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Effects of different dietary selenium sources on growth performance, liver and muscle composition, antioxidant status, stress response and expression of related genes in gilthead seabream (*Sparus aurata*)

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ABSTRACT

The present study aimed to determine the effects of dietary inclusion of selenium (Se) in the form of either inorganic Se (sodium selenite, NaSe) or organic Se (hydroxy-selenomethionine, OH-SeMet) on gilthead seabream (*Sparus aurata*). Triplicate groups of 150 fish each (6.2 ± 0.04 g initial body weight) were distributed in 15 tanks and fed one of five experimental diets for 63 days. A control diet (C) without Se supplementation (0.8 mg Se/kg diet), was formulated and served as a basal diet for the other 4 experimental feeds, supplemented with either 0.2 or 0.5 mg Se/kg supplied in the form of NaSe or OH-SeMet. Fish growth performance, biochemical composition of liver and muscle and Se content, malondialdehyde (MDA) concentration in muscle and hepatopancreas, hepatocyte morphology and lysozyme activity in the serum were studied. Furthermore, expression of related hepatic genes, such as manganese superoxide dismutase (Mn-*sod*), catalase (*cat*), heat shock protein 70 (*hsp70*), and glutathione peroxidase (*gpx*) was also analyzed. After the trial, fish were exposed to an acute and chronic confinement stress. Blood samples for cortisol analysis were taken at 0 and 2 h after the acute stress and after 7 days of chronic stress.

The highest growth rate was observed in fish fed OH-SeMet at a level of 0.2 mg/kg, but with no significant difference with fish fed the control diet with no-added Se. The lowest growth was observed in fish fed NaSe up to 0.5 mg/kg. Increase in dietary Se, particularly in the form of OH-SeMet, led to an increase in Se contents in liver and muscle. Furthermore, dietary inclusion of OH-SeMet, led to a significant ($p < .05$) reduction in MDA in both liver and muscle. Se inclusion as NaSe at 0.2 mg/kg was not as effective as organic Se to improve muscle oxidative status. Dietary inclusion of Se at 0.2 mg/kg significantly reduced plasma cortisol after 2 h of acute stress, regardless of the Se form fed. Lysozyme activity measured in the serum was decreased with the increase in dietary Se supplementation.

In summary, supplementation of Se up to 0.2 mg/kg (1–1.1 mg/kg analyzed dietary Se), particularly in the form of OH-SeMet, led to a beneficial effect on growth, maintenance of hepatic morphology and improved protection of juvenile gilthead seabream from acute or chronic stress. Besides, OH-SeMet was found to be more effective than NaSe in protection against oxidative stress in fish muscle.

1. Introduction

Selenium (Se) is an essential trace element for fish (Watanabe et al., 1997; NRC, 2011), important for growth. The central role of Se is the conservation of biological compounds, especially DNA, proteins and lipids, exerting protection against free radicals resulting from normal metabolism (Rider et al., 2009). Se is essential for the functioning of selenoproteins, especially for Se-dependent glutathione peroxidase (GPX) (Antony Jesu Prabhu et al., 2014), an enzyme involved in protecting cells against oxidative damage (Toppo et al., 2008). This enzyme complex made of several isoenzymes, uses the reduced glutathione to catalyze the reduction of peroxides and hydroperoxides and converts it into more stable alcohols and water (Halliwell and Gutteridge, 2007). Thus, the hepatic GPX activity has been suggested as

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a relevant indicator of the Se status in fish (Antony Jesu Prabhu et al., 2014). For instance, a linear regression relationship was found between hepatic GPX activity and hepatic or whole body Se concentration in channel catfish, *Ictalurus punctatus* (Gatlin III and Wilson, 1984), Atlantic salmon, *Salmo salar* (Bell et al., 1987), grouper, *Epinephelus malabaricus* (Lin and Shiau, 2005), Gibel carp, *Carassius gibelio* (Lin and Shiau, 2007) or African catfish, *Clarias gariepinus* (Abdel-Tawwab et al., 2007). Se is also involved in maintaining adequate skeletal development. Thus, the expression of bone morphogenetic protein (*bmp*) and osteocalcin (*oc*) genes, which encode for two important proteins involved in bone cells differentiation and mineralization has been shown to be strongly dependent on dietary Se inclusion (Saleh et al., 2014; Terova et al., 2018). Moreover, Se is known to be involved in the regulation of expression of different genes involved in phospholipid synthesis, redox reactions, and immune response (Persson-Moschos et al., 2000; Behne and Kyriakopoulos, 2001; Schweizer et al., 2011).

Se deficiency can induce loss of appetite, reduced growth, increased mortality, decreased muscular tone, oxidative damage to cells and membranes by decreasing GPX activity and fish defense functions (Felton et al., 1996; Watanabe et al., 1997; Poston and Combs, 1979; Lin and Shiau, 2005; Deng et al., 2007; Wang et al., 2007; Jaramillo Jr et al., 2009). However, like in terrestrial domestic animals, high Se levels may also cause toxicity in fish. Se toxicity has been associated to changes in the intracellular concentration of GPX (Dickson and Tappel, 1969; Han et al., 2011). Nevertheless, the consequences of Se excess or deficiency depend on various factors, such as the species of fish, Se oxidation state and Se concentration in the feed (Pedrero and Madrid, 2009; Han et al., 2011).

Se is contained in different dietary ingredients, it may be specifically supplemented in different forms in the diet and its bioavailability depends on the dietary form of supply (Fairweather-Tait et al., 2010). In fish, Se supplementation through organic forms showed higher bioavailability and tissue retention rate than the inorganic forms (Antony Jesu Prabhu et al., 2014; Le and Fotedar, 2014a, 2014b; Fontagne-Dicharry et al., 2015). Moreover, an increase of dietary Se in organic form showed higher bioavailability and effectiveness in regulating the expression of oxidative stress related genes in seabream (Domínguez et al., 2017). Therefore, the European Food Safety Authority (EFSA), considering the value of other dietary antioxidant nutrient levels (vitamin E and C) and to maintain a good antioxidant status in the fish avoiding excessive levels, recommends a maximum Se content in animal feeds of 0.5 mg/kg (EFSA, 2012) and a maximum supplementation of selenomethionine (Se-Met) as an additive of 0.2 mg/kg (EFSA, 2013).

Sodium selenite (NaSe) is the most common and traditional inorganic source of Se added to animal feeds, including fish feeds (Surai, 2006). Nevertheless, new organic forms such as hydroxy-selenomethionine (OH-SeMet) have been recently developed to improve Se bioavailability. The efficacy of this new organic Se source has been studied in poultry (Briens et al., 2013; Jlali et al., 2013) as well as in pigs (Jlali et al., 2014), but so far, no studies have been undertaken with marine fish. Therefore, in the present study we investigated the effect of OH-SeMet in comparison to NaSe on growth performance, antioxidant status, and resistance to stress of gilthead seabream (*Sparus aurata*).

2. Materials and methods

2.1. Experimental animals and trial conditions

Seven hundred fifty fish (6.16 ± 0.04 g body weight and 7.65 ± 0.01 cm total length) were randomly distributed into 15 tanks (250 l each). Tanks were supplied with fresh seawater (36 ppt) with continuous aeration and kept under a natural photoperiod (approximately 12 L:12D). Temperature and dissolved O_2 were measured daily with a multiparametric probe (Oxy Guard, Zeigler Bros, Gardners, USA), whereas pH was weekly measured with pH-meter (GLP 21+, CRISON, Barcelona, Spain). Water temperature, dissolved oxygen and pH along the whole trial were 23.3 ± 0.5 °C, 5.6 ± 0.0 mg/l and 7.8 ± 0.1, respectively. Seawater from the tanks was sampled and collected in small tubes to determine Se concentration at four different points during the feeding trial. At the beginning of the experiment, water samples were taken from the 15 tanks. One week after starting the feeding trial, 15 other water samples were collected by filling small tubes at 8:00 am before starting to feed the fishes, and 30 min after feeding them 15 other samples were collected. In addition, at the end of the experiment, 15 other samples were taken. In total, the 60 water samples (15 samples ∗ 4) were stored in a cool dark room until analysis.

Table 1
Formulation and analyzed composition of experimental diets containing different Se sources.

Ingredients	Diet				
	C	NaSe 0.2	NaSe 0.5	OH-SeMet 0.2	OH-SeMet 0.5
Peruvian fishmeal	10.0	10.0	10.0	10.0	10.0
Squid meal[a]	2.0	2.0	2.0	2.0	2.0
Blood meal[b]	4.5	4.5	4.5	4.5	4.5
Rapeseed meal[c]	12.7	12.7	12.7	12.7	12.7
Corn gluten meal	12.2	12.2	12.2	12.2	12.2
Soy protein concentrate[d]	18.3	18.3	18.3	18.3	18.3
Wheat meal	5.7	5.7	5.7	5.7	5.7
Wheat gluten	12.2	12.2	12.2	12.2	12.2
Fish oil	6.0	6.0	6.0	6.0	6.0
Rapeseed oil	4.0	4.0	4.0	4.0	4.0
Linseed oil	4.0	4.0	4.0	4.0	4.0
Soy lecithin[e]	1.5	1.5	1.5	1.5	1.5
Vitamin mix[f]	2.0	2.0	2.0	2.0	2.0
Mineral mix[g]	2.0	2.0	2.0	2.0	2.0
NaSe	–	0.2	0.5	–	–
OH-SeMet	–	–	–	0.2	0.5
Ca $(H_2PO_4)_2$	1.6	1.6	1.6	1.6	1.6
L-Lysine	0.46	0.5	0.5	0.5	0.5
DL-Methionine	0.06	0.06	0.06	0.06	0.06
L-Histidine	0.1	0.1	0.1	0.1	0.1
Cholesterol[h]	0.1	0.1	0.1	0.1	0.1
Carboxymethylcellulose	0.5	0.5	0.5	0.5	0.5
α-Cellulose	6.9	6.9	6.9	6.9	6.9
Analytical composition % dry matter (DM)					
Selenium (mg /kg diet)	0.8	1.0	1.3	1.1	1.4
Protein, % DM	43.0	44.6	43.2	43.4	43.6
Lipids, % DM	16.9	16.3	17.8	18.2	17.8
Ash, % DM	7.3	7.4	7.0	7.4	7.4

[a] Squid meal (Skretting, Spain).
[b] Blood meal (Porcine origen, ceded by Dibaq, Spain.
[c] Rapeseed meal (0.0 glucosinalates, ceded by Dibaq, Spain).
[d] Soycomil PC (Sopropeche, France).
[e] Soy lecithin (Deliply, Spain).
[f] Vitamin mixture (% premix); Thiamine (B1), 0.04; Riboflavin (B2), 0.05; Pyridoxine (B6), 0.04; Calcium pantothenate, 0.1169; Nicotinic acid, 0.2; Biotin (H), 0.001; Folic acid, 0.01; Cyanocobalamine (B12), 0.0005, Choline, 2.7; Myo-Inosiol, 2; Ascorbic acid (Sigma Aldrich, free form) (C), 5; γ-tocopherol (E), 0.02; Menadione (K3), 0.005; Cholecalciferol (D3), 0.025; Retinol acetate (A), 0.1, α-Cellulose, qsp.
[g] Mineral mixture (% premix) (Sigma, USA); $(H_2PO)_2Ca$, 1.605; $CaCO_3$, 4; $FeSO_47H_2O$, 1.5; $MgSO_47H_2O$, 1.605; K_2HPO_4, 2.8; $Na_2PO_4H_2O$, 1; Al $(SO_4)_36H_2O$, 0.02; $ZnSO_47H_2O$, 0.28; $CuSO_45H_2O$, 0.12; $MnSO_4H_2O$, 0.08; KI, 0.02; $CoSO_47H_2O$, 0.08; α-Cellulose, qsp.
[h] Cholesterol (96.3%, C27H46O; Acofarma, Spain).

2.2. Diets and feeding

Five isonitrogenous and isoenergetic practical diets were formulated with low levels of fish meal (10% FM) and fish oil (6% FO) and supplemented or not with one of two different Se sources (organic and inorganic). Two different inclusion levels of Se (0.2 and 0.5 mg/kg)

(Table 1) were tested according to the maximum levels of total Se in animal feeds (including fish feed) allowed by European legislation (EFSA, 2013). Thus, the control diet had no added Se (C), two diets were supplemented with NaSe at 0.2 or 0.5 mg Se/kg, and other two diets with OH-SeMet (Selisseo®, Adisseo, France) at 0.2 and 0.5 mg Se/kg. The diets were pelleted using a California Pellet Mill (CPM, 2HP mod 8.3 USA). The diets were afterward dried at 40 °C for 24 h and then stored in a cool dark room.

Fish were manually fed with approximately 25% of the daily ration four times per day (9:00 am, 12:00 pm, 2:00 pm and 4:00 pm, 6 days per week) for 63 days. The fish were fed slowly to ensure that all animals would have access to feed and no uneaten feed would remain in the tanks. The amount of feed consumed was daily recorded to determine the feed intake. During the trial, dead fish, if any, were collected, identified and weighed to calculate the survival rate.

2.3. Growth performance

Fish were anesthetized with clove oil (4%) (Guinama S.L.U., Valencia, Spain) and weighed every 3 weeks, and daily rations were adjusted accordingly. Weight gain (WG), Feed Efficiency (FE), Thermal Growth Coefficient (TGC), Condition Factor (K), Hepatosomatic Index (HSI) and survival were calculated using the following formulae: TGC = (W2^1/3 – W1^1/3)/ temp * days * 1000; WG = [(W2-W1)/W1] * 100, K = (W/L^3) * 100, FE = wet weight gain / dry feed intake, Survival (%) = (N° final fish/ N° initial fish) * 100; where W1: initial weight (g), W2: final weight (g), L: length (cm), Temp: temperature (°C). At the end of the feeding trial 5 fish/tank were randomly selected, anesthetized and sacrificed with an excess dose of clove oil. Samples of liver and muscle were collected to perform the following analyses: proteins, lipid, ash, moisture, fatty acid methyl esters (FAMEs), thiobarbituric acid reactive substances (TBARs), and total Se concentration. Furthermore, prior to stress challenge, liver samples were taken from 6 fish/tank for the molecular study. In addition, blood samples were sampled from 5 fish/tank for the analyses of immune parameters and livers were collected from 5 fish/tank for histological studies.

2.4. Stress resistance

After the feeding trial (63 days), gilthead seabream juveniles were subjected to three different stressful situations by persecution, handling and confinement (2 h), followed by a chronic confinement stress (7d). A total of 18 fish per tank (6 fish/ tank/ measurement point: 0 h–2 h and 7d) were handled and confined in 2.4 l plastic net cages to be sampled for blood at 0 h, 2 h and 7 days. At the point 0 h, six fishes from each tank were captured and blood samples were taken for the cortisol measurement, in the meantime, 12 other fishes from each tank (6 fish/net cage) were captured and confined in small cages (for 2 h and 7 days sampling). After two hours from the confinement, cages were collected one by one and fishes were taken out from the cage and blood samples were taken. Same after 7 days of confinement. Fish were fed twice per day during the stress challenge. Blood was obtained from all the 6 fish of each cage in < 4 min to avoid further increase in plasma cortisol and immediately stored in iced cold heparinized tubes. Plasma obtained by centrifugation (5000 rpm, 10 min) was used for cortisol analyses by immunoassay (Access Immunoassays system, Cortisol ref.: 33600, Beckman Coulter, Inc., USA), whereas serum (3000 rpm, 10 min) was used for lysozyme activity measurements (Parry Jr et al., 1965).

2.5. Biochemical analysis

Crude protein content was determined as N x 6.25, according to the Kjeldahl technique (AOAC, 2000), total crude lipid content following the method of Folch et al. (1957), ash content by incineration at 600 °C (AOAC, 2000), and moisture level by drying at 110 °C until constant weight (AOAC, 2000). Transmethylation (Christie, 1989) was used to obtain fatty acid methyl esters of the lipids, which were then separated by gas liquid chromatography according to the method described by Izquierdo et al. (1990). Malondialdehyde (MDA) concentration in muscle and hepatopancreas (liver) was determined following the method of Burk et al. (1980).

2.6. Selenium determination

Total Se concentration was determined in water, liver and muscle, and analysis was carried out in the UT2A (Helioparc Pau, France), using the method described by Mester et al. (2006). For the digestion, samples were acidified with 7 ml of high purity nitric acid and 0.2 ml hydrogen peroxides (ASC grade) and digested afterwards in the CEM MDS-2100 microwave oven. A combination of a ThermoFinnigan Element2 sector field ICP-MS (SF-ICP-MS; Bremen, Germany) with a Scott-type double-pass glass spray chamber fitted with a PFA self-aspirating nebulizer (Elemental Scientific, Omaha, NE), along with a plug-in quartz torch and a sapphire was used for total Se content measurement.

2.7. Liver morphology

Prior to the stress challenge, five fish per tank were sampled for the histological study of the livers, which were kept in 10% buffered formaldehyde and stained with hematoxylin-eosin (Martoja and Martoja-Pierson, 1970). Samples were checked for: nucleus size, necrosis, lipid accumulation, hepatocytes dimorphism and pyknosis (Table 2). A double evaluation was conducted based on a range from 0 to 3 assigned to each feature, considering the score 0 as normal liver histomorphology, and score 3 as severely affected (Caballero et al., 2004).

2.8. Gene expression

Prior to the stress challenge, 6 seabream juveniles per tank were selected and liver samples were collected and conserved in RNA Later (Sigma-Aldrich, Madrid, Spain) overnight at 4 °C then RNA Later was removed, and samples were kept at 80 °C until RNA extraction. Total RNA extraction was realized using the RNeasy Mini Kit (Qiagen) following the manufacturer's protocol. Firstly, the six livers were divided in two groups of 3 samples each. Then, the 3 livers were pooled and homogenized, to end up with 2 samples of one hundred milligrams from each tank. Afterwards, total tissues were homogenized using the TissueLyser-II (Qiagen, Hilden, Germany) by adding 1 ml of tri-reagent per each 50–100 mg of tissue. Samples were centrifuged with chloroform to separate the RNA from the remains of DNA, proteins and reagents (12,000 G, 15 min, 4 °C). The upper aqueous phase containing RNA was mixed with 70% ethanol (ETOH 70%) (600 µl RNA + 600 µl ETOH 70%), then transferred into a RNeasy spin column. From this point, a series of buffers (RW1 and RPE) have been added to obtain RNA without contaminants. Total purified RNA concentration and its quality was measured by NanoDrop 1000 spectrophotometer (ThermoScientific, Wilmington, DE, EE.UU.) at 260 and 280 nm of absorbance and by electrophoresis (1.4% agarose gel).

Synthesis of cDNA was carried out using the iScript cDNA Synthesis Kit (Bio-Rad) following manufacturer's instructions. First, the RNA was diluted to obtain a concentration of 0.5 µg/µl (CiVi = CfVf) and a volume of 10 µl Afterwards, the denaturation of the purified RNA was realized with the thermal cycler iCycler (Bio-Rad, Hercules, CA, EU.UU.) by adding 13 µl of RNase-free water for each 2 µl of samples. Subsequently, cDNA synthesis was performed in a thermal cycler, using the iScript cDNA synthesis kit (Bio-Rad). Once the samples were synthesized, they were diluted with sterile Milli-Q water at a 1:10.

Real-time quantitative PCR reactions were all performed in an iQ™5 Multicolor Real-Time PCR detection system (Bio-Rad, Hercules, CA, USA) using ribosomal protein L27 (*rpl27*) as the housekeeping gene in a final volume of 20 ml per reaction. PCR primer sequences used for the transcripts quantification encoding rpl27, and the target genes such as

Table 2
Evaluation of histological changes in livers from sea bream juveniles.

Range	Lipid accumulation	Pyknosis	Necrosis	Sinusoids dilation	Hepatocytes dimorphism
0	Lack of intracellular fat	Normal nucleus form in a central position	Normal cells with vacuolated cytoplasm and characteristic nucleus	Lack of enlargement of the hepatic capillaries	Homogeneity in hepatocytes size
1	Discrete accumulation of intracytoplasmic fat	Presence of minor proportion of shrunken nucleus	Few dead cells were present with defined cytoplasm margin	Proportion between plasma and cells	A few differences in cells size
2	Nucleus slightly displaced to the cellular periphery	Slight degenerative condition of cell's nucleus	Minor number of broken cells and contracted cytoplasm	Slight presence of sinusoids dilation	Slight heterogeneity in cells size
3	Marked nuclear displacement, transparent cytoplasm and severity of lipid infiltration	Severe chromatin condensing and shrinkage of the nucleus	Higher magnification of degenerated area, necrosis clearly differentiated	Severe enlargement of the hepatic capillaries	Severe heterogeneity in hepatocytes size

0 = normal liver histomorphology, 1 = few alterations, 2 = medium alterations and 3 = severe alterations.

glutathione peroxidase (*gpx*), manganese superoxide dismutase (*Mn-sod*), heat shock protein 70 (*hsp70*) and catalase (*cat*) are reported in Table 3. The efficiency of the primer sequences was elaborated and evaluated: each gene sample was analyzed once per gene and each assay was performed in duplicate. For *gpx*, *Mn-sod* and *cat*, cycling conditions for enzyme denaturation were the followings: 95 °C for 3 min and 30 s, followed by 40 cycles of 95 °C for 15 s, 58.1 °C for 30 s, and 72 °C for 30 s; 95 °C for 1 min and final step 58 °C for 10 s. The efficiency of the primers sequences was previously evaluated. The Ct values obtained by real time PCR amplification were used to create standard curves for target genes and Livak method ($2^{-\Delta\Delta CT}$) was used to determine relative mRNA expression.

2.9. Statistical analyses

Data were analyzed using SPSS v21 software (IBM Corp., Chicago, IL, USA) and the results are presented as mean and standard deviation (SD) of three replicates. All data were tested for normality and homogeneity of variance. The effects of dietary Se source were analyzed by one-way ANOVA. To determine differences between treatments, means were compared using post hoc Tukey test if variances were the same, or Games-Howell test whenever variances were different. Differences were considered significant when $p < .05$. In addition, two-way ANOVA was undertaken to check the individual effect of Se source and Se level as well as the interactions between both and *p* values are presented as part of the results tables.

3. Results

Analysis of Se levels in the water showed no significant differences between tanks with fish fed different Se sources and along the feeding trial (average value 3.4 μg/l) (Table 6). After 63 days of feeding, no significant differences in the survival rate were found. Final body weight was higher in fish fed OH-SeMet at 0.2 mg Se/kg than in fish fed NaSe at 0.5 mg Se/kg, without significant differences with fish fed the control diet (Table 4). Relative growth rates followed a trend similar to that of the final body weight: fish fed OH-SeMet at 0.2 mg Se/kg showed higher values than the group of fish fed NaSe at 0.5 mg Se/kg. In terms of feed efficiency, feed intake, and condition factor, no significant differences were found between fish fed the different diets (Table 4). HSI was significantly increased ($p < .05$) by dietary Se supplementation, showing higher values in fish fed OH-SeMet 0.5 diet as compared to the other groups. Liver lipid content was significantly ($p < .05$) lower in fish fed the C diet (18.7 ± 2% fresh weight) than in those fed OH-SeMet (24.1 ± 2.0% fresh weight) (Table 5). No significant differences were found in moisture, protein, and ash levels of liver and muscle from seabream juveniles (Table 5).

The accumulation of Se in muscle and liver after 63 days of feeding depended on the Se concentration in feed and its form of supplementation (Table 6). Indeed, fish fed basal diet with no added Se (containing 0.8 mg Se/kg dry matter), showed a similar value of Se concentration in liver of fish fed basal diet supplemented with NaSe or OH-SeMet at 0.2 and to the group of fish fed NaSe at 0.5 in the case of muscle. In liver, Se concentration was increased by dietary Se supplementation either as NaSe or OH-SeMet, following similar exponential regressions ($y = 0.82\times + 0.52$, $R^2 = 0.97$ for NaSe, and $y = 0.83\times + 0.52$, $R^2 = 0.99$ for OH SeMet). However, higher levels were found in muscle of fish fed diets supplemented with OH-SeMet ($y = 0.83x - 0.28$, $R^2 = 0{,}99$), than in fish fed diets supplemented with NaSe ($y = 0.21x + 0.22$, $R^2 = 0{,}84$). Thus, fish fed diets with OH-SeMet showed significantly higher Se concentrations in muscle than fish fed NaSe.

MDA content was significantly ($p < .05$) reduced by the increase in dietary Se, following an inverse exponential regression ($y = -51.89 \ln(x) + 24.46$, $R^2 = 0.78$) and being higher in liver of fish fed 0.5 NaSe or OH-SeMet than in those fed the C diet (Table 6). Moreover, MDA

Table 3
Sequences of forward and reverse primers (5′-3′) used for PCR quantification of selected genes and accession numbers for *Sparus aurata*.

Gene	Product size (pb)	GenBank accession n°	Forward primer 5′ – 3′	Reverse primer 5′ – 3′
gpx	1156	KC201352.1	CTGACGGGACTCCAAATGAT	GCTTTGAGCCAAAGATCCAG
Mn-sod	133	JQ3088331	AGTGCCTCCTGATATTTCTCCTCTG	CCTGACCTGACCTACGACTATGG
cat	145	JQ308823	ATGGTGTGGGACTTCTGGAG	AGTGGAACTTGCAGTAGAAAC
hsp70	127	EU805481.1	TCC TTC TTG TAC TTG CGC TTG	TTG ACC ATT GAG GAT GGC ATC
rpl27		DQ629167.1	CTGACGGGACTCCAAATGAT	GCTTTGAGCCAAAGATCCAG

Table 4
Growth performance, feed intake, feed efficiency, condition factor, and hepatosomatic index of gilthead seabream fed for 63 days diets with different sources of selenium at two different supplementation levels.

Diet	C	NaSe 0.2	NaSe 0.5	OH-SeMet 0.2	OH-SeMet 0.5	Two-way ANOVA (*p*-value)		
						Se source	Se level	Source * level
Initial weight (g)	6.2 ± 0.5	6.2 ± 0.5	6.2 ± 0.6	6.2 ± 0.6	6.1 ± 0.5	0.6	0.5	0.4
Final weight (g)	23.1 ± 3.2ab	22.7 ± 3.8ab	21.9 ± 3.3b	23.4 ± 3.8a	22.7 ± 3.8ab	0.04	0.7	0.2
Initial length (cm)	7.6 ± 0.3	7.7 ± 0.3	7.7 ± 0.3	7.7 ± 0.3	7.6 ± 0.3	0.5	0.23	0.7
Final length (cm)	11.5 ± 1.0	11.6 ± 1.0	11.4 ± 1.0	11.5 ± 1.3	11.4 ± 0.6	0.8	0.4	0.2
Weight gain (%)	275 ± 6.0a	267 ± 4.5ab	254 ± 11.3b	276 ± 7.4a	272 ± 6.0ab	0.01	0.1	0.4
K	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.0	1.6 ± 0.1	1.6 ± 0.0	0.9	0.7	0.7
Feed efficiency (FE)	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8	0.8	0.7
TGC	0.7 ± 0.0ab	0.7 ± 0.0 ab	0.7 ± 0.0b	0.7 ± 0.0a	0.7 ± 0.0ab	0.01	0.05	0.4
FI (g feed fish − 1 day-1)	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.2	0.3	0.7
HSI (%)	1.6 ± 0.2b	1.8 ± 0.1ab	1.7 ± 0.0ab	1.9 ± 0.2ab	2.0 ± 0.2a	0.02	0.5	0.2
Survival (%)	98.7 ± 1.2	100 ± 0.0	98.7 ± 2.3	98.7 ± 1.2	98.7 ± 2.3	0.5	0.7	0.8

Data presented as means ± SD (n = 3) different letters denote significant differences between fish groups fed different diets ($p < .05$). TGC = (W2ˆ1/3 – W1ˆ1/3)/ temp * days * 1000; FE = wet weight gain / dry feed intake; WG = [(W2-W1)/W1] * 100; K = (W/Lˆ3) * 100; Survival (%) = (N° final fish/ N° initial fish) * 100.

content was also significantly related to Se contents in liver ($y = 798.78e^{-2.618x}$, $R^2 = 0.870$, $p = .021$). Similarly, MDA content in the muscle was significantly reduced by the increase of dietary Se supplementation and was inversely related to the levels found in both, diet, and muscle. However, dietary inclusion of OH-SeMet at 0.2 mg Se/kg led to a significant reduction in MDA, whereas the NaSe at 0.2 mg Se/kg was not sufficient to significantly reduce MDA, in comparison to fish fed the control diet. On the contrary, no significant differences ($p > .05$) were found in lysozyme activity between fish fed the different experimental diets (Table 7).

The histological examination of hepatic tissue (Table 8) showed a significantly ($p < .05$) higher degree of lipid accumulation, pyknosis, necrosis, erythrocytes dimorphism, and sinusoids dilation, in the liver of fish fed OH-SeMet at 0.5 mg Se/kg diet in comparison to fish fed the other diets.

The gene expression of the antioxidant enzymes as well as the *hsp70* in liver of seabream juveniles did not show significant differences between the different groups (Table 9). Nevertheless, increased supplementation of Se as OH-SeMet up-regulated *gpx* expression following a linear regression ($y = 1.88\times - 0.31$, $R^2 = 0.95$, $p < .05$) and down-regulated Mn-*sod* ($y = -0.45\times + 1.61$, $R^2 = 0.91$). On the contrary, no relation was found between NaSe supplementation and the expression of any of the studied genes.

No significant differences were found in the basal plasma cortisol concentrations measured before the stress challenge (0 h) between fish fed the different diets (Table 10). However, an increase in plasma cortisol levels was observed 2 h after capture and confinement (acute stress). The highest values were found in fish fed diet C, followed by those in fish fed OH-SeMet 0.5, and NaSe 0.5 diets, whereas the lowest values were found in fish fed OH-SeMet up to 0.2 mg Se/kg, and NaSe up to 0.2 mg Se/kg. After 7 days of confinement (chronic stress), there were no significant differences in plasma cortisol concentrations, with slightly high values in fish fed the C diet.

4. Discussion

Survival of gilthead seabream fed either control diet or supplemented diet with Se in the form of NaSe or OH-SeMet (0.8–1.4 mg Se/kg diet) was not affected after 63 days of feeding, in agreement with previous studies conducted in the same or other fish species. For

Table 5
Overview of protein, lipid, ash and moisture content (%) in the liver and muscle of gilthead sea bream fed experimental diets (% fresh weight) (mean ± SD, *n* = 3).

	NC	NaSe 0.2	NaSe 0.5	OH-SeMet 0.5	OH-SeMet 0.2	Two-way ANOVA (*p*-value)		
						Se source	Se level	Source * level
Liver								
Protein	11.0 ± 0.6	11.2 ± 0.7	10.9 ± 0.4	10.8 ± 0.4	10.7 ± 0.3	0.3	0.6	0.4
Lipid	18.7 ± 2.0c	21.6 ± 1.0bc	20.2 ± 2.0bc	24.1 ± 2.0a	23.2 ± 2.0ab	0.003	0.7	0.2
Ash	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.4 ± 0.3	0.4	0.08	0.4
Moisture	66.6 ± 2.0	64.4 ± 3.0	65.9 ± 4.0	62.3 ± 1.0	65.0 ± 3.0	0.2	0.7	0.5
Muscle								
Protein	19.6 ± 0.5	19.2 ± 0.4	19.5 ± 0.3	18.9 ± 1.0	19.2 ± 0.8	0.5	0.4	0.5
Lipid	4.6 ± 0.6	4.3 ± 1	5.1 ± 0.4	5.9 ± 1.0	5.5 ± 0.6	0.1	0.07	0.5
Ash	1.6 ± 0.4a	1.5 ± 0.1b	1.5 ± 0.5ab	1.5 ± 0.1b	1.5 ± 0.1ab	0.02	0.5	0.9
Moisture	76.9 ± 1.0a	75.5 ± 3.0ab	73.5 ± 1.0b	73.5 ± 1.0b	72.9 ± 1.0b	0.04	0.3	0.04

Table 6
Selenium content in water, in the liver and muscle (mg/kg) of gilthead seabream juveniles fed experimental diets for 63 days.

Diet	C	NaSe 0.2	NaSe 0.5	OH-SeMet 0.2	OH-SeMet 0.5	Two-way ANOVA (*p*-value)		
						Se source	Se level	Source * level
Dietary Se (mg/kg DM)	0.8	1.0	1.3	1.1	1.4			
Water Se (µg/L)	3.0 ± 0.1	3.6 ± 0.2	3.4 ± 0.4	3.2 ± 0.2	3.4 ± 0.2	0.5	0.38	0.2
Liver Se (mg/kg fresh weight)	1.2 ± 0.1c	1.3 ± 0.0cb	1.6 ± 0.1ab	1.4 ± 0.2abc	1.7 ± 0.2a	0.2	0.004	0.9
Muscle Se (mg/kg fresh weight)	0.4 ± 0.0c	0.4 ± 0.0c	0.5 ± 0.0c	0.6 ± 0.0b	0.9 ± 0.0a	0.00	0.05	0.01

Data are presented as means ± SD (n = 3), different letters denote significant differences between fish groups fed different diets ($p < .05$).

Table 7
Lysozyme activity (units/ml serum) and TBARs (MDA nmol/g) in liver and muscle of gilthead seabream fed five experimental diets for 63 days.

Diet	C	NaSe 0.2	NaSe 0.5	OH-SeMet 0.2	OH-SeMet 0.5	Two-way ANOVA (p-value)		
						Se source	Se level	Source * level
Lysozyme 0 h	47.8 ± 24.3	44.6 ± 20.0	44.2 ± 15.8	43.8 ± 20.1	35.0 ± 20.7	0.7	0.5	0.5
MDA in liver	39.2 ± 3.3c	22.0 ± 4.5b	9.5 ± 2.6a	16.1 ± 1.5b	11.2 ± 0.9a	0.02	0.004	0.002
MDA in muscle	3.9 ± 0.4b	3.6 ± 0.2b	3.0 ± 0.4a	2.7 ± 0.2a	2.4 ± 0.4a	0.01	0.07	0.2

Data presented as means ± SD (n = 3), different letters denote significant differences between fish groups fed different diets ($p < .05$).

instance, increase in dietary Se levels from 0.45 to 1.70 mg Se/kg supplemented as NaSe, did not affect survival in gilthead sea bream juveniles (Dominguez et al., accepted). Similarly, survival rate was not affected by increased dietary Se levels supplemented as SeMet in white sturgeon (*Acipenser transmontanus*, 0.04–19.1 mg Se/g diet, Tashjian et al., 2006), cutthroat trout (*Oncorhynchus clarki*, Hardy et al., 2009), and yellowtail kingfish (*Seriola lalandi,* 2.3–20.87 mg Se/kg diet, Le and Fotedar, 2014a, 2014b) or as NaSe (0.97–2.06 mg Se/kg) in largemouth bass, *Micropterus salmoides* (Zhu et al., 2011). In contrast, survival of juveniles of cobia (*Rachycentron canadum L*) was increased by dietary Se supplementation, (0.21–0.8 mg Se/kg, Liu et al., 2010). In the present study, neither feed intake nor feed utilization were significantly affected by dietary Se level or source, in agreement with studies conducted in crucian carp (*Carassius carassius*) (0.5 mg Se kg − 1 as sodium selenite or selenomethionine, Wang et al., 2007), hybrid striped bass (0–3.2 mg kg − 1 as SelPlex or 0.2 and 0.4 mg kg − 1 as sodium selenite, Cotter et al., 2008) or largemouth bass (0.97, 1.17, 1.42, 1.60, 1.85 and 2.06 mg Se kg − 1 diet as sodium selenite, Zhu et al., 2011). The basal levels obtained in the control diet without Se supplementation (0.8 mg Se/kg) were close to the values on requirements reported for gilthead seabream (0.9 mg Se/kg, Domínguez et al., pers. comm), which can possibly explain the lack of effect of dietary supplementation up to 1 mg Se/kg as NaSe on gilthead sea bream performance.

The increase in dietary Se content contributed to markedly increase the Se concentration in liver and to reduce oxidation, as denoted by the reduction in MDA. These results are in line with the positive relation between dietary and hepatic Se content reported in seabream (Dominguez et al., accepted) and in other fish species (Hilton et al., 1980; Gatlin III and Wilson, 1984; Liu et al., 2010). Furthermore, MDA content was effectively decreased with increasing dietary Se level in the different forms, with higher reduction in the hepatic tissue. These results are in agreement with a reduction in seabream larval MDA content when dietary Se was increased up to 11.65 mg Se/kg diet (Saleh et al., 2014). Similarly, Se supplementation in diets for European sea bass (*Dicentrarchus labrax*) markedly enhanced larvae growth performance, improved cell antioxidant capacity, decreased the oxidative risk denoted by the MDA reduction, as well as the incidence of muscular injury (Betancor et al., 2012).

Interestingly, in the present study supplementation with 0.2 mg/kg OH-SeMet (total Se content: 1.1 mg Se/kg) maintained fish growth and increased Se content in the muscle and reduced muscle oxidative stress to a larger extent than NaSe supplemented at 0.2 mg Se/kg. These results denote a higher bioavailability and tissue accumulation when Se is supplemented as OH-SeMet than as NaSe, in agreement with previous studies in other species (Fontagne-Dicharry et al., 2015). Similarly, in yellowtail kingfish, supplementation with 2 mg/kg of seleno-methionine (SeMet) or Se-yeast increased Se content in the muscle in a higher extent than selenocysteine (SeCys) or NaSe (Le and Fotedar, 2014a, 2014b). Also, a higher deposition of Se supplemented as SeMet than as an inorganic form (NaSe) has been found in whole body of hybrid striped bass (Jaramillo Jr et al., 2009). In Atlantic salmon, feeding organic Se as selenium yeast (Se-yeast) also led to a higher deposition of Se in muscle in comparison to NaSe, and it was demonstrated that in organic Se fed fish, SeMet accounts for 90% of the total Se, whereas in fish fed NaSe, SeMet only accounts for 30% (Sele et al., 2018). Because of the higher Se deposition in gilthead sea bream muscle in the present study, oxidative risk was reduced in this tissue, in line with the higher reduction of MDA found in rainbow trout fed SeMet in comparison to selenite forms (Küçükbay et al., 2009).

Several studies have shown that an increase in dietary Se levels is

Table 8
Different microscopic evaluation of liver sections of gilthead seabream fed the five experimental diets for 63 days.

Diet	C	NaSe 0.2	NaSe 0.5	OH-SeMet 0.2	OH-SeMet 0.5	Two-way ANOVA (p-value)		
						Se source	Se level	Source * level
Lipid accumulation	0.8 ± 1.2a	0.9 ± 0.8a	1.1 ± 0.9a	1.3 ± 1.1a	2.1 ± 1.0b	0.04	0.7	0.9
Pyknosis	0.9 ± 0.8a	1.0 ± 0.7a	1.2 ± 0.7a	1.5 ± 0.5ab	2.4 ± 0.5b	0.04	0.07	0.3
Necrosis	0.9 ± 0.7a	1.3 ± 0.8ab	1.4 ± 0.7ab	1.7 ± 0.5ab	2.1 ± 0.6b	0.03	0.1	0.2
Sinusoids dilation	0.5 ± 0.5a	1.1 ± 0.8ab	1.2 ± 0.9ab	1.4 ± 1.0ab	2.1 ± 0.8b	0.009	0.07	0.03
Hepatocytes dimorphism	1.3 ± 0.8a	1.7 ± 0.5a	1.7 ± 0.8a	2.2 ± 0.4ab	2.7 ± 0.5b	0.01	0.7	0.2

Data presented as means ± SD (n = 3), different letters denote significant differences between fish groups fed different diets ($p < .05$)

Table 9
Glutathione peroxidase (*gpx*), manganese superoxide dismutase (Mn-*sod*), catalase (*cat*) and heat shock protein (*hsp70*) genes expression levels (means ± SD, *n* = 3) measured in liver of gilthead seabream fed five experimental diets for 63 days.

Diet	C	NaSe 0.2	NaSe 0.5	OH-SeMet 0.2	OH-SeMet 0.5	Two-way ANOVA (p-value)		
						Se source	Se level	Source * level
gpx	1.3 ± 0.9	1.3 ± 0.9	1.3 ± 1.0	1.6 ± 1.2	2.4 ± 1.1	0.3	0.3	0.5
sod	1.3 ± 0.9	1.7 ± 1.1	0.8 ± 0.3	1.1 ± 0.4	1.0 ± 0.7	0.3	0.6	0.4
cat	0.9 ± 0.5	1.0 ± 0.5	1.2 ± 0.9	1.5 ± 0.9	1.0 ± 0.6	0.3	0.7	0.9
hsp70	1.3 ± 0.9	1.1 ± 0.9	1.4 ± 1.1	1.3 ± 0.0	1.5 ± 0.6	0.06	0.3	0.5

directly related to GPX activity (Gatlin III and Wilson, 1984; Daniels, 1996; Watanabe et al., 1997; Lin and Shiau, 2005; Abdel-Tawwab et al., 2007; Antony Jesu Prabhu et al., 2014; Terova et al., 2018). However, the expression of the gene that codifies for this enzyme (*gpx*) does not show such a strong direct relationship with dietary Se and only very low dietary Se levels in fish larvae (Penglase et al., 2010) or a large difference in dietary Se levels in juveniles seems to significantly up-regulate *gpx* expression (Pacitti et al., 2015; Wang et al., 2018). In the present study, increase of dietary Se from 0.8 to 1.3 mg/kg supplemented as NaSe did not affect liver expression of *gpx*. These results are in agreement with the lack of significant effect of dietary Se (supplemented as NaSe) from 0.45 to 1.7 mg/kg diet on *gpx* expression in gilthead seabream (Dominguez et al., accepted). Interestingly, despite large deviations did not allow to obtain significant differences between means, increased dietary Se levels supplemented as OH-SeMet were linearly related to increased *gpx* expression.

An increase in supplemented OH-SeMet led to an increase in stress tolerance, as shown by the decreased cortisol levels during the stress challenge and disease resistance with reduced mortality of seabream juveniles submitted to stress. Se plays an important role to prevent oxidative stress and, in chinook salmon (*Oncorhynchus tshawytscha*) transport-causing stress has been found to induce a loss of whole body Se (Halver et al., 2004). The levels of plasma cortisol found in the present study were in the range of those previously found for gilthead seabream (Rotllant et al., 2001) or other fish species (Barton, 2002). Although basal cortisol levels were not affected by dietary Se level or source, after a crowding stress, plasma cortisol concentrations increased in all treatments, but progressively reduced along with the increase in dietary Se. Se supplementation has been reported to allow salmonids to better cope with stressful conditions caused by handling (Rider et al., 2009). Dietary organic selenium (in form of selenomethionine) has been shown to be related with amelioration of overcrowding-related deleterious stress effects on rainbow trout (Küçükbay et al., 2009). In the present study, Se supplementation had no significant effect ($p > .05$) on lysozyme activity measured in pre-challenged gilthead seabream juveniles, in line with hybrid striped bass exposed to *Streptococcus iniae* in the study of Jaramillo Jr et al. (2009). Similar results were also obtained in pre-challenged yellowtail kingfish with *Vibrio anguillarum* fed diets supplemented with SeMet. However, post-challenged fish showed an increase in lysozyme activity with increasing dietary Se levels (Le and Fotedar, 2014a, 2014b).

The margin line between required and toxic Se can be very narrow for some species (Khan et al., 2017) and the toxicity signs include reduced growth and feed intake, increased oxidative stress or disturbance of fatty acid metabolism (Berntssen et al., 2017). Increased supplementation with SeMet up to 4.2 mg/kg causes histopathological changes in white sturgeon liver such as hepatocellular vacuolar degeneration and necrosis (Tashjian et al., 2006). In a previous study, undertaken with gilthead seabream, an increase in dietary Se as NaSe from 1.1 to 1.70 mg Se/kg significantly reduced growth (Dominguez et al., accepted). In the present study, supplementation with 0.5 OH-SeMet (providing 1.4 mg Se/kg) led to an increased in HSI, liver lipid content and altered liver morphology, but did not negatively affect growth. These results agree well with the liver histopathological alteration found in Atlantic salmon fed 5.4 mg selenite kg^{-1} or 21 mg SeMet kg^{-}, as denoted by the presence of hepatocyte degeneration and focal necrosis (Berntssen et al., 2018). Further studies must be conducted to determine Se toxicity levels supplemented either as inorganic or organic forms in diets for gilthead sea bream juveniles.

In summary, the present study has shown the importance of dietary Se supplementation on health and welfare in gilthead seabream. Thus, although fish fed a non-supplemented diet (0.8 mg Se/kg) maintained good growth and high survival rates as the other groups fed diets supplemented with two different sources and levels of Se, this dietary Se level was insufficient to protect fish from oxidation and stressful conditions. Supplementation with 0.2 mg/kg OH-SeMet (total Se content: 1.1 mg/kg) provided good fish growth, raised Se contents in muscle and reduced oxidative stress in this tissue in a more effective way than NaSe.

Author contributions

Marwa Mechlaoui prepared the diets with the help of Lidia E. Robaina, ran the feeding trial and the stress challenge experiment, analyzed the samples and the data, prepared the tables and wrote the paper.
David Dominguez helped in fish sampling, analyzed the data and reviewed paper.
Reda Saleh helped in sampling and reviewed the paper.
Sachi Kaushik, Pierre-Andre Geraert and Marisol Izquierdo designed the experiments, analyzed the data, reviewed the paper.
Mickael Briens analyzed Se contents.
Montero Daniel contributed to the stress challenge test.
Lidia E. Robaina formulated and prepared the diets.

Table 10
Plasma cortisol concentrations (ng/dL, means ± SD, n = 3) in gilthead seabream during stress challenge for 15 days at three different sampling points (0 h, 2 h and 7 days).

Diet	C	NaSe 0.2	NaSe 0.5	OH-SeMet 0.2	OH-SeMet 0.5	Two-way ANOVA (p-value)		
						Se source	Se level	Source * level
0 h	20.2 ± 1.4	10.8 ± 1.8	10.2 ± 0.8	20.3 ± 0.7	10.8 ± 0.0	0.4	0.8	0.6
2 h	200.1 ± 12.4a	60.1 ± 3.6b	80.4 ± 9.1ab	50.5 ± 2.8b	90.2 ± 4.3ab	0.01	0.7	0.8
7d	30.2 ± 4.0	17.0 ± 0.1	20.1 ± 0.6	10.0 ± 1.2	16.1 ± 0.0	0.8	0.1	0.9

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References

Abdel-Tawwab, M., Mousa, M., Abbass, F., 2007. Growth performance and physiological response of African catfish, *Clarias gariepinus* (B.) fed organic selenium prior to the exposure to environmental copper toxicity. Aquaculture 272 (1–4), 335–345. https://doi.org/10.1016/j.aquaculture.2007.09.004.

Antony Jesu Prabhu, P., Schrama, J., Kaushik, S., 2014. Mineral requirements of fish: a systematic review. Rev. Aquac. 8 (2), 172–219. https://doi.org/10.1111/raq.12090.

Association of Official Analytical Chemists (AOAC), 2000. Official Methods of Analysis, 17th edition. Association of Official Analytical Chemists, Gaithersburg.

Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Bio. 42 (3), 517–525. https://doi.org/10.1093/icb/42.3.517.

Behne, D., Kyriakopoulos, A., 2001. Mammalian selenium-containing proteins. Annu. Rev. Nutr. 21 (1), 453–473. https://doi.org/10.1146/annurev.nutr.21.1.453.

Bell, J.G., Cowey, C.B., Adron, J.W., Pirie, B.J.S., 1987. Some effects of selenium deficiency on enzyme activities and indices of tissue peroxidation in Atlantic salmon parr (*Salmo* salar). Aquaculture 65 (1), 43–54. https://doi.org/10.1016/0044-8486(87)90269-9.

Berntssen, M., Sundal, T., Olsvik, P., Amlund, H., Rasinger, J., Sele, V., Hamre, K., Hillestad, M., Buttle, L., Ørnsrud, R., 2017. Sensitivity and toxic mode of action of dietary organic and inorganic selenium in Atlantic salmon (Salmo salar). Aquat. Toxicol. 192, 116–126. https://doi.org/10.1016/j.aquatox.2017.09.014.

Berntssen, M., Betancor, M., Caballero, M., Hillestad, M., Rasinger, J., Hamre, K., Sele, V., Amlund, H., Ørnsrud, R., 2018. Safe limits of selenomethionine and selenite supplementation to plant-based Atlantic salmon feeds. Aquaculture 495, 617–630. https://doi.org/10.1016/j.aquaculture.2018.06.041.

Betancor, M.B., Caballero, M., Terova, G., Saleh, R., Atalah, E., Benítez-Santana, T., Izquierdo, M., 2012. Selenium inclusion decreases oxidative stress indicators and muscle injuries in sea bass larvae fed high-DHA microdiets. Br. J. Nutr. 108 (12), 2115–2128. https://doi.org/10.1017/S0007114512000311.

Briens, M., Mercier, Y., Rouffineau, F., Vacchina, V., Geraert, P., 2013. Comparative study of a new organic selenium source v. seleno-yeast and mineral selenium sources on muscle selenium enrichment and selenium digestibility in broiler chickens. Br. J. Nutr. 110 (04), 617–624. https://doi.org/10.1017/S0007114512005545.

Burk, R.F., Trumble, M.J., Lawrence, R.A., 1980. Rat hepatic cytosolic glutathione-dependent enzyme protection against lipid peroxidation in the NADPH-microsomal lipid peroxidation system. Biochim. Biophys. Acta 618 (1), 35–41. https://doi.org/10.1016/0005-2760(80)90051-X.

Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Fernandez, A.J., Rosenlund, G., 2004. Histological alterations in the liver of sea bream, Sparus aurata L., caused by short-or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. J. Fish Dis. 27, 531–541. https://doi.org/10.1111/j.1365-2761.2004.00572.x.

Christie, William W., 1989. Gas Chromatography and Lipids. The Oily Press, Glasgow, UK.

Cotter, P.A., Craig, S.R., McLean, E., 2008. Hyperaccumulation of selenium in hybrid striped bass: a functional food for aquaculture? Aquac. Nutr. 14 (3), 215–222. https://doi.org/10.1111/j.1365-2095.2007.00520.x.

Daniels, L.A., 1996. Selenium metabolism and bioavailability. Biol. Trace Elem. Res. 54 (3), 185–199. https://doi.org/10.1007/BF02784430.

Deng, D.F., Hung, S.S.O., Teh, S.J., 2007. Selenium depuration: residual effects of dietary selenium on Sacramento splittail (*Pogonichthys macrolepidotus*). Sci. Total Environ. 377, 224–232. https://doi.org/10.1016/j.scitotenv.2007.02.025.

Dickson, R.C., Tappel, A.L., 1969. Effects of selenocystine and selenomethionine on activation of sulfhydryl enzymes. Arch. Biochem. Biophys. 131 (1), 100–110. https://doi.org/10.1016/0003-9861(69)90109-X.

Domínguez, D., Rimoldi, S., Robaina, L.E., Torrecillas, S., Terova, G., Zamorano, M.J., Karalazos, V., Hamre, K., Izquierdo, M., 2017. Inorganic, organic, and encapsulated minerals in vegetable meal based diets for *Sparus aurata* (Linnaeus, 1758). PeerJ 5, e3710. https://doi.org/10.7717/peerj.3710.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2012. Scientific opinion on safety and efficacy of selenium in the form of organic compounds produced by the selenium-enriched yeast Saccharomyces cerevisiae NCYC R646 (Selemax 1000/2000) as feed additive for all species. EFSA J. 10 (7), 2778. [17 pp.]. https://doi.org/10.2903/j.efsa.2012.2778.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2013. Scientific opinion on safety and efficacy of hydroxy-analogue of selenomethionine as feed additive for all species. EFSA J. 11 (1), 3046. (30 pp.). https://doi.org/10.2903/j.efsa.2013.3046.

Fairweather-Tait, S.J., Collings, R., Hurst, R., 2010. Selenium bioavailability: current knowledge and future research requirements. Am. J. Clin. Nutr. 91 (5), 1484S–1491S. https://doi.org/10.3945/ajcn.2010.28674J.

Felton, S.P., Landolt, M.L., Grace, R., Palmisano, A., 1996. Effects of selenium dietary enhancement on hatchery-reared coho salmon, *Oncorhynchus kisutch* (Walbaum), when compared with wild coho: hepatic enzymes and seawater adaptation evaluated. Aquac. Res. 27 (2), 135–142. https://doi.org/10.1111/j.1365-2109.1996.tb00977.x.

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226 (1), 497–509.

Fontagne-Dicharry, S., Godin, S., Liu, H.K., Prabhu, P.A.J., Bouyssiere, B., Bueno, M., Tacon, P., Medale, F., Kaushik, S.J., 2015. Influence of the forms and levels of dietary selenium on antioxidant status and oxidative stress-related parameters in rainbow trout (*Oncorhynchus mykiss)* fry. Br. J. Nutr. 113, 1876–1887. https://doi.org/10.1017/S0007114515001300.

Gatlin III, D.M., Wilson, R.P., 1984. Dietary selenium requirement of fingerling channel catfish. J. Nutr. 114 (3), 627–633. https://doi.org/10.1093/jn/114.3.627.

Halliwell, B., Gutteridge, J.M.C., 2007. Reactive species can be poisonous. In: Halliwell, B., Gutteridge, J.M.C. (Eds.), Free Radicals in Biology and Medicine, 4th ed. Oxford University Press, New York, pp. 440–487.

Halver, J.E., Felton, S.M., Zbanyszek, R., 2004. Carcass selenium loss as an indicator of stress in barge transported chinook salmon (*Oncorhynchus tshawytscha Walbaum*) smolts. Aquac. Res. 35 (11), 1099–1103. https://doi.org/10.1111/j.1365-2109.2004.01146.x.

Han, D., Xie, S., Liu, M., Xiao, X., Liu, H., Zhu, X., Yang, Y., 2011. The effects of dietary selenium on growth performances, oxidative stress and tissue selenium concentration of gibel carp (*Carassius auratus gibelio*). Aquac. Nutr. 17 (3), e741–e749. https://doi.org/10.1111/j.1365-2095.2010.00841.x.

Hardy, R., Oram, L., Möller, G., 2009. Effects of dietary selenomethionine on cutthroat trout (*Oncorhynchus clarki bouvieri*) growth and reproductive performance over a life cycle. Arch. Environ. Contam. Toxicol. 58 (1). https://doi.org/10.1007/s00244-009-9392-x.

Hilton, J.W., Hodson, P.V., Slinger, S.J., 1980. The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). J. Nutr. 110, 2527–2535. Options Mediterraneénnes 63, 91–102. https://doi.org/10.1093/jn/110.12.2527.

Izquierdo, M., Watanabe, T., Takeuchi, T., Arakawa, T., Kitajima, C., 1990. Optimum EFA levels in Artemia to meet the EFA requirements of red seabream (*Pagrus major*). In: Takeda, M., Watanabe, T. (Eds.), The Current Status of Fish Nutrition in Aquaculture. Tokyo Univ. Fisheries, Tokyo, pp. 221–232.

Jaramillo Jr., F., Peng, L.I., Gatlin Iii, D.M., 2009. Selenium nutrition of hybrid striped bass (Morone chrysops× M. saxatilis) bioavailability, toxicity and interaction with vitamin E. Aquac. Nutr. 15 (2), 160–165. https://doi.org/10.1111/j.1365-2095.2008.00579.x.

Jlali, M., Briens, M., Rouffineau, F., Mercerand, F., Geraert, P., Mercier, Y., 2013. Effect of 2-hydroxy-4-methylselenobutanoic acid as a dietary selenium supplement to improve the selenium concentration of table eggs1. J. Anim. Sci. 91 (4), 1745–1752. https://doi.org/10.2527/jas2012-5825.

Jlali, M., Briens, M., Rouffineau, F., Geraert, P., Mercier, Y., 2014. Evaluation of the efficacy of 2-hydroxy-4-methylselenobutanoic acid on growth performance and tissue selenium retention in growing pigs1. J. Anim. Sci. 92 (1), 182–188. https://doi.org/10.2527/jas2013-6783.

Khan, K.U., Zuberi, A., Nazir, S., Ullah, I., Jamil, Z., Sarwar, H., 2017. Synergistic effects of dietary nano selenium and vitamin C on growth, feeding, and physiological parameters of mahseer fish (*Tor putitora*). Aquacult. Rep. 5, 70–75. https://doi.org/10.1016/j.aqrep.2017.01.002.

Küçükbay, F.Z., Yazlak, H., Karaca, I., Sahin, N., Tuzcu, M., Cakmak, M.N., Sahin, K., 2009. The effects of dietary organic or inorganic selenium in rainbow trout (*Oncorhynchus mykiss)* under crowding conditions. Aquac. Nutr. 15 (6), 569–576. https://doi.org/10.1111/j.1365-2095.2008.00624.x.

Le, K., Fotedar, R., 2014a. Bioavailability of selenium from different dietary sources in yellowtail kingfish (*Seriola lalandi*). Aquaculture 420-421, 57–62. https://doi.org/10.1016/j.aquaculture.2013.10.034.

Le, K., Fotedar, R., 2014b. Toxic effects of excessive levels of dietary selenium in juvenile yellowtail kingfish (*Seriola lalandi*). Aquaculture 433, 229–234. https://doi.org/10.1016/j.aquaculture.2014.06.021.

Lin, Y.H., Shiau, S.Y., 2005. Dietary selenium requirement of grouper, *Epinephelus malabaricus*. Aquaculture 250, 356–363. https://doi.org/10.1016/j.aquaculture.2005.03.022.

Lin, Y., Shiau, S., 2007. The effects of dietary selenium on the oxidative stress of grouper, *Epinephelus malabaricus*, fed high copper. Aquaculture 267 (1–4), 38–43. https://doi.org/10.1016/j.aquaculture.2006.12.015.

Liu, K., Wang, X., Ai, Q., Mai, K., Zhang, W., 2010. Dietary selenium requirement for juvenile cobia*, Rachycentron canadum* L. Aquac. Res. https://doi.org/10.1111/j.1365-2109.2010.02562.x. no-no.

Martoja R., Martoja-Pierson M. 1970. Tecnicas de Histologıa Animal Toray-Masson S.A., (Barcelona, 350 pp.).

Mester, Z., Willie, S., Yang, L., Sturgeon, R., Caruso, J., Fernández, M., Fodor, P., Goldschmidt, R., Goenaga-Infante, H., Lobinski, R., Maxwell, P., McSheehy, S., Polatajko, A., Sadi, B., Sanz-Medel, A., Scriver, C., Szpunar, J., Wahlen, R., Wolf, W., 2006. Certification of a new selenized yeast reference material (SELM-1) for methionine, selenomethinone and total selenium content and its use in an intercomparison exercise for quantifying these analytes. Anal. Bioanal. Chem. 385 (1), 168–180. https://doi.org/10.1007/s00216-006-0338-0.

NRC, 2011. Nutrient Requirements of Fish and Shrimp. National Academy Press, Washington.

Pacitti, D., Lawan, M.M., Sweetman, J., Martin, S.A., Feldmann, J., Secombes, C.J., 2015. Selenium supplementation in fish: a combined chemical and biomolecular study to understand Sel-Plex assimilation and impact on selenoproteome expression in rainbow trout (Oncorhynchus mykiss). PLoS One 10 (5), e0127041. https://doi.org/10.1371/journal.pone.0127041.

Parry Jr., R.M., Chandan, R.C., Shahani, K.M., 1965. A rapid and sensitive assay of muramidase. Proc. Soc. Exp. Biol. Med. 119 (2), 384–386. https://doi.org/10.3181/00379727-119-30188.

Pedrero, Z., Madrid, Y., 2009. Novel approaches for selenium speciation in foodstuffs and biological specimens: a review. Anal. Chim. Acta 634 (2), 135–152. https://doi.org/10.1016/j.aca.2008.12.026.

Penglase, S., Nordgreen, A., van der Meeren, T., Olsvik, P., Sæle, Ø., Sweetman, J., Baeverfjord, G., Helland, S., Hamre, K., 2010. Increasing the level of selenium in rotifers (*Brachionus plicatilis* 'Cayman') enhances the mRNA expression and activity of glutathione peroxidase in cod (*Gadus morhua* L.) larvae. Aquaculture 306 (1–4), 259–269. https://doi.org/10.1016/j.aquaculture.2010.05.011.

Persson-Moschos, M.E., Stavenow, L., Åkesson, B., Lindgärde, F., 2000. Selenoprotein P in plasma in relation to cancer morbidity in middle-aged Swedish men. Nutr. Cancer 36 (1), 19–26. https://doi.org/10.1207/S15327914NC3601_4.

Poston, H.A., Combs, G.F., 1979. Interrelationships between requirements for dietary selenium, vitamin E, and L-ascorbic acid by Atlantic salmon (*Salmo salar*) fed a semipurified diet. Fish Health News 8 (4), VI–VII.

Rider, S., Davies, S., Jha, A., Fisher, A., Knight, J., Sweetman, J., 2009. Supra-nutritional dietary intake of selenite and selenium yeast in normal and stressed rainbow trout (*Oncorhynchus mykiss*): implications on selenium status and health responses. Aquaculture 295 (3–4), 282–291. https://doi.org/10.1016/j.aquaculture.2009.07.003.

Rotllant, J., Balm, P.H.M., Perez-Sanchez, J., Wendelaar-Bonga, S.E., Tort, L., 2001. Pituitary and interrenal function in gilthead seabream (*Sparus aurata* L., Teleostei) after handling and confinement stress. Gen. Comp. Endocrinol. 121 (3), 333–342. https://doi.org/10.1006/gcen.2001.7604.

Saleh, R., Betancor, M.B., Roo, J., Montero, D., Zamorano, M.J., Izquierdo, M., 2014. Selenium levels in early weaning diets for gilthead seabream larvae. Aquaculture 426–427, 256–263. https://doi.org/10.1016/j.aquaculture.2014.02.011.

Schweizer, U., Dehina, N., Schomburg, L., 2011. Disorders of selenium metabolism and selenoprotein function. Curr. Opin. Pediatr. 23 (4), 429–435. https://doi.org/10.1097/MOP.0b013e32834877da.

Sele, V., Ørnsrud, R., Sloth, J., Berntssen, M., Amlund, H., 2018. Selenium and selenium species in feeds and muscle tissue of Atlantic salmon. J. Trace Elem. Med. Biol. 47, 124–133. https://doi.org/10.1016/j.jtemb.2018.02.005.

Surai, P.F., 2006. Selenium in Nutrition and Health. Nottingham Univ. Press, Nottingham, UK.

Tashjian, D.H., Teh, S.J., Sogomonyan, A., Hung, S.S., 2006. Bioaccumulation and chronic toxicity of dietary l-selenomethionine in juvenile white sturgeon (*Acipenser transmontanus*). Aquat. Toxicol. 79 (4), 401–409. https://doi.org/10.1016/j.aquatox.2006.07.008.

Terova, G., Rimoldi, S., Izquierdo, M., Pirrone, C., Ghrab, W., Bernardini, G., 2018. Nano-delivery of trace minerals for marine fish larvae: influence on skeletal ossification, and the expression of genes involved in intestinal transport of minerals, osteoblast differentiation, and oxidative stress response. Fish Physiol. Biochem. 44, 1375–1391. https://doi.org/10.1007/s10695-018-0528-7.

Toppo, S., Vanin, S., Bosello, V., Tosatto, S.C., 2008. Evolutionary and structural insights into the multifaceted glutathione peroxidase (Gpx) superfamily. Antioxid. Redox Signal. 10 (9), 1501–1514. https://doi.org/10.1089/ars.2008.2057.

Wang, Y., Han, J., Li, W., Xu, Z., 2007. Effect of different selenium source on growth performances, glutathione peroxidase activities, muscle composition and selenium concentration of allogynogenetic crucian carp (*Carassiusauratus gibelio*). Anim. Feed Sci. Technol. 134, 243–251. https://doi.org/10.1016/j.anifeedsci.2006.12.007.

Wang, L., Zhang, X., Wu, L., Liu, Q., Zhang, D., Yin, J., 2018. Expression of selenoprotein genes in muscle is crucial for the growth of rainbow trout (Oncorhynchus mykiss) fed diets supplemented with selenium yeast. Aquaculture 492, 82–90. https://doi.org/10.1016/j.aquaculture.2018.03.054.

Watanabe, T., Kiron, V., Satoh, S., 1997. Trace minerals in fish nutrition. Aquaculture 151 (1–4), 185–207. https://doi.org/10.1016/S0044-8486(96)01503-7.

Zhu, Y., Chen, Y., Liu, Y., Yang, H., Liang, G., Tian, L., 2011. Effect of dietary selenium level on growth performance, body composition and hepatic glutathione peroxidase activities of largemouth bass *Micropterus salmoide*. Aquac. Res. 43 (11), 1660–1668. https://doi.org/10.1111/j.1365-2109.2011.02972.x.