	Glutathione peroxidase
LC-PUFA	Long-chain polyunsaturated fatty acid
NTC	No-template control
PIn	Peroxidation index
qPCR	Quantitative PCR
RO	Rapeseed oil
ROS	Reactive oxygen species
SGR	Specific growth rate
SEM	Standard error of the mean
SECP43	tRNA selenocysteine 1-associated protein 1b
SEP15	15 kDa Selenoprotein
SEPP1a	Selenoprotein P1

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SEPW	Selenoprotein W
SPS2	Selenophosphate synthetase 2
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TXNRD1	Thioredoxin reductase 1

Introduction

The evolution of aerobic metabolic processes unavoidably leads to the production of reactive oxygen species (ROS) that can result in oxidative damage to proteins, DNA and lipids (Halliwell 2006). Fish are at high risk of experiencing peroxidative attack to their membranes as they can be naturally rich in the essential long-chain polyunsaturated fatty acids (LC-PUFA) from both the n-6 (arachidonic acid; ARA, 20:4n-6) and n-3 series (eicosapentaenoic acid; EPA, 20:5n-3 and docosahexaenoic acid; DHA, 22:6n-3). Among these, DHA is particularly susceptible to ROS attack due to the high degree of unsaturation (Nagaoka et al. 1990). An array of antioxidant defense mechanisms has evolved to counteract the potential deleterious effects of activated oxygen consisting of enzymatic or non-enzymatic scavengers (Yu 1994). However, certain circumstances can increase the production of ROS in an organism which will lead to an imbalance between the production and removal of ROS and the consequent alteration of oxidative status. Previous studies in fish, both in vivo and in vitro (e.g., Todorćević et al. 2009; Betancor et al. 2011; Izquierdo et al. 2013), have shown that high dietary levels of DHA without adequate amounts of antioxidant nutrients can lead to oxidative stress and thus, increased dietary levels of these nutrients are necessary under these circumstances (Puangkaew et al. 2005; Kjær et al. 2008; Betancor et al. 2012).

Selenium (Se) is an essential micronutrient that has been linked to many health benefits in humans and other vertebrates (Hatfield et al. 2014) and has been shown to be an essential antioxidant (Tinggi 2008). Se is a ubiquitous element present in both inorganic and organic forms in a wide range of feed ingredients, although organic Se bioavailability is higher than that of inorganic sources such as selenate or selenite (Dumont et al. 2006). The biological functions of this micronutrient are mediated in part by selenoproteins which contain selenocysteine (Sec) in their active site.

Zebrafish (*Danio rerio*) have 37 selenoprotein genes (Mariotti et al. 2012) in contrast to the 25 that have been reported for humans (Hatfield et al. 2014), with this difference possibly due to genome duplication (Meyer and Schartl 1999). Both inorganic and organic forms of Se can be converted to Sec due to the existence of a specific Sec tRNA where biosynthesis occurs. A latter step in Sec biosynthesis is the production of selenophosphate from selenide in the presence of ATP by selenophosphate synthetase 2 (SPS2), a selenoprotein essential for selenoprotein synthesis in mammals (Xu et al. 2007) that may also be involved in the autoregulation of selenoprotein synthesis (Guimarães et al. 1996). In addition, binding tRNA^{[ser]sec} (SECP43) has been identified as a key factor in orchestrating the interactions and localizations of the other factors involved in selenoprotein biosynthesis by increasing Sec incorporation and selenoprotein mRNA levels (Small-Howard et al. 2006).

Of the selenoprotein genes characterized in humans, only half have a known function. Glutathione peroxidases (GPX) catalyze glutathione-dependent reduction in hydrogen peroxide or other peroxides; thioredoxin reductases catalyze the reduction in thioredoxin or other proteins utilizing NADPH, and thyroid hormone deiodinases (DIO) can activate or inactivate the thyroid hormones (reviewed in Kryukov et al. 2003). Furthermore, selenoprotein P (SEPP) is a plasma protein primarily synthesized by the liver (Schweizer et al. 2005) that delivers Se to other organs (Burk and Hill 2009). The detailed functions of other selenoproteins have not been elucidated although the 15 kDa selenoprotein (SEP15) is an endoplasmic reticulum protein involved in quality control of protein folding, whereas SEPW is characterized by a thioredoxin-like fold, and SEPN mutations have been linked to muscle disorders (Hatfield et al. 2014).

Little information exists regarding the roles of selenoproteins in fish (Deniziak et al. 2007; Mariotti et al. 2012; Pacitti et al. 2013) or their modulation by dietary Se (Benner et al. 2010; Penglase et al. 2010, 2014). Furthermore, there are no reports concerning how altered oxidative status may affect selenoprotein expression in different tissues or by which mechanisms Se functions as an antioxidant under these circumstances. Thus, the aim of the present study was to evaluate the selenoprotein response in muscle and liver of zebrafish exposed to a nutritional oxidative stress caused by high dietary DHA, with or without

supplementation of Se. In addition to fish growth performance, Se content, fatty acid profiles and TBARS of whole-body zebrafish were determined.

Materials and methods

Fish maintenance

The experiment was performed using zebrafish of 8 months of age belonging to a resident colony established in 2009 at the Institute of Aquaculture, University of Stirling (for colony conditions, see Almáida-Pagán et al. 2014). A total of 224 adult zebrafish (total length 3.05 ± 0.46 cm; body weight 0.21 ± 0.04 g) were randomly placed in eight tanks ($61 \times 25 \times 20$ cm; 28 fish per tank), containing 30.5 l of chlorine-free and permanently aerated water. After being transferred to the experimental tanks and prior to the start of the experiment, fish were fed a commercial diet (TetraMin Tropical Fish Food Flakes, Tetra, Blacksburg, Virginia, USA) for 2 weeks during acclimation. Throughout the experiment, the water temperature was maintained at 26.0 ± 1.0 °C with a constant 12-h-light/12-h-dark photoperiod. Tanks were cleaned daily with approximately one-third of the water replaced. Fish were fed four times per day with the four experimental diets (duplicate tanks per treatment) at a level corresponding to 2 % of the fish wet weight for 2 months.

Experimental diets

Four isolipidic and isoproteic practical diets were formulated differing only in the oil used as lipid source (Table 1). Rapeseed oil was used as the sole lipid source in the two low-DHA diets (low oxidation risk; diets RO and RO + Se), whereas rapeseed oil in combination with fish oil concentrate was used as a DHA source to formulate the two high-DHA diets (high oxidation risk; diets DHA and DHA + Se). Two of the diets were supplemented with 7 mg/kg of organic Se (Sel-Plex 2000, Alltech) (diets RO + Se and DHA + Se). The protein source, fish meal, was defatted three consecutive times with chloroform to enable further control of the fatty acid profile of the diets (defatted meal final lipid content 2.3 %). Pellets resulting from all mixed ingredients were dried overnight at 30 °C and subsequently ground and sieved to obtain a particle size between 500 and 790 µm.

Sample collection

Prior to final sampling, fish were starved for 24 h to ensure digestive tracts were free of feed or feces to avoid any interference with the analysis. Twelve fish from each tank ($n = 24$ per treatment) were killed by overdose with benzocaine hydrochloride (400 mg/l) and quickly frozen at -20 °C for total lipid determination. An additional six fish were euthanized and frozen at -20 °C until total Se determination. Three fish per tank ($n = 6$ per treatment) were also killed by anesthetic overdose and quickly dissected to remove the muscle and liver which were fixed in RNALater® (Sigma, Poole, UK) prior to total RNA extraction.

Proximate composition

Diets were ground before determination of proximate composition according to standard procedures (AOAC 1995). Moisture contents were obtained after drying in an oven at 110 °C for 24 h and ash content determined after incineration at 600 °C for 16 h. Crude protein content was measured by determining nitrogen content ($N \times 6.25$) using automated Kjeldahl analysis (Tecator Kjeltex Auto 1030 analyzer, Foss, Warrington, UK) and crude lipid content determined gravimetrically after Soxhlet lipid extraction (Tecator Soxtec system 2050 Auto Extraction apparatus).

Lipid content, lipid class composition and fatty acid analysis

All lipid analyses were performed following methodologies approved by the American Oil Chemists' Society (based on the AOCS Official Method CE11-07). Samples of 12 whole zebrafish per tank were prepared as two pools (six fish per pool; $n = 4$ per treatment) by homogenizing in chloroform/methanol (2:1, v/v) using an Ultra-Turrax tissue disrupter (Fischer Scientific, Loughborough, UK), and the lipid content determined gravimetrically (Folch et al. 1957). Separation of individual lipid classes was performed by double-development high-performance thin-layer chromatography (HPTLC) as described previously (Olsen and Henderson 1989). Lipid classes were visualized by charring at 160 °C for 25 min after spraying with 3 % (w/v) aqueous cupric acetate containing 8 % (v/v) phosphoric acid and quantified by densitometry using a CAMAG-3 TLC scanner

Table 1 Formulation of the experimental diets (expressed as g/kg of dry matter) fed to zebrafish for 8 weeks

	DHA + Se	DHA	RO + Se	RO
Vitamin free casein ^a	480	480	480	480
Potato starch ^b	150	150	150	150
Defatted fish meal ^c	50	50	50	50
Vitamin mix ^d	10	10	10	10
Mineral mix ^e	47	47	47	47
Arginine	4	4	4	4
Leucine	4	4	4	4
Methionine	3	3	3	3
Cystine	2	2	2	2
Orange G	1	1	1	1
α -cellulose	135.1	138.6	135.1	138.6
INCROMEGA DHA 500 TG ^f	44	44	0	0
Rapeseed oil ^g	66	66	110	110
Sel-Plex 2000 ^h	3.5	0	3.5	0
Antioxidant mix ⁱ	0.4	0.4	0.4	0.4

^a Vitamin-free micropulverized (ICN Biomedical Ltd, High Wycombe, UK)

^b Passeli WA4 (Avebe, UK) Ltd, Ulceby, South Humberside, UK

^c Norse-LT94, Norsidmel AS, Fyllingsdalen, Norway

^d Supplied (mg/kg diet): ascorbic acid, 1000; myo-inositol, 400; nicotinic acid, 150; calcium pantothenate, 44; all-rac- α -tocopheryl acetate, 40; riboflavin, 20; pyridoxine hydrochloride, 12; menadione, 10; thiamine hydrochloride, 10; retinyl acetate, 7.3; folic acid, 5; biotin, 1; cholecalciferol, 0.06; cyanocobalamin, 0.02

^e Supplied (per kg diet): KH_2PO_4 , 22 g; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 g; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.13 g; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 52.8 mg; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 12 mg; $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 2 mg

^f Croda Chemicals Europe

^g The Cooperative Food, UK

^h Alltech, Lexington, KY, USA

ⁱ Dissolved in propylene glycol and contained (g/l): butylated hydroxyl anisole, 60; propyl gallate, 60; citric acid, 40

(Version Firmware 1.14.16; CAMAG, Muttenz, Switzerland) with winCATS Planar Chromatography Manager. The identities of individual lipid classes were confirmed by comparison with reference to the Rf values of authentic standards run alongside the experimental samples. Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalyzed transesterification at 50 °C for 16 h (Christie 2003), and FAME extracted and purified as described previously (Tocher and Harvie 1988).

FAME were separated and quantified by gas–liquid chromatography using a Fisons GC-8160 (thermo Scientific, Milan, Italy) equipped with a 30 m \times 0.32 mm i.d. \times 0.25 μm ZB-wax column (Phenomenex, Cheshire, UK), “on-column” injection and flame ionization detection. Data were collected and processed using Chromcard for Windows (version 2.01; Thermoquest Italia S.p.A., Milan, Italy). Individual FAME were identified by comparison with known standards and published data.

Thiobarbituric acid reactive substances (TBARS)

TBARS were determined in total lipid extracts (10 mg/ml) according to a modification of the protocol of Burk et al. (1980). Briefly, 50 μl of 0.2 % (w/v) butylated hydroxytoluene (BHT) in ethanol was added to the lipid samples followed by 0.5 ml of 1 % (w/v) TBA and 0.5 ml 10 % (w/v) trichloroacetic acid, both solutions freshly prepared. The reagents were mixed in a stoppered test tube and heated at 100 °C for 20 min. After cooling, particulate matter was removed by centrifugation at 2000g, and absorbance in the supernatant determined in a spectrophotometer at 532 nm against a blank sample. The concentration of TBARS, expressed as mmol MDA/g lipid, was calculated using the absorption coefficient 0.156 $\mu\text{M}^{-1} \text{cm}^{-1}$.

Selenium determination

Total Se concentration was measured in whole zebrafish and diets in triplicates according to the method established in Betancor et al. (2012). Samples were acidified and digested in Teflon vessels in a microwave digester (MarsXpress, CEM, Kamp-Lintfort, Germany) with 5 ml of 69 % pure nitric acid and then poured into a 10-ml volumetric flask and made up to volume with distilled water. A total of 0.4 ml of this solution was then placed in a 10-ml sample tube, 10 μl of the internal standard (Ga and Sc, 10 ppm) included and 0.3 ml of methanol added. The tubes were made up to volume with distilled water and total Se measured by collision/reaction ICP-MS (Thermo Scientific, Cheshire, UK) using argon and hydrogen as carrier gas. To ensure the good quality of the Se determination, serial dilutions of a known Se concentration (10, 20 and 30 ppb) were injected at the start of the analysis preceded by a blank.

Gene expression analysis

Liver and muscle from six individual fish per dietary treatment were homogenized in 1 ml of TriReagent® (Sigma-Aldrich, Dorset, UK) RNA extraction buffer using a bead tissue disruptor (Bio Spec, Bartlesville, Oklahoma, USA). The two tissues were chosen because liver is the main metabolic tissue and muscle is the most abundant tissue in whole fish. Total RNA was isolated following manufacturer's instructions and quantity and quality determined by spectrophotometry using a Nanodrop ND-1000 (Labtech Int., East Sussex, UK) and electrophoresis using 200 ng of total RNA in 1 % agarose gel. Expression of genes of interest (Table 2) was determined by quantitative PCR (qPCR) from fish fed all diets. Results were normalized using reference genes, beta-2 microglobulin and beta-actin, which were chosen as the most stable according to GeNorm. cDNA was synthesized using 2 µg of total

RNA and random primers in 20 µl reactions and the high-capacity reverse transcription kit without RNase inhibitor according to the manufacturer's protocol (Applied Biosystems, Warrington, UK). The resulting cDNA was diluted 20-fold with milliQ water. The efficiency of the primers for each gene was previously evaluated to ensure that it was close to 100 %. qPCR was performed using a Biometra TOptical Thermocycler (Analytik Jena, Goettingen, Germany) in 96-well plates in duplicate 20 µl reaction volumes containing 10 µl of Luminaris Color HiGreen qPCR Master Mix (Thermo Scientific, Hemel Hempstead, UK), 1 µl of the primer corresponding to the analyzed gene (10 pmol), 3 µl of molecular biology grade water and 5 µl of cDNA, with the exception of the reference genes, which were determined using 2 µl of cDNA. In addition, amplifications were carried out with a systematic negative control (NTC-non-template control) containing no cDNA. Standard amplification

Table 2 Details of PCR primer used in the present study for real-time quantitative PCR (qPCR)

Transcript	Primer sequence (5'-3')	Amplicon (bp)	Ta (°C)	Accession No.
<i>gpx3</i>	F: TCCAGGAAATGGATTCGTTTC R: TCTCTCTACAGGCGGACAT	116	60	NM_001137555.1
<i>gpx4b</i>	F: GGAAGTGGATGAAGGAGCAG R: GCCGCAGCATAACACTCTA	181	60	BC165898.1
<i>txnrd1</i>	F: GGAGCAACCATGAAGTGTGA R: TGATCTTGGCGTAGCACTTG	101	60	NM_183072.1
<i>dio2</i>	F: ACTCGGACACTTGGCTTGAC R: CAAATGAGCGACTGCCTATG	128	60	NM_212789.3
<i>sepp1a</i>	F: ATCCTGAGCCACCCACAT R: ACTCTTCAAGTTGCGCTGAA	100	60	BC159240.1
<i>sepw</i>	F: GCTGGTTGGAGGTGGAAGTA R: CTACCCAGGAGTCCCACAAA	181	60	NM_178287.5
<i>sep15</i>	F: CTTTGGTTGCTGCCGTTATT R: GCTCCTGGGTAGAGCTTCTCT	209	60	NM_178294.4
<i>sps2</i>	F: GGGTTGAGCAGCTTTCTCTG R: AGAGCCAGGTGAATGAATGG	197	60	NM_001004295.2
<i>secp43</i>	F: GGGCGATTTAGACCCATACA R: CCAGCTGAGCCTCCAGTAAC	117	60	NM_001029966.2
<i>b2m</i>	F: CCACTCCGAAAGTTCATGTGT R: ATCTCCTTTCTCTGGGGTGAA	221	60	NM_131163.2
<i>bact</i>	F: CTCTTCCAGCCTTCCTCCT R: CACCGATCCAGACGGAGTAT	246	60	AF057040.1

The data include sequences and annealing temperatures (Ta) for primer pairs, amplicon sizes and accession numbers

gpx glutathione peroxidase, *txnrd1* thioredoxin reductase 1, *dio2* deiodinase type II, *sepp1a* selenoprotein P1, *sepw* selenoprotein w, *sep15* 15 kDa selenoprotein, *sps2* selenophosphate synthetase 2, *secp43* tRNA selenocysteine 1-associated protein 1b, *b2m*, beta-2-microglobulin, *bact* beta-actin

parameters contained an UDG pre-treatment at 50 °C for 2 min, an initial denaturation step at 95 °C for 10 min, followed by 35 cycles: 15 s at 95 °C, 30 s at the annealing T_m and 30 s at 72 °C.

Calculations

The peroxidation index (PI_n) was used as an estimate of susceptibility of lipids to oxidation and was calculated using the formula: $PI_n = 0.025 \times (\text{percentage of monoenoics}) + 1 \times (\text{percentage of dienoics}) + 2 \times (\text{percentage of trienoics}) + 4 \times (\text{percentage of tetraenoics}) + 6 \times (\text{percentage of pentaenoics}) + 8 \times (\text{percentage of hexaenoics})$ (Witting and Horwitt, 1964). Specific growth rate (SGR) = $100 \times [\ln(W_f) - \ln(W_o)] / t$, where W_o and W_f are the initial and the final weights (g), respectively, and t the number of days.

Statistical analysis

Data are presented as mean \pm SE unless otherwise specified. Percentage data from lipid content and fatty acid composition were subjected to arcsin transformation prior to statistical analyses. Data were tested for normality and homogeneity of variances with Levene's test prior to one-way analysis of variance followed by a Tukey–Kramer HSD multiple comparisons of means. All statistical analyses were performed using SPSS software (IBM SPSS Statistics 19; SPSS Inc., Chicago, IL, USA). Gene expression results were analyzed using the relative expression software tool (REST 2009; <http://www.gene-quantification.info/>), which employs a pairwise fixed reallocation randomization test (10000 randomizations) with efficiency correction (Pfaffl et al. 2002) to determine the statistical significance of expression ratios (gene expression fold changes) between two treatments. Pearson's correlation coefficients were calculated to determine correlations between genes.

Results

Diet composition

All diets contained similar lipid, protein and ash contents, although they differed in Se contents. The supplemented RO and DHA diets contained more than four times the level of Se than the non-supplemented

Table 3 Fatty acid composition (percentage of total fatty acids by weight) of experimental diets containing high-DHA oil (DHA) or rapeseed oil (RO), with or without selenium supplementation (Se)

	Experimental diets			
	RO	RO + Se	DHA	DHA + Se
<i>Gross composition</i>				
Dry matter (%)	88.8	90.1	87.8	88.4
Crude protein (%)	49.4	49.4	49.7	49.7
Crude fat (%)	11.0	11.1	10.9	10.4
Ash (%)	6.2	6.4	6.4	6.4
Selenium (mg/kg)	1.7	7.0	1.3	6.3
<i>Fatty acids %</i>				
16:0	5.1	5.2	4.2	5.1
20:0	0.6	0.6	0.6	0.6
Total SAFA ^a	8.2	8.2	7.9	9.3
16:1n-9	0.4	0.4	0.3	0.3
18:1n-9	55.9	56.7	39.7	39.4
Total MUFA ^b	62.1	62.8	46.0	45.3
18:2n-6	19.5	19.5	13.1	13.7
18:3n-6	n.d.	n.d.	0.1	0.1
20:2n-6	0.1	0.1	0.2	0.1
20:4n-6	n.d.	n.d.	0.8	0.7
22:4n-6	n.d.	n.d.	0.1	0.1
22:5n-6	n.d.	n.d.	0.9	0.9
Total n-6 PUFA ^c	19.5	19.5	15.4	15.6
18:3n-3	9.3	9.1	6.3	6.3
18:4n-3	n.d.	n.d.	0.1	0.1
20:3n-3	n.d.	n.d.	0.1	n.d.
20:4n-3	n.d.	n.d.	0.2	0.1
20:5n-3	0.1	n.d.	3.7	3.6
22:5n-3	n.d.	n.d.	1.1	1.0
22:6n-3	0.7	0.2	19.0	18.5
Total n-3 PUFA ^d	10.1	9.4	30.6	29.6
Total PUFA ^e	29.7	29.0	46.0	45.3
PI _n	45.9	41.0	218.5	212.7

Different superscript letters within a row denote significant differences among diets. Statistical differences were determined by one-way ANOVA with Tukey's comparison test ($p < 0.05$)

n.d. not detected, SAFA saturated fatty acid, MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid

^a Includes 14:0, 15:0, 18:0, 22:0 and 24:0

^b Includes 16:1n-7, 17:1, 18:1n-7, 20:1n-9, 20:1n-11, 20:1n-7, 22:1n-11, 22:1n-9 and 24:1n-9

^c Includes 18:3n-6, 20:3n-6, 22:4n-6

^d Includes 18:4n-3, 20:3n-3 and 20:4n-3

^e Includes 16:2, 16:3 and 16:4

diets (Table 3). The fatty acid profiles reflected the oil employed in the formulation of the diets. Thus, RO feeds were characterized by high contents of 18:1n-9, 18:2n-6 and 18:3n-3 and reduced n-3 LC-PUFA, whereas DHA feeds contained enhanced levels of EPA, DHA and total n-3 LC-PUFA. DHA levels were more than 20 times higher in the DHA enriched diets than in the RO alone diet (Table 3). This higher content in LC-PUFA was reflected in the peroxidation indices of the diets (43.4 average for the RO feed vs. 215.6 for DHA feeds).

Growth and survival

All experimental feeds were well accepted by the zebrafish. Fish performance differed among the dietary treatments after 8 weeks of feeding. Growth in terms of total length was lower when high levels of DHA were added to the feeds, although increased dietary Se contents improved this parameter (Table 4). Similarly, the SGR was reduced by high-DHA levels, with dietary Se maintaining this parameter at a value similar to that obtained in RO-fed fish. However, no significant differences were observed in fish final total length. Survival was high throughout the experimental period and did not differ among the dietary treatments.

Zebrafish lipid contents, lipid classes and fatty acid profiles

No significant differences in total lipid contents or lipid classes were observed between dietary treatments,

although DHA + Se-fed fish showed the highest percentage of lipid (Table 5). Triacylglycerol (TAG) was the major lipid class in whole fish accounting for over 75 % of total lipid, whereas phosphatidylcholine (PC) was the most prominent polar lipid at almost 6 %. Fatty acid profiles from total lipids of adult zebrafish generally reflected dietary fatty acid compositions. In this respect, low-DHA and total n-3 PUFA levels and high monoenes and n-6 PUFA were found in zebrafish fed rapeseed oil and therefore low oxidation risk diets (RO and RO + Se). Conversely, DHA-fed fish had higher contents of total PUFA and n-3 PUFA (Table 5). In addition, some differences were observed among fish fed the dietary treatments with the same lipid source (Table 5). For instance, higher proportions of 18:3n-6 and 18:4n-3 were found in fish fed the RO + Se diet than in those fed RO. Higher levels of DHA were found in fish fed both diets with a high-DHA inclusion, with Se supplementation having no effect on the deposition of this fatty acid. Furthermore, differences were found in the PIn among RO and DHA-fed fish, reflecting the fatty acid contents in the diets independent of the Se levels of the diets.

Zebrafish selenium and TBARS contents

Whole-body Se contents were directly correlated with dietary Se ($y = 0.0588x + 0.1562$; $R^2 = 0.9957$). Fish fed RO + Se and DHA + Se diets displayed the highest Se contents regardless of the origin of the lipid source (Table 4). Non-supplemented diets showed similar amounts of Se, around 0.20 mg/kg, derived from the fish meal employed in the

Table 4 Growth performance, survival, TBARS and selenium contents in whole zebrafish fed the four experimental diets for 8 weeks

	RO	RO + Se	DHA	DHA + Se
Final weight (g)	0.40 ± 0.01 ^a	0.39 ± 0.02 ^a	0.29 ± 0.02 ^b	0.37 ± 0.03 ^{ab}
Final length (cm)	2.90 ± 0.07	2.83 ± 0.04	2.92 ± 0.08	2.96 ± 0.06
SGR	1.13 ± 0.07 ^a	1.07 ± 0.09 ^a	0.50 ± 0.15 ^b	0.92 ± 0.15 ^{ab}
Survival (%)	96.4 ± 0.0	94.6 ± 1.7	92.8 ± 3.5	96.4 ± 0.0
TBARS (mmol MDA/g lipid)	1889.7 ± 118.8 ^b	1600.4 ± 10.7 ^b	2756.8 ± 165.2 ^a	1641.8 ± 75.3 ^b
Selenium (mg/kg; WW)	0.27 ± 0.03 ^b	0.57 ± 0.03 ^a	0.22 ± 0.00 ^b	0.53 ± 0.06 ^a

Different superscript letters within a row denote significant differences among diets. Statistical differences were determined by one-way ANOVA with Tukey's comparison test ($p < 0.05$)

Data are mean ± SE. ($n = 12$ for TBARS; $n = 6$ for selenium)

TBARS thiobarbituric acid reactive substance, SGR specific growth rate

Table 5 Total lipid fatty acid compositions of young zebrafish fed one of the experimental diets. (mean \pm SE, $n = 4$)

Fatty acid	RO	RO + Se	DHA	DHA + Se
Lipid content (%)	8.8 \pm 0.4	8.0 \pm 1.5	9.0 \pm 2.8	10.7 \pm 0.5
Phosphatidylcholine	7.0 \pm 0.3	8.1 \pm 2.4	5.9 \pm 2.5	8.2 \pm 1.5
Phosphatidylethanolamine	4.4 \pm 0.4	4.7 \pm 1.2	4.3 \pm 1.4	4.9 \pm 0.9
Phosphatidylserine	1.2 \pm 0.4	1.0 \pm 0.4	1.4 \pm 0.2	1.2 \pm 0.5
Phosphatidylinositol	0.7 \pm 0.2	1.0 \pm 0.3	1.0 \pm 0.3	1.3 \pm 0.4
Sphingomyelin	1.2 \pm 0.4	1.3 \pm 0.3	1.3 \pm 0.2	1.1 \pm 0.3
Phosphatidylglycerol/cardiolipin	1.2 \pm 0.3	1.2 \pm 0.4	1.3 \pm 0.3	1.4 \pm 0.3
Lysophosphatidylcholine	0.3 \pm 0.2	0.3 \pm 0.2	0.3 \pm 0.2	0.4 \pm 0.2
Glycolipid	2.3 \pm 0.2	2.1 \pm 0.2	1.5 \pm 0.3	2.1 \pm 0.5
Unknown polar lipid	1.3 \pm 0.3	1.3 \pm 0.4	0.5 \pm 0.1	1.1 \pm 0.4
Total polar lipids	21.1 \pm 3.2	23.7 \pm 4.9	21.5 \pm 3.7	23.0 \pm 4.4
Total neutral lipids	78.9 \pm 3.2	76.3 \pm 4.9	78.5 \pm 3.7	77.0 \pm 4.4
Triacylglycerols	54.6 \pm 4.6	49.5 \pm 5.5	50.3 \pm 1.7	52.3 \pm 5.1
Cholesterol/sterols	9.9 \pm 0.7	10.6 \pm 1.0	10.0 \pm 1.0	9.8 \pm 0.5
Free fatty acids	9.6 \pm 1.2	10.5 \pm 1.5	12.3 \pm 2.3	8.6 \pm 2.1
Sterol esters	1.6 \pm 0.6	2.1 \pm 1.4	2.3 \pm 0.6	3.0 \pm 1.1
Unknown neutral lipid	3.3 \pm 0.2	3.6 \pm 0.4	3.6 \pm 0.8	3.4 \pm 0.6
16:0	15.5 \pm 0.7 ^b	17.0 \pm 0.9 ^{ab}	19.5 \pm 1.5 ^a	19.8 \pm 2.2 ^a
20:0	0.2 \pm 0.0 ^{ab}	0.1 \pm 0.0 ^b	0.2 \pm 0.0 ^{ab}	0.2 \pm 0.0 ^a
Total SAFA	19.9 \pm 1.0 ^c	21.1 \pm 0.9 ^{bc}	24.0 \pm 1.7 ^{ab}	24.9 \pm 1.9 ^a
16:1n-9	1.1 \pm 0.2 ^{ab}	1.2 \pm 0.3 ^{ab}	0.8 \pm 0.1 ^b	1.3 \pm 0.3 ^a
18:1n-9	47.8 \pm 1.1	47.4 \pm 3.3	39.0 \pm 0.5	37.0 \pm 2.0
Total MUFA	56.5 \pm 1.2 ^a	56.5 \pm 1.4 ^a	46.9 \pm 0.7 ^b	47.2 \pm 0.4 ^b
18:2n-6	11.8 \pm 0.2 ^a	10.8 \pm 0.7 ^{ab}	9.2 \pm 0.3 ^b	9.1 \pm 1.4 ^b
18:3n-6	0.4 \pm 0.0 ^b	0.6 \pm 0.1 ^a	0.2 \pm 0.0 ^b	0.2 \pm 0.0 ^b
20:2n-6	0.2 \pm 0.0	0.3 \pm 0.1	0.3 \pm 0.0	0.3 \pm 0.0
20:3n-6	0.8 \pm 0.1 ^a	0.7 \pm 0.1 ^a	0.4 \pm 0.1 ^b	0.4 \pm 0.0 ^b
20:4n-6	1.2 \pm 0.1	1.3 \pm 0.3	1.2 \pm 0.1	1.1 \pm 0.0
22:4n-6	0.1 \pm 0.0	0.1 \pm 0.0	0.1 \pm 0.0	0.1 \pm 0.0
22:5n-6	0.1 \pm 0.0	0.1 \pm 0.0	0.4 \pm 0.0	0.3 \pm 0.0
Total n-6 PUFA	14.7 \pm 0.2 ^a	13.9 \pm 0.7 ^a	11.8 \pm 0.4 ^b	11.4 \pm 1.4 ^b
18:3n-3	3.7 \pm 0.2	3.5 \pm 0.3	3.3 \pm 0.3	3.7 \pm 0.5
18:4n-3	0.4 \pm 0.0 ^b	0.5 \pm 0.1 ^a	0.2 \pm 0.0 ^c	0.2 \pm 0.0 ^c
20:3n-3	0.1 \pm 0.0	0.1 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.0
20:4n-3	0.2 \pm 0.0 ^{ab}	0.2 \pm 0.0 ^b	0.3 \pm 0.0 ^a	0.3 \pm 0.0 ^{ab}
20:5n-3	0.8 \pm 0.1 ^b	0.8 \pm 0.3 ^b	2.3 \pm 0.3 ^a	2.2 \pm 0.2 ^a
22:5n-3	0.3 \pm 0.0 ^b	0.3 \pm 0.1 ^b	0.8 \pm 0.1 ^a	0.9 \pm 0.1 ^a
22:6n-3	3.5 \pm 0.6 ^b	3.2 \pm 0.5 ^b	10.4 \pm 1.2 ^a	9.1 \pm 0.7 ^a
Total n-3 PUFA	9.0 \pm 0.3 ^b	8.5 \pm 1.1 ^b	17.4 \pm 2.0 ^a	16.5 \pm 0.6 ^a
Total PUFA	23.7 \pm 0.4 ^b	22.4 \pm 1.4 ^b	29.2 \pm 2.3 ^a	28.0 \pm 1.5 ^a
PI _n	63.9 \pm 2.1 ^b	59.9 \pm 3.8 ^b	128.3 \pm 6.8 ^a	118.2 \pm 2.4 ^a

Different superscript letters within a row denote significant differences among diets. Statistical differences were determined by one-way ANOVA with Tukey's comparison test ($p < 0.05$)

formulation of the experimental feeds. In contrast, the TBARS values, an indicator of lipid peroxidation, were increased in zebrafish fed the high-DHA diets,

although the addition of organic Se reduced this parameter to levels similar to those observed for RO-fed fish (Table 4).

Liver and muscle gene expression

In general terms, the highest gene expression was consistently found in fish fed the RO diet, both liver and muscle (Figs. 1, 2), although not always statistically significant. Furthermore, all studied genes showed differential expression in liver, whereas *gpx*s did not vary among dietary treatments in the muscle. The use of rapeseed oil as a lipid source enhanced the relative expression of *sepw* in muscle, whereas no differences were observed in liver between fish fed the DHA diets and RO + Se diet. Irrespective of the dietary lipid source, the inclusion of Se decreased the expression of *dio2* in muscle (Fig. 2), whereas this effect was not observed in liver where non-supplemented Se DHA-fed fish also showed low expression of this gene (Fig. 1).

In muscle, the lowest expression levels of *txnr1*, *sep15*, *sps2*, *sepp1a* and *secp43* were found in fish fed the DHA + Se diet, although there were no differences in *sep15* and *txnr1* with DHA-fed fish, and *sps2* for DHA and RO + Se-fed fish (Fig. 2). Conversely, in liver, RO-fed fish showed the highest relative expression of *gpx3*, *gpx4b*, *dio2* and *sepp1a*. The same trend was observed in the other genes, although the response was similar between fish fed diets RO and RO + Se for *sep15*, *sepw*, *secp43* and *sps2* (Fig. 1).

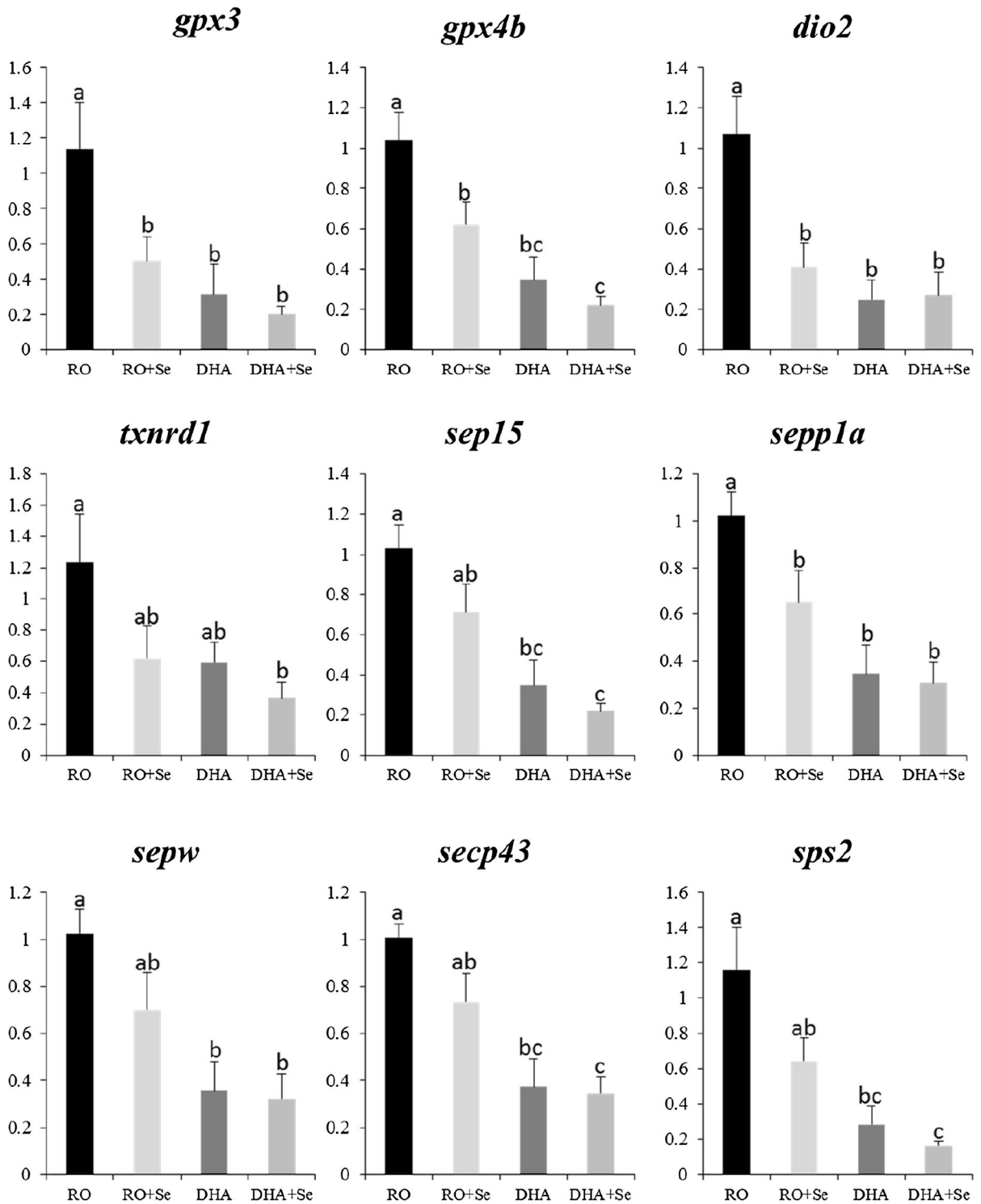
Calculations of Pearson's correlation coefficients (Table 6) for the two selenoprotein biosynthesis-related genes (*secp43* and *sps2*) with the other seven studied selenoproteins genes indicated strong correlations in all genes in the liver. Many of the correlations were also observed in muscle, although the amounts of *gpx3* mRNA were not correlated with those of *secp43* or *sps2*. In contrast *gpx4b* in muscle was correlated with both biosynthesis-related genes although not as significantly (Table 6; $P = 0.018$ and $P = 0.022$, respectively). In addition, a low significance ($P = 0.033$) was found between the biosynthetic gene *sps2* and *sepw* in muscle tissue, although a moderate positive correlation was still observed ($R = 0.42$).

Discussion

Despite zebrafish being a common model organism for toxicology, developmental biology, neurobiology and genetic studies (Ulloa et al. 2011), most of its nutritional requirements remain unknown. DHA is

an essential fatty acid that plays key roles in numerous metabolic and physiological processes ensuring normal cellular function and high levels of this fatty acid are generally included in fish feeds. However, excessively high amounts of dietary DHA have been shown to cause adverse effects in teleosts, especially when accompanied by insufficient amounts of antioxidants in the feeds. Symptoms of an inadequate balance between dietary DHA and antioxidant nutrients include reduced growth (Betancor et al. 2011, 2012) muscle and hepatic lesions (Betancor et al. 2011), accelerated osteoblast differentiation (Villeneuve et al. 2006) and decreased oxidative stability of the fillet (Stéphan et al. 1995). In the present study, high dietary DHA levels (around 19 % of total fatty acids) without supplementation of organic Se altered the oxidative status of zebrafish as observed by reduced growth and increased TBARS contents. However, the addition of 7 mg/kg of yeast-derived organic Se proved effective as an antioxidant as evidenced by decreased whole-body zebrafish TBARS contents and enhanced growth. Previously, dietary Se was shown to reduce TBARS contents in fish under normal conditions (Hao et al. 2014; Saleh et al. 2014) or when subjected to oxidative stress (Betancor et al. 2011; Özkan-Yilmaz et al. 2014). Nevertheless, the mechanisms by which Se exerts antioxidant protection in fish are not clear nor whether these vary between tissues.

When the presence of Se is limiting, a hierarchy within selenoproteins appears in mammals (Schomburg and Schweizer 2009) and thus not all tissues and not all selenoproteins are equally well supplied under those conditions. Although all of the diets employed in the present study contained Se as a result of including fish meal in the feed formulations, the peroxidation promoted by high dietary DHA contents may have altered the distribution of Se within the tissues. Thus, an unequal distribution of this trace element could have occurred among organs, without altering whole-body Se levels. Previous studies in rainbow trout (*Oncorhynchus mykiss*) showed tissue-specific accumulation of Se, with efficient retention in liver, kidney and spleen, whereas muscle was a tissue with one of the lowest Se content (Misra et al. 2012). In addition, when dietary Se levels are low, there is a tendency for selective accumulation in specific organs including brain, testes and endocrine tissues (Behne et al. 1988) which may account for why Se deficiencies can cause both cardiac and skeletal muscle diseases (Rederstorff



◀ **Fig. 1** Expression of selenoproteins and related genes in zebrafish liver after 8 weeks of feeding the experimental diets. Results are normalized expression ratios (average \pm SE, $n = 6$) of the expression of these genes in fish fed the different diets in relation to fish fed RO diet. Diets contain either rapeseed oil alone (RO), in combination with concentrated fish oil (DHA) and extra supplementation of Se (RO + Se and DHA + Se). *gpx* glutathione peroxidase, *txnr1* thioredoxin reductase 1, *dio2* deiodinase type II, *sepp1a* selenoprotein P1, *sepw* selenoprotein w, *sep15* 15 kDa selenoprotein, *sps2* selenophosphate synthetase 2, *secp43* tRNA selenocysteine 1-associated protein 1b, *b2m* beta-2-microglobulin, *bact* beta-actin

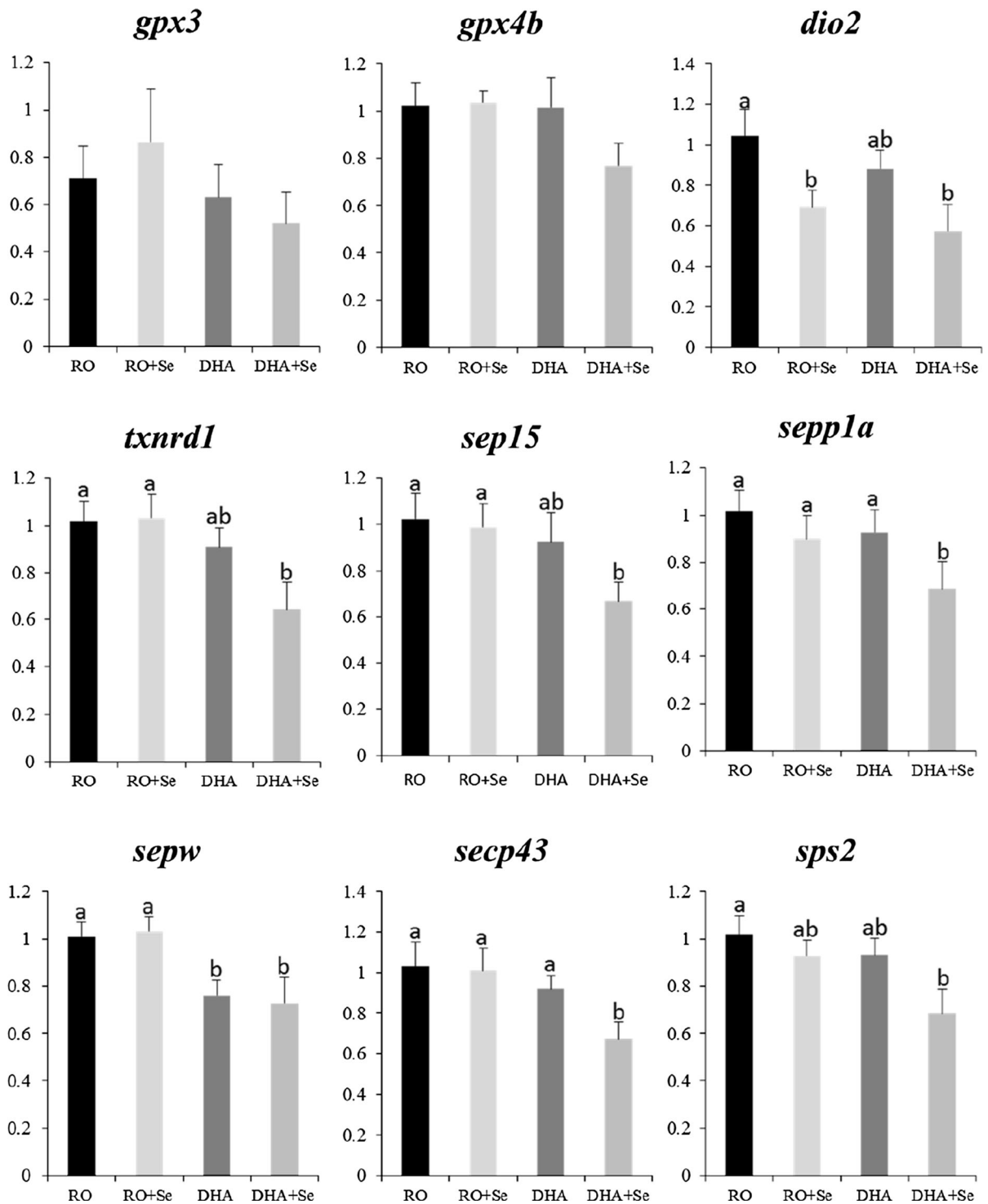
et al. 2006). In fact, it has been suggested that the activity of a selenoprotein in tissues may depend on the ability of that tissue to maintain its local Se concentration (Bates et al. 2000). In the present experiment, the Se content of individual tissues could not be evaluated due to the small size and limited number of fish. Nevertheless, Se content in whole fish was not diminished when fish were subjected to an oxidative stress, suggesting that enough Se was provided through the feeds, but not excluding a mobilization of Se to certain tissues like brain or liver.

GPXs are the most studied selenoprotein family and have been traditionally used as biomarkers of Se requirements in vertebrates since they normally respond to changes in Se status (Sunde et al. 2009). Dietary Se supplementation did not elicit transcriptional changes in *gpx3* or *gpx4* in zebrafish muscle, whereas differences were found in liver. In agreement with observed values for muscle in the present study, brain *gpx3* expression did not respond to selenomethionine supplementation zebrafish in a previous study (Benner et al. 2010). Liver *gpxs* expression followed an unexpected pattern, with lower expression found in fish fed high-DHA contents, irrespective of Se supplementation and independent of TBARS contents. A similar pattern was also observed in Manchurian trout (*Brachymystax lenok*) larvae fed high dietary lipids, where the activity of GPX varied depending on tissue and was not directly related to the oxidation risk of the diets or to TBARS levels (Zhang et al. 2009). Thus, results obtained in the present study indicated that the use of these selenoproteins as indicators may depend on the tissue used to perform the analysis. On the other hand, these results contrast with previous studies performed on zebrafish, where a good correlation was shown to exist between Se status and *gpx1a* and *b* expression (Penglase et al. 2014). However,

those authors examined whole body of zebrafish to perform the gene expression analysis, rather than individual tissues, which may explain differences between the studies.

SEPP has also been considered as a possible indicator of Se status of an organism as the homeostasis of this essential micronutrient is largely maintained by this selenoprotein (Hill et al. 2003). The main role of SEPP is to transport and deliver Se to the tissues although additional roles as a heavy metal chelator and antioxidant have been postulated (Burk and Hill 2009). *Sepp1a* expression was not affected by dietary Se in zebrafish muscle but was down-regulated by the combination of high dietary DHA levels and Se. Conversely, in liver, the addition of Se altered oxidative status, and the combination of both conditions led to a down-regulation of this selenoprotein. Variable results have been reported regarding the regulation of *sepp1a* in teleosts. For example, a recent study in zebrafish showed a differential transcriptional response in zebrafish *sepp1a* when fed higher than 0.3 Se mg/kg in selenomethionine form (Penglase et al. 2014), while neither selenomethionine nor sodium selenite supplementation triggered a response in laboratory strains of the same fish species (Benner et al. 2010). These conflicting results suggest that SEPP1 is not a good indicator of Se status in teleosts. In agreement with this, Sunde and Raines (2011) also reported that *sepp* mRNA may not be a reliable indicator of Se deficiency in rats as its levels are only regulated by Se levels of 0.04 mg/kg diet.

Of all the selenoproteins studied in the current study, only DIO 2 in the muscle of zebrafish displayed a strong response to Se supplementation regardless of oxidative status. Three DIOs have been identified: DIO1 and 2 are termed as the outer ring deiodinases and catalyze the deiodination of T_4 , whereas DIO3 converts T_4 into reverse T_3 . In addition, DIOs have specific tissue localization, with DIO2 being mainly located in the brain, pituitary, thyroid, skeletal muscle and brown adipose tissue in mammals (reviewed in Lu and Holmgren 2009), which could explain the different expression pattern between liver and muscle observed in the present study. In addition, studies in DIO2 knock-out mice have shown that DIO2 regulates the transcription of many physiologically relevant genes in muscle, including MyoD, the master regulator of the myogenic developmental and regeneration program (Muscat et al. 1994). Although a decrease in



dio2 expression has been associated with a decrease in dietary Se in mammals (Arthur et al. 1993), in the present study, supplementary dietary Se decreased the

expression of this selenoprotein. As previously stated, the diets employed in the present report contained Se from the inclusion of fish meal and thus deficiency

Fig. 2 Expression of selenoproteins and related genes in zebrafish liver after 8 weeks of feeding the experimental diets. Results are normalized expression ratios (average \pm SE, $n = 6$) of the expression of these genes in fish fed the different diets in relation to fish fed RO diet. Diets contain either rapeseed oil alone (RO), in combination with concentrated fish oil (DHA) and extra supplementation of Se (RO + Se and DHA + Se). *gpx* glutathione peroxidase, *txnr1* thioredoxin reductase 1, *dio2* deiodinase type II, *sepp1a* selenoprotein P1, *sepw* selenoprotein w, *sep15* 15 kDa selenoprotein, *sps2* selenophosphate synthetase 2, *secp43* tRNA selenocysteine 1-associated protein 1b, *b2m* beta-2-microglobulin, *bact* beta-actin

conditions were not induced by any of the dietary treatments. Therefore, this may suggest that supranutritional Se intakes do not increase the activity/expression of selenoproteins as these are homeostatically controlled. Similarly, a study in rats showed that the activities of both DIO1 and GPX were not increased when additional selenomethionine was added in their feeds, but resulted in somewhat lower values (Behne and Kyriakopoulos 1993). Furthermore, feeding cod larvae with rotifers with increased organic Se did not increase *dio3* expression, although *gpx* mRNA expression and activity were enhanced (Penglase et al. 2010). Although increased dietary Se supplementation did not generally affect the expression of the studied selenoproteins, fish were capable of retaining these essential micronutrients. This could simply reflect the non-specific incorporation of this trace mineral in place of methionine and could serve as a storage pool for periods of suboptimal supply.

Limited information exists regarding the regulation of selenoprotein expression under altered oxidative status, which was one of the aims of the present study. A clear pattern was not observed regarding gene expression in either tissue (liver and muscle), and

generally, few differences were found in fish fed the RO + Se diet. *Secp43* and *sps2*, two enzymes involved in selenoprotein synthesis, showed a correlation with most of the selenoproteins evaluated, whereby the lowest expression found was in fish fed the high oxidation risk feeds. For instance, muscle *sepw* was down-regulated in fish fed high-DHA diets regardless of Se supplementation. These results are in agreement with Papp et al. (2006) who demonstrated that HeLa cells subjected to oxidative stress displayed an inhibition in the synthesis of selenoproteins due to a translocation of the SECIS-binding protein 2 from the cytoplasm to the nucleus. However, in the present study, RO + Se-fed fish showed down-regulation in some of these genes. In accordance, Benner et al. (2010) found that adding selenomethionine to feeds did not enhance the expression of *sepp1a*, *secp43* or *dio2* in the brain of certain strains of zebrafish when compared to the control treatment when using similar levels of Se to those used in the present study. This appears to indicate that supernutritional dietary levels of Se do not further increase the mRNA transcripts of the studied selenoproteins and that when there is an adequate pool of selenomethionine, down-regulation in selenoprotein synthesis occurs. Similarly, *gpx1*, *gpx4*, *txnr1*, *sepw* and *selh* expression was not enhanced when dietary Se was supplemented to rats (Sunde and Raines 2011).

Another factor to take into account is the possible transcriptional and post-transcriptional regulation of selenoproteins by DHA. LC-PUFA have been reported to affect a large number of cellular systems and functions such as regulation of ion channels, membrane trafficking or protein acylation (Pégorier et al. 2004). In this sense, previous in vitro studies have shown regulation of activity and gene expression of

Table 6 Pearson's correlation coefficients of *secp43* and *sps2* with the seven selenoprotein genes in the two studied tissues

	<i>sepp1</i>	<i>gpx3</i>	<i>gpx4b</i>	<i>dio2</i>	<i>sepw</i>	<i>sep15</i>	<i>txnr1</i>
Liver							
<i>secp43</i>	0.79**	0.65**	0.84**	0.74**	0.75**	0.86**	0.57**
<i>sps2</i>	0.70**	0.72**	0.68**	0.74**	0.71**	0.82**	0.81**
Muscle							
<i>secp43</i>	0.77**	0.32	0.48*	0.62**	0.60**	0.71**	0.84**
<i>sps2</i>	0.60**	0.19	0.46*	0.61**	0.42*	0.55**	0.84**

Correlation values (≥ 0.5 = high correlation; 0.35–0.49 = medium correlation). Data from the four dietary treatments were used

Asterisks indicate statistical significances: * $P < 0.05$; ** $P < 0.01$

both *gpx1* (Crosby et al. 1996; Wahle and Rotondo 1999) and *gpx4* (Sneddon et al. 2003) by DHA, but also by arachidonic and eicosapentaenoic acids. In the present study, there was not an obvious regulation of *gpx3* or *gpx4b* gene expression in liver or muscle by DHA, but it would be feasible to think that DHA may regulate other selenoproteins. In fact, DHA was only shown to differentially regulate *sepw* in muscle, where a down-regulation was observed when comparing DHA- to RO-fed fish. *sepw* is highly expressed in skeletal muscle, and although its role has not been clearly elucidated, it is thought to protect muscle from oxidative damage due to its ability to bind glutathione (Stoytcheva and Berry 2009). Furthermore, *sepw* is a target gene of the transcription factors MTF-1 and Sp1, and it is known that LC-PUFA can reduce the DNA-binding activities of Sp1 (Clarke 2001) which could lead to a reduction in the abundance of their target genes.

Overall, the present study demonstrated that high dietary DHA levels (around 19 %) increased zebrafish oxidative risk, although Se addition mitigated this situation. However, analysis of the expression of eight different selenoprotein genes in liver and muscle did not clearly identify the mechanisms by which Se exerts its antioxidant protection. Studies in mammals showed that selenoprotein transcripts were not regulated by supranutritional Se dietary levels, and in the present study, the diets may exceed the Se requirements of zebrafish, estimated to be 0.3 mg/kg for juvenile zebrafish (Penglase et al. 2014). Thus, the data from the current study suggest that the oxidative stress protection associated with high dietary intake of Se may not be mediated by transcriptional changes but that regulation could be at the protein level. In addition, a possible role for the regulation of selenoprotein expression by dietary DHA in teleost appears possible. These results further indicate that additional study of the selenoproteome of teleost species with several levels of dietary Se supplementation would be necessary to elucidate the mechanisms of action of Se antioxidant protection in fish.

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Conflict of interest The authors report no conflicts of interest.

Ethical standard The authors confirm that there are no potential conflicts of interests. Fish were treated in accordance with British national ethical requirements and the experiments conducted under the UK Government Home Office Project licence number PPL 60/03969 in accordance with the amended Animals Scientific Procedures Act 1986 implementing EU directive 2010/63.

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