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Selenium affects the expression of GPx4 and catalase in the liver of chicken

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ABSTRACT

A total of 128 chickens (*Gallus gallus*, broilers) were used to investigate the effect of organic selenium (Se) in expression of catalase (CAT) and phospholipid hydroperoxidase 4 (GPx4) genes. There were 4 replicates of 4 dietary treatments: T1 (basal diet with no added Se), T2 (T1 with 0.15 ppm Se added), T3 (T1 with 0.3 ppm Se) and T4 (T1 with 3.0 ppm Se). At 4th and 6th week, 2 chickens per replicate pen were sacrificed for whole blood and liver sample collections. Samples were analyzed for total Se by ICP-MS and gene expression by RT-PCR. Dietary supplementation with organic Se (Se-yeast) readily elevated its concentration in the tissues. GPx4 mRNA levels, pooled for both ages, of chickens fed T3 and T4 diets were significantly reduced compared to those fed diet T1 by 47% and 77% respectively, while that of T2 did not differ. Liver CAT mRNA levels at 4th week were significantly decreased as Se supplementation increased, while at 6th week, were not significantly affected by Se. The study showed that liver GPx4 mRNA levels could be down-regulated by excess of Se. It is possible that reserves built by excess of Se meet antioxidant requirements and no additional GPx4 transcription is necessary.

1. Introduction

Selenium (Se) is a trace element that plays a key role in the antioxidant defense system. It is the integral part of at least 25 selenoproteins and via their actions protects the organism from harmful actions of free radicals (Pappas et al., 2008). In plants, Se occurs as part of an organic compound predominantly selenomethionine (Schrauzer, 2003). Selenium is added to the diet of animals either as an inorganic salt (sodium selenite or sodium selenate) or as an organo-Se compound more often in the form of Se-yeast (Navarro-Alarcon and Cabrera-Vique, 2008). Selenium from Se-yeast is more thoroughly absorbed and more efficiently metabolized than the inorganic salts, which are poorly absorbed (Schrauzer, 2000, 2003). Absorbed selenomethionine can be incorporated into tissue proteins in place of methionine or can be metabolized in liver yielding hydrogen selenide (H_2Se) which is further used for synthesis of specific selenoproteins (Schrauzer, 2003; Pappas et al., 2008; Behne et al., 2009). Unlike metals that interact with proteins in form of cofactors, Se becomes cotranslationally incorporated into the polypeptide chains of selenoproteins as part of the amino acid selenocysteine (Pappas et al., 2008).

The selenoprotein phospholipid hydroperoxidase (glutathione peroxidase 4) (PHGPx, GPx4, EC 1.11.1.12) together with the non-Se containing enzyme catalase (CAT, EC 1.11.1.6) belongs to the primary antioxidant defense system of the organism which role is mainly preventive against lipid peroxidation and damage from hydrogen peroxide (H_2O_2) (Surai, 2006). Catalase is a tetrameric heme-containing enzyme, located primarily in the peroxisomes, consisting of four identical tetrahedrally arranged subunits of 60 kDa. Therefore, it contains four ferriprotoporphyrin groups per molecule, and its molecular mass is about 240 kDa (Nishikawa et al., 2009). It detoxifies H_2O_2 by catalyzing its breakdown to water and divalent oxygen (Lledías et al., 1998). GPx4 is located in both the cytosol and the membrane of the cells and is a member of the GPx superfamily (Pappas et al., 2008). Structurally, the major difference between GPx4 and other GPxs is that GPx4 is a 20–22 kDa monomer protein, whereas the other GPxs are tetramers (Imai and Nakagawa, 2003). Functionally, GPx4 is different from other GPxs; while all GPxs reduce hydrogen peroxide, alkyl peroxide and fatty acid hydroperoxides, only GPx4 can reduce hydroperoxides in lipoproteins and complex lipids such as those derived from cholesterol, cholesteryl esters and phospholipids (Liang et al., 2007). Because of its unique enzymatic property in reducing hydroperoxides in membrane lipids, GPx4 is believed to play a key role in protecting bio-membranes against oxidative insults (Brigelius-Flohe, 1999). GPx4 can also reduce thymidine peroxides (Bao et al., 1997; Yant et al., 2003) and catalyzes the reduction of H_2O_2 while simultaneously oxidizing GSH to GSSG (Matés, 2000). The glutathione redox cycle is a major source of protection against low levels of oxidant stress, whereas CAT becomes more significant in protecting against severe oxidative stress (Yan and Harding, 1997; Aggarwal et al., 2009). Selenoproteins have been identified for over 20 years however; the physiological role for some of them became clear with studies on transgenic mice models (Cheng

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et al., 1997a) or knock-out mice (Cheng et al., 1997b, 1998). It seems that a relative position within the hierarchical system of selenoprotein mRNA translation exists. This ranking reflects the physiological importance of the respective gene product during development and early life (Schneider, et al., 2006; Schomburg and Schweizer, 2009). For GPx4, it was demonstrated in knock-out mice, that it is essential for life, while this was not the case for GPx1 and GPx2 (Ho, et al., 1997; Yant et al., 2003; Schweizer and Schomburg, 2005).

Three issues were taken into account during the design of this study. Firstly, most of the previous work on GPx4 and CAT focused on the relation between Se supplementation in the diet and the activity of these enzymes (Liang et al., 2007; Piršljin et al., 2008). Secondly, the dietary Se manipulation of GPx1 and GPx2 expressions is thoroughly studied, while data on the housekeeping GPx4 are scarcely (Toyoda et al., 1989; Lei et al., 1998; Zhou et al., 2009). Thirdly, most of these dietary Se studies were depletion–repletion experiments, conducted on mammals or cell cultures (Weiss et al., 1996; Imai and Nakagawa, 2003). The present study was designed to investigate whether excess of Se-yeast added to the diet of chicken affects the gene expression of an Se dependent and a non-Se dependent enzyme, namely GPx4 and CAT.

2. Materials and methods

2.1. Animals, diets and design

One hundred twenty eight (128) as hatched Cobb chickens (*Gallus gallus*; broilers) were used in total. The chickens were obtained from a commercial hatchery. All chickens had the same Se status since the broiler breeder flock was fed a commercial diet with 0.20 ppm Se. There were 4 replicate pens of 4 dietary treatments namely T1, T2, T3 and T4. Pen was the experimental unit. There were 8 chickens per pen, 32 per treatment. The study was designed considering that a concentration of 0.15 mg Se per kg of diet is recommended by the National Research Council (NRC, 1994), that the maximum allowed Se inclusion level in the United States is 0.3 ppm (Payne et al., 2005) and that current total maximum EU authorized level of Se in complete feed is 0.5 ppm. In T1, chickens were fed a basal diet without Se supplementation; the only Se present was that of feed ingredients (Table 1). In treatments T2, T3 and T4, the chickens were fed the same basal diet supplemented with additional Se from a yeast source, Sel-Plex® (Alltech Inc, Nicholasville, KY, USA) to provide 0.15, 0.3 and 3 ppm Se respectively. Recent findings regarding chemical or enzymatic yeast protein hydrolysis revealed that selenomethionine accounts for 90% of total Se, while selenocystine, γ-glutamylselenocysteine, selenocystathione, methylselenocysteine and other compounds represent the rest of the total Se (Schrauzer, 2006).

The duration of the trial was 6 weeks with housing and care of chickens to conform the guidelines of the Faculty of Animal Science and Aquaculture of the Agricultural University of Athens. The chickens were raised, according to Cobb's management manual, in a house where light and ventilation were controlled. The chickens were fed a starter diet to the 2nd week of their life, a grower diet to the 4th week and a finisher diet thereafter. Feed and water were provided *ad libitum*. Each growing phase diet (starter, grower and finisher) contained the same level of added Se which was the appropriate for each experimental treatment (Table 1). The lighting program was 23 h of light and 1 h of dark. Stocking density was according to EU legislation. At weekly intervals, chickens were weighted and the body weight was recorded. Furthermore, feed intake was measured weekly and feed to gain ratio was calculated. At the end of the 4th and 6th week, two chickens per pen were sacrificed by neck dislocation and liver and whole blood samples were collected for determination of Se concentration. Expression of GPx4 and CAT genes was investigated in the same liver samples.

Table 1
Composition (g kg^{-1}) of control and Se-supplemented diets for chickens.

Ingredients (g/kg)	Starter (0–2 wk)	Grower (2–4 wk)	Finisher (4–6 wk)
Maize	579.4	618.5	666.5
Soybean meal	332.8	287.2	251.5
Soya oil	42.7	52.7	43.0
Dicalcium phosphate	16.2	15.3	14.2
Limestone	13.6	11.1	10.8
Lysine	2.6	3.1	2.6
Methionine	3.7	3.8	3.4
NaCl	5.0	4.3	4.0
Premix[a]	4.0	4.0	4.0
Treatment	Se added[b] (μg kg^{-1})	Se determined (μg kg^{-1})	
T1	–	210 ± 14	
T2	150	443 ± 19	
T3	300	685 ± 29	
T4	3000	3683 ± 210	

[a] Premix supplied per kg of diet: 12000 IU vitamin A (retinyl acetate), 4000 IU vitamin D_3 (cholecalciferol), 80 mg vitamin E (DL-α-tocopheryl acetate), 9 mg vitamin K_3, 3 mg thiamin, 7 mg riboflavin, 6 mg vitamin B_6, 25 μg vitamin B_{12}, 15 mg pantothenic acid, 1.5 mg folic acid, 0.15 mg biotin, 400 mg choline, 1.5 mg iodine, 50 mg iron, 20 mg copper, and 100 mg zinc. No Se was provided in the vitamin–mineral premix.
[b] Se was added in the form of Sel-Plex (Alltech Inc, Nicholasville, KY, USA) and only in the Se-supplemented diets.

2.2. Selenium determination

Selenium in feed and tissue samples was determined using inductively coupled plasma mass spectrometry, ICP-MS (Perkin Elmer, Elan 9000, PerkinElmer Life and Analytical Sciences Inc, Waltham, MA, USA). The instrumental parameters of the equipment used were: Nebuliser flow 0.775 L/min, vacuum pressure 1.5×10^{-5} torr, lens voltage 950 W, analogue stage voltage −1900 V, pulse stage voltage 950 V, sweeps/reading 20, readings/replicate 1, number of replicates 3, time per sample 83 s. Feed samples were collected prior to feeding and milled prior to analysis through a 1 mm sieve (Cyclotec, 1093 sample mill, Tecator, Höganäs, Sweden). For total Se determination, complete digestion of the samples was performed with a microwave digestion system (CEM, Mars X-Press, NC, USA). Samples (0.5 g) of wet tissue or feed were soaked in 10 mL concentrated HNO_3 (65% w/v, Suprapur, Merck, Darmstadt, Germany). The samples were heated in the microwave accelerated digestion system according to the following program: the power was ramped during 20 min from 100 W to 1200 W and held for 15 min. The temperature reached a maximum of 200 °C and followed by a cool down cycle for 15 min. Losses of volatile element compounds do not occur as the tubes are sealed during heating. The samples were then filtered with disposable syringe filters (Chromafil, Macherey-Nagel, Duren, Germany) and diluted 50 times with reversed osmosis water (Milli-Q Water Purification Systems, Billerica, MA, USA) prior to injection in the ICP-MS instrument. Standard solutions used for calibration curves were prepared from high purity standards (Multi-element standard solution, Fluka Analytical, Sigma-Aldrich, St Louis, USA). The analytical procedure was validated using standard reference material (NIST-RM 8414 -bovine muscle powder — LGC Standards Promochem, Wesel, Germany) and a recovery procedure (Georgiou and Koupparis, 1990). The bovine muscle reference material was certified to contain 0.076 ± 0.010 ppm of Se and the ICP-MS determined 0.078 ± 0.010 ppm of Se. The recovery procedure was as follows: 4 samples from the same liver tissue (1 g each) were spiked with 250, 500 and 750 μL of Se standard solution of 4 ppm, respectively. The spiked samples were analyzed following the aforementioned analytical procedure.

2.3. RNA isolation

Total RNA was isolated from (80–100 mg) frozen liver homogenates using NucleoSpin RNA II (Macherey-Nagel, Dueren, Germany)