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Effects of Dietary Selenium Against Lead Toxicity on mRNA Levels of 25 Selenoprotein Genes in the Cartilage Tissue of Broiler Chicken

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Abstract The interactions between the essential element selenium (Se) and the toxic element lead (Pb) have been reported extensively; however, little is known about the effect of Se on Pb toxicity and the expression pattern of selenoproteins in the cartilage of chicken. To investigate the effects of Se on Pb toxicity and the messenger RNA (mRNA) expressions of selenoproteins in cartilage tissue, an in vitro study was performed on 1-day-old broiler chickens (randomly allocated into four groups) with diet of different concentration of Se and Pb. After 90 days, the meniscus cartilage and sword cartilage tissue were examined for the mRNA levels of 25 selenoprotein genes. The results showed that Se and Pb influenced the expression of selenoprotein genes in the chicken cartilage tissue. In detail, Se could alleviate the downtrend of the expression of Gpx1, Gpx2, Gpx4, Txnrd2, Txnrd3, Dio1, Dio2, Seli, Selu, Sepx1, Selk, Selw, Selo, Selm, Sep15, Sepnn1, Sels, and Selt induced by Pb exposure in the meniscus cartilage. In the sword cartilage, Se alleviated the downtrend of the expression of Gpx2, Gpx3, Gpx4, Txnrd1, Txnrd2, Dio2, Dio3, Seli, Selh, SPS2, Sepx1, Selk, Selw, Selo, Selm, Sep15, Selpb, Sepn1, and Selt induced by Pb exposure. The present study provided some compensated data about the roles of Se against Pb toxicity in the regulation of selenoprotein expression.

Keywords Selenium · Lead · Chicken cartilage · Selenoprotein

All other authors have read the manuscript and have agreed to submit it in its current form for consideration for publication in the journal.

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Introduction

Lead (Pb) is one of the most toxic metals, and exposure to it could induce a wide range of biochemical and physiological dysfunctions in humans and laboratory animals [1, 2], such as negative effects on the birds' general health, reproduction, and behavior, potentially leading to death [3]. It has been reported that the major mechanism of lead-induced toxicity is related to oxidative stress. Two different pathways is associated with Pb-induced oxidative stress; first comes the generation of ROS, such as hydroperoxides (HO2•), singlet oxygen and hydrogen peroxide (H_2O_2), and second, the antioxidant reserves becomes depleted [4]. Another study proved that Pb-induced oxidative stress could lead to lipid peroxidation in red blood cell membranes and, as a consequence, results in hemolysis and anemia [5]. Most animal studies reported that Pb could circulate in the body via blood and accumulate in bones and other vital organs (liver, kidneys, etc.) [6, 7]. Normally, the bones contain approximately 90 % of the total amount of Pb in the body [8, 9]. Pb deposition in bones is mainly by replacing

calcium. Therefore, exposure to Pb can result in a decrease in bone mineral density (BMD) [10].

Selenium (Se), a necessary trace mineral in the diet of animals including birds, has a wide variety of functions (the biological functions of Se are primarily implemented through selenoproteins) in many aspects of health, especially in maintaining the physiological functions of the skeletal muscle system [11]. Se deficiency could cause exudative diathesis and skeletal myodegeneration in avian which would be rapidly recovered by Se supplementation [12]. So far, 25 selenoprotein genes in chickens have been identified [13], which are essential for the normal growth, development, and metabolism of kinds of organisms [14]. The levels of several selenoproteins in different chicken tissues are modulated by the levels of dietary Se [15]. Dietary Se deficiency mainly influences the expressions of antioxidative selenoproteins in chicken muscles [16] and decreases the messenger RNA (mRNA) levels of seven common selenoprotein genes (Gpx1, Gpx4, Sepw1, Sepn1, Sepp1, Selo, and Selk) in broiler chicken muscle and liver [17]. Thus, it can be seen that certified Se could regulate the mRNA expression levels of selenoproteins.

Some of the interactions between Se and Pb have already been reported. Pb inhibits the expression of Se in the kidney of rats and acts as an antagonist in the process of Se metabolism [18]. Prolonged exposure to Se and Pb influences the ion profiles in chicken liver [19]; for example, Se can attenuate the alterations in APP expression and Aβ production as well as Bcl-2 family proteins induced by lead exposure in cells and animals [20]. However, too little is known about possible interactions between Pb and Se in the expression of selenoprotein genes. In the present study, based on the model of chicken fed with Se or Pb and the compound treatment of Se and Pb supplemental diet, we have detected the expression of 25 selenoprotein genes in cartilage tissue. The aim of the present study was to explore the effects of dietary Se against Pb toxicity on mRNA levels of selenoprotein in broiler chickens.

Materials and Methods

Birds and Diets

Three hundred and sixty 1-day-old male broiler chickens were purchased from Weiwei Co. Ltd. (Harbin, China) and were randomly allocated to four groups (Se-adequate group, Pb-supplemented group, Se and Pb compound group, and the control group). Each treatment was replicated six times with 15 chickens each. The experimental chickens were given free access to feed and water, and the chickens were maintained either on a basic diet (control group) containing 0.2 mg/kg Se and 0.5 mg/kg Pb, a Se-adequate (sodium selenite) diet (+Se group) containing 1 mg/kg Se and 0.5 mg/kg Pb, a Pb-supplemented (lead acetate) diet (+Pb group) containing 0.2 mg/kg Se and 350 mg/kg Pb, or a Se and Pb compound diet (Se + Pb group) containing 1 mg/kg Se and 350 mg/kg Pb. In this experiment, the content of Pb according to the median lethal dose (LD50) of Pb acetate for cocks is 320 mg/kg body weight/day [21]. The feeding experiment lasted for 90 days and the experimental chickens were given free access to feed and water. All experiments were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. On 90 days, chicken tibial cartilage (the meniscus cartilage) and chest cartilage (sword cartilage) were collected after euthanized with sodium pentobarbital. The tissues were rinsed with ice-cold sterile deionized water and frozen immediately in liquid nitrogen and then stored at −80 °C until analysis.

Quantification of Selenoproteins mRNA

The total RNA of chicken tissues (30-mg tissue; n = 6/diet group) was isolated by using a TRIzol reagent according to the manufacturer's instructions (Invitrogen, China). Then the dried RNA pellets were resuspended in 50 μl of diethylpyrocarbonate-treated water. After determining the concentration and purity of the total RNA at 260/280 nm, first-strand complementary DNA (cDNA) was synthesized from 5 μg of total RNA using oligo dT primers and Superscript II reverse transcriptase according to the manufacturer's instructions (Invitrogen, China). Finally, synthesized cDNA was diluted five times with sterile water and stored at −80 °C before use.

After quantification, the expression levels of selenoprotein genes were determined by real-time quantitative reverse transcription PCR with SYBR Premix ExTaq TM (Takara, China), and the real-time PCR was performed with ABI PRISM 7500 (Applied Biosystems). The PCR primers (Table 1) were designed using Oligo Primer Analysis software (version 6.0) and synthesized by Invitrogen (Shanghai, China).

Reactions consisted of the following: 10 μl of 2× SYBR Green I PCR Master Mix (TaKaRa, China), 0.4 μl of 50× ROX reference Dye II, 0.4 μl of each primer (10 μM), 2 μl of either diluted cDNA, and 6.8 μl of PCR-grade water. The PCR procedure for selenoproteins and GAPDH consisted of 95 °C for 30 s followed by 40 cycles at 95 °C for 15 s and 60 °C for 30 s. Results (fold changes) were expressed as $2^{-\Delta\Delta Ct}$ in which $\Delta\Delta Ct = (Ct\ \text{selenoprotein} - Ct\ GADPH)_{B/C/D} - (Ct\ \text{selenoprotein} - Ct\ GADPH)_A$. A is the control group, B is the +Pb group, C is the +Se group, and D is the Se + Pb group.

Determination of Pb Concentration in Cartilage Tissue

The concentration of Pb was determined in the acid digest of the samples according to the method of Xutong et al. [19].

Table 1 Primers used for quantitative real-time PCR

Target gene	Primer Sequence (5′-3′)
Gpx1	Forward 5′-ACGGCGCATCTTCCAAAG-3′ Reverse 5′-TGTTCCCCCAACCATTTCTC-3′
Gpx2	Forward 5′-ATCGCCAAGTCCTTCTACGA-3′ Reverse 5′-ACGTTCTCGATGAGGACCAC-3′
Gpx3	Forward 5′-CCTGCAGTACCTCGAACTGA-3′ Reverse 5′-CTTCAGTGCAGGGAG GATCT-3′
Gpx4	Forward 5′-CTTCGTCTGCATCATCACCAA-3′ Reverse 5′-TCGACGAGCTGAGTGTAATTCAC-3′
Txnrd1	Forward 5′-TACGCCTCTGGGAAATTCGT-3′ Reverse 5′-CTTGCAAGGCTTGTCCCAGTA-3′
Txnrd2	Forward 5′-GCTCTTAAAGATGCCCAGCACTAC-3′ Reverse 5′-GAACAGCTTGAGCCATCACAGA-3′
Txnrd3	Forward 5′-CCTGGCAAAACGCTAGTTGT G-3′ Reverse 5′-CGCACCATTACTGTGACATCTAGAC-3′
Dio1	Forward 5′-GCGCTATACCACAGGCAGTA-3′ Reverse 5′-GGTCTTGCAAATGTCACCAC-3′
Dio2	Forward 5′-ATTTGCTGATCACGCTTCAG-3′ Reverse 5′-GCTCAGAAACAGCACCATGT-3′
Dio3	Forward 5′-CTGTGCATTCGCAAGAAGAT-3′ Reverse 5′-GCCGACTTGAAGAAGTCCAG-3′
SPS2	Forward 5′-CGTTGGGTATCGGAACTGAC-3′ Reverse 5′-CGTCCACCAGAGGGTAGAAA-3′
Sepp1	Forward 5′-CCAAGTGGTCAGCATTCACATC-3′ Reverse 5′-ATGACGACCACCCTCACGAT-3′
SelPb	Forward 5′-AGGCCAACAGTACCATGGAG-3′ Reverse 5′-GTGGTGAGGATGGAGATGGT-3′
Sep15	Forward 5′-ACTTGGCTTCTCCAGTAACTTGCT-3′ Reverse 5′-GCCTACAGAATGGATCCAACTGA-3′
Selh	Forward 5′- CATCGAGCACTGCCGTAG-3′ Reverse 5′-GACACCTCGAAGCTGTTCCT -3′
Seli	Forward 5′-TGCCAGCCTCTGAACTGGAT-3′ Reverse 5′-TGCAAACCCAGACATCACCAT-3′
Selm	Forward 5′-AAGAAGGACCACCCAGACCT-3′ Reverse 5′-GCTGTCCTGTCTCCCTCATC-3′
Selo	Forward 5′-CCAGCGTTAACCGGAATGAT-3′ Reverse 5′-ATGCGCCTCCTGGATTTCT-3′
Sels	Forward 5′-GCCTGCGTCGCCATCTATCTCA-3′ Reverse 5′-TTCTGCCTTCGCTTCTGTTCTTCAA-3′
Sepx1	Forward 5′-TGGCAAGTGTGGCAATGG-3′ Reverse 5′-GAATTTGAGCGAGCTGCTGAAT-3′
Selu	Forward 5′-GATGCTTTCAGGCTTCTTCC-3′ Reverse 5′-CTGTCTTCCTGCTCCAATCA-3′
Selk	Forward 5′- GAAGAGGGCCTCCAGGAAAT-3′ Reverse 5′- CAGCCATTGGTGGTGGACTAG-3′
Selw	Forward 5′- TGGTGTGGGTCTGCTTTACG-3′ Reverse 5′- CCAAAGCTGGAAGGTGCAA-3′
Seln	Forward 5′- CAGGATCCATGCTGAGTTCCA-3′ Reverse 5′- GAGAGGACGATGTAACCCGTAAAC-3′
Selt	Forward 5′- AGGAGTACATGCGGGTCATCA-3′ Reverse 5′- GACAGACAGG AAGGATGCTATGTG-3′
GAPDH	Forward 5′-AGAACATCATCCCAGCGT-3′ Reverse 5′-AGCCTTCACTACCCTCTTG-3′

One gram of each sample was digested with 5 mL of HNO3 (65 %) and 2 mL of H2O2 (30 %) in a microwave digestion system and diluted to 10 ml with deionized water. A blank digest was carried out in the same way. All of the sample solutions were clear. The digestion conditions for the microwave system were applied at 3 min for 1800 W at 100 °C, 10 min for 1800 W at 150 °C, and 45 min for 1800 W at 180 °C. The digested samples were filled with ultrapure water to the final volume before analysis by ICP-MS.

Statistical Analyses

Statistical analysis was performed using SPSS (SPSS, Chicago, IL, USA). Data were expressed as the mean ± standard deviation. All data showed a normal distribution and passed equal variance testing. Differences between means were assessed using Tukey's honestly significant difference test for post hoc multiple comparisons.

Results

The mRNA levels of 25 selenoprotein genes in broiler chickens' meniscus and sword cartilage were detected. As shown in Table 2 and Fig. 1, Pb decreased the mRNA levels of 24 selenoprotein genes (Gpx1, Gpx2, Gpx3, Txnrd1, Txnrd2, Txnrd3, Dio1, Dio2, Dio3, Seli, Selh, Selu, SPS2, Sepx1, Selk, Sepp1, Selw, Selo, Selm, Sep15, Selpb, Sepn1, Sels, and Selt) in the +Pb group of the meniscus cartilage tissue ($p < 0.05$). However, there was no significant difference between the +Pb group and control group in the mRNA levels of Gpx4 ($p > 0.05$). The mRNA levels of 20 selenoprotein genes (Gpx4, Txnrd1, Txnrd2, Txnrd3, Dio1, Dio2, Dio3, Seli, Selh, SPS2, Sepx1, Selk, Sepp1, Selw, Selo, Selm, Sep15, Selpb, Sepn1, Sels, and Selt) were higher in the +Se group than those in the control group ($p < 0.05$), and there was no significant difference between the +Se group and control group in the mRNA levels of Gpx1, Gpx2, Gpx3, Selh and Selu ($p > 0.05$). In the Se + Pb group, the mRNA levels of Gpx1, Gpx3, Txnrd1, Txnrd2, Txnrd3, Dio1, Dio2, Dio3, Seli, Selh, Selu, SPS2, Sepx1, Selk, Sepp1, Selo, Selm, Sep15, Selpb, and Sepn1 were decreased but the decreased degree is less than that in the +Pb group, and the mRNA levels of Sels were increased compared with the control group ($p < 0.05$) but the increased degree is also less than that in +Se group, however, Gpx2, Gpx4, and Selt were not influenced ($p > 0.05$).

Likewise in Table 3 and Fig. 2, Pb decreased the mRNA levels of 24 selenoprotein genes (Gpx1, Gpx2, Gpx3, Gpx4, Txnrd1, Txnrd2, Txnrd3, Dio2, Dio3, Seli, Selh, Selu, SPS2, Sepx1, Selk, Sepp1, Selw, Selo, Selm, Sep15, Selpb, Sepn1, Sels and Selt) in the +Pb group of the sword cartilage tissue ($p < 0.05$), except Dio1. In the +Se group, Se supplementation

Table 2 Effects of dietary Se against Pb toxicity on mRNA levels of 25 selenoprotein genes in the tibial cartilage of broiler chickens

Gene	Control group	+Pb group	+Se group	Se + Pb group
Gpx1	1.000 ± 0.000^{a}	0.528 ± 0.021^{b}	1.057 ± 0.14^{a}	0.753 ± 0.058^{c}
Gpx2	1.000 ± 0.000^{a}	0.374 ± 0.034 ^{b}	1.342 ± 0.0240^{a}	0.898 ± 0.089^{a}
Gpx3	1.000 ± 0.000^{a}	0.405 ± 0.014^{b}	1.102 ± 0.192^{a}	0.546 ± 0.035^{b}
Gpx4	1.000 ± 0.000^{a}	0.865 ± 0.121^{a}	1.790 ± 0.241^{b}	0.958 ± 0.054^{a}
Txnrd1	1.000 ± 0.000^{a}	0.250 ± 0.040^{b}	1.526 ± 0.187^{c}	0.345 ± 0.037^{b}
Txnrd2	1.000 ± 0.000^{a}	0.779 ± 0.105^{b}	1.778 ± 0.067^{c}	0.893 ± 0.015^{d}
Txnrd3	1.000 ± 0.000^{a}	0.384 ± 0.075^{b}	1.726 ± 0.211^{c}	0.745 ± 0.018^{d}
Dio1	1.000 ± 0.000^{a}	0.151 ± 0.045^{b}	1.359 ± 0.457^{c}	0.577 ± 0.052^{d}
Dio2	1.000 ± 0.000^{a}	0.236 ± 0.062^{b}	1.505 ± 0.104^{c}	0.482 ± 0.016^{d}
Dio3	1.000 ± 0.000^{a}	0.155 ± 0.023^{b}	3.234 ± 0.379^{c}	0.238 ± 0.007^{b}
SPS2	1.000 ± 0.000^{a}	0.664 ± 0.062^{b}	1.206 ± 0.249^{c}	0.774 ± 0.046^{b}
Sepp1	1.000 ± 0.000^{a}	0.116 ± 0.035^{b}	1.213 ± 0.190^{c}	0.172 ± 0.032^{b}
Selpb	1.000 ± 0.000^{a}	0.247 ± 0.079^{b}	1.395 ± 0.128^{c}	0.348 ± 0.023^{b}
Sep15	1.000 ± 0.000^{a}	0.192 ± 0.049^{b}	1.950 ± 0.089^{c}	0.538 ± 0.115^{d}
Selh	1.000 ± 0.000^{a}	0.169 ± 0.011^{b}	1.128 ± 0.042^{a}	0.282 ± 0.048^{b}
Seli	1.000 ± 0.000^{a}	0.415 ± 0.013^{b}	2.969 ± 0.254^{c}	0.829 ± 0.034^{d}
Selm	1.000 ± 0.000^{a}	0.273 ± 0.147^{b}	1.434 ± 0.200^{c}	0.437 ± 0.002^{d}
Selo	1.000 ± 0.000^{a}	0.122 ± 0.056^{b}	1.870 ± 0.241^{c}	0.435 ± 0.056^{d}
Sels	1.000 ± 0.000^{a}	0.764 ± 0.037^{b}	2.649 ± 0.091^{c}	1.133 ± 0.033^{d}
Sepx1	1.000 ± 0.000^{a}	0.243 ± 0.055^{b}	1.250 ± 0.545^{c}	0.426 ± 0.017^{d}
Selu	1.000 ± 0.000^{a}	0.108 ± 0.003^{b}	1.148 ± 0.064^{a}	0.597 ± 0.009^{c}
Selk	1.000 ± 0.000^{a}	0.612 ± 0.219^{b}	1.892 ± 0.035^{c}	0.849 ± 0.057^{d}
Selw	1.000 ± 0.000^{a}	0.883 ± 0.312^{b}	1.210 ± 0.138^{c}	1.049 ± 0.006^{a}
Seln	1.000 ± 0.000^{a}	0.483 ± 0.180^{b}	1.547 ± 0.586^{c}	0.791 ± 0.017^{d}
Selt	1.000 ± 0.000^{a}	0.780 ± 0.125^{b}	1.558 ± 0.123^{c}	1.04 ± 0.145^{a}

increased the mRNA levels of all detective selenoprotein genes ($p < 0.05$). In the Se + Pb group, the mRNA levels of Gpx1, Gpx2, Gpx3, Gpx4, Txnrd2, Txnrd3, Dio2, Dio3, Selu, Sepx1, Selk, Selw, Selm, Selpb, and Sels were lower than in the control group, but the decreased degree is less than those in the +Pb group, and the mRNA levels of Txnrd1, Seli,

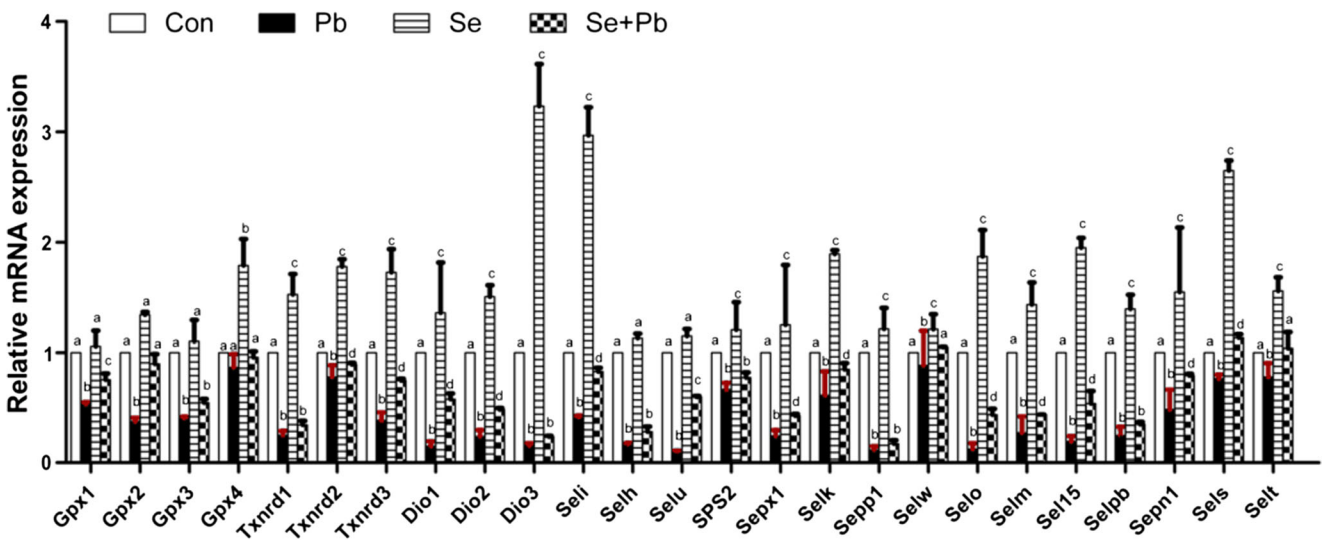

Fig. 1 Effects of dietary Se against Pb toxicity on mRNA levels of selenoprotein genes in the meniscus cartilage tissue of chicken. Values (mean ± SD) bearing *different letters in a row* differ significantly ($p < 0.05$). $n = 6$

Table 3 Effects of dietary Se against Pb toxicity on mRNA levels of 25 selenoprotein genes in the chest cartilage of broiler chickens

Gene	Control group	+Se group	+Pb group	Se + Pb group
Gpx1	1.000 ± 0.000^{a}	0.420 ± 0.390^{b}	1.570 ± 0.138^{c}	0.637 ± 0.007^{b}
Gpx2	1.000 ± 0.000^{a}	0.430 ± 0.010^{b}	1.283 ± 0.096^{c}	0.732 ± 0.339^{d}
Gpx3	1.000 ± 0.000^{a}	0.263 ± 0.130^{b}	1.132 ± 0.030^{c}	0.633 ± 0.177^{d}
Gpx4	1.000 ± 0.000^{a}	0.308 ± 0.035^{b}	2.961 ± 0.160^{c}	0.742 ± 0.010^{d}
Txnrd1	1.000 ± 0.000^{a}	0.366 ± 0.010^{b}	1.960 ± 0.300^{c}	1.440 ± 0.294^{d}
Txnrd2	1.000 ± 0.000^{a}	0.648 ± 0.070^{b}	2.297 ± 0.231^{c}	0.839 ± 0.035^{d}
Txnrd3	1.000 ± 0.000^{a}	0.708 ± 0.043^{b}	2.189 ± 0.171^{c}	0.763 ± 0.031^{b}
Dio1	1.000 ± 0.000^{a}	0.470 ± 0.052^{a}	3.271 ± 0.298^{b}	0.971 ± 0.033^{a}
Dio2	1.000 ± 0.000^{a}	0.438 ± 0.011^{b}	3.271 ± 0.284^{c}	0.624 ± 0.012^{d}
Dio3	1.000 ± 0.000^{a}	0.167 ± 0.015^{b}	1.972 ± 0.475^{c}	0.775 ± 0.170^{d}
SPS2	1.000 ± 0.000^{a}	0.207 ± 0.037^{b}	2.770 ± 0.211^{c}	0.920 ± 0.011^{a}
Sepp1	1.000 ± 0.000^{a}	0.788 ± 0.058^{b}	1.972 ± 0.327^{c}	1.119 ± 0.092^{b}
Selpb	1.000 ± 0.000^{a}	0.211 ± 0.013^{b}	3.226 ± 0.312^{c}	0.620 ± 0.191^{d}
Sep15	1.000 ± 0.000^{a}	0.384 ± 0.031^{b}	1.682 ± 0.033^{c}	1.407 ± 0.124^{d}
Selh	1.000 ± 0.000^{a}	0.125 ± 0.022^{b}	1.751 ± 0.256^{c}	1.038 ± 0.042^{a}
Seli	1.000 ± 0.000^{a}	0.707 ± 0.028^{b}	1.424 ± 0.015^{c}	1.292 ± 0.069^{d}
Selm	1.000 ± 0.000^{a}	0.228 ± 0.019^{b}	1.241 ± 0.073^{c}	0.861 ± 0.119^{d}
Selo	1.000 ± 0.000^{a}	0.543 ± 0.090^{b}	1.828 ± 0.024^{c}	0.895 ± 0.019^{a}
Sels	1.000 ± 0.000^{a}	0.664 ± 0.012^{b}	4.257 ± 0.456^{c}	0.734 ± 0.060^{b}
Sepx1	1.000 ± 0.000^{a}	0.245 ± 0.126^{b}	1.705 ± 0.141^{c}	0.836 ± 0.450^{d}
Selu	1.000 ± 0.000^{a}	0.486 ± 0.072^{b}	3.986 ± 0.020^{c}	0.561 ± 0.047^{b}
Selk	1.000 ± 0.000^{a}	0.501 ± 0.097^{b}	1.157 ± 0.002^{c}	0.799 ± 0.022^{d}
Selw	1.000 ± 0.000^{a}	0.247 ± 0.012^{b}	1.277 ± 0.279^{c}	0.521 ± 0.006^{d}
Seln	1.000 ± 0.000^{a}	0.460 ± 0.087^{b}	7.542 ± 0.037^{c}	1.14 ± 0.003^{d}
Selt	1.000 ± 0.000^{a}	0.582 ± 0.340^{b}	2.418 ± 0.271^{c}	1.174 ± 0.238^{d}

Sepp1, Sep15, Sepn1, and Selt were higher than those in the control group ($p < 0.05$), but the increased degree is also less than in +Se group. However, there was no change in the expression of Dio1, Selh, SPS2, and Selo ($p > 0.05$).

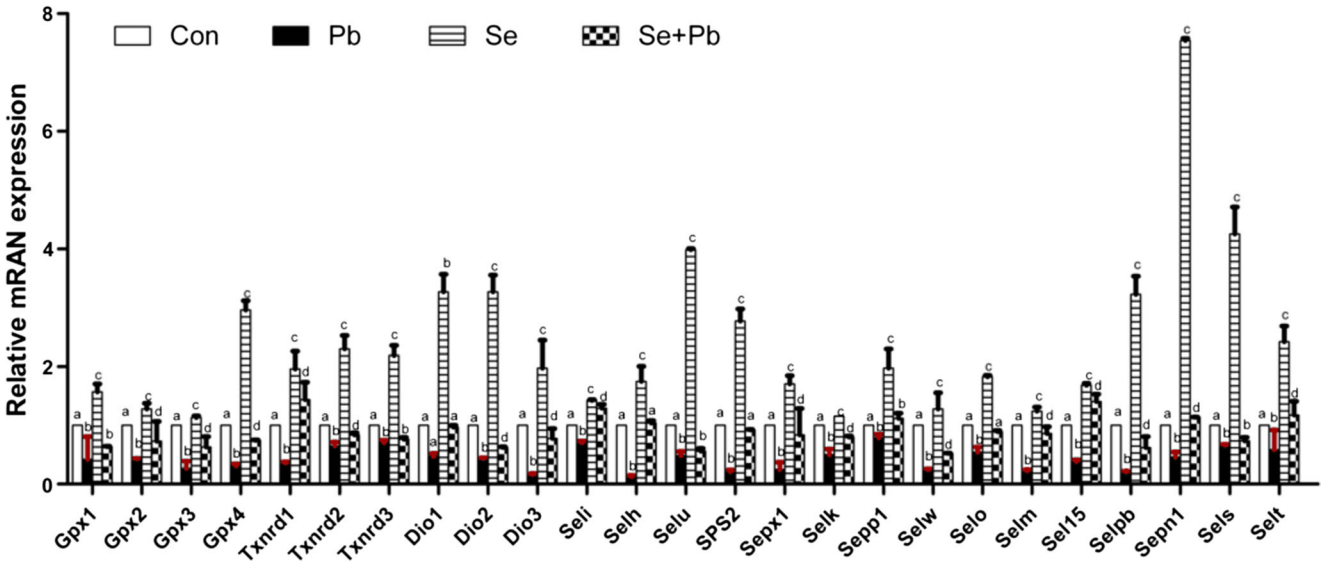

Fig. 2 Effects of dietary Se against Pb toxicity on mRNA levels of selenoprotein genes in the sword cartilage tissue of chicken. Values (mean ± SD) bearing *different letters in a row* differ significantly ($p < 0.05$). $n = 6$

From Figs. 3 and 4, we could see that in the meniscus cartilage, the Pb content in the +Pb group and the Se + Pb group was higher than that in the control group ($p < 0.05$). And the Pb content in Se + Pb group was lower than that in +Pb group. The Pb content in the sword cartilage had the same variation trend. The result shows that Se could help to alleviate the Pb toxicity.

Discussion

The antagonistic function of Se against typical toxic elements such as Cd, Hg, and Pb has been shown in plants and animals in previous studies. However, the effects of Se against Pb toxicity on mRNA levels of selenoprotein genes have been little reported in chickens. In the present study, we detected the effects of dietary Se against Pb toxicity on mRNA levels of 25 selenoprotein genes in the cartilage tissue of broiler chicken. The results showed that the treatment of Se and Pb influenced the gene expression of selenoprotein in chicken cartilage tissue.

Se is an essential micronutrient and plays an important role in antioxidation [22–24]. One of its main functions is an antioxidant action involved in protection against damage caused by free radicals and oxidative stress [25]. The physiological functions of Se are considered to be mediated through selenoproteins. Selenoproteins are extensively expressed in animals, and the levels of several selenoproteins are modulated by the levels of dietary Se in different chicken tissues. GPx, the first identified selenoprotein, is most abundant in the liver. GPx family including GPxl, GPx2, GPx3, and GPx4 reserve kinds of enzymatic properties, and most of them are involved in the catabolism of peroxides [26]. The thioredoxin reductase gene (TrxR) is one essential selenoprotein gene in different kinds of animals, and our previous study indicated that TrxR1, TrxR2, and TrxR3 were decreased in layer chicken liver by Se deficiency [14]. Iodothyronine deiodinase (DIO) family plays an important role in thyroid metabolism [27]. The Sep15 is located mainly in the endoplasmic reticulum and is believed to be involved in protein folding. Biosynthesis of selenoproteins

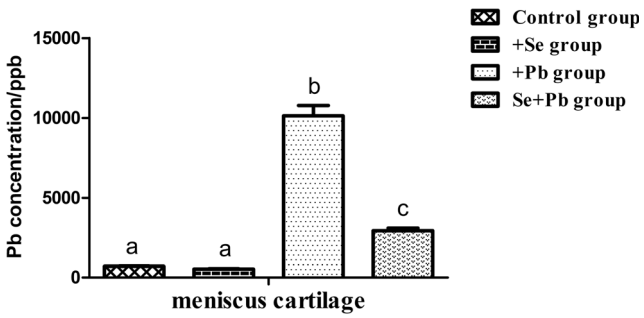

Fig. 3 Effects of dietary Se against Pb toxicity on the Pb content in the meniscus cartilage tissue of chicken. Values (mean ± SD) bearing *different letters in a row* differ significantly ($p < 0.05$). $n = 6$

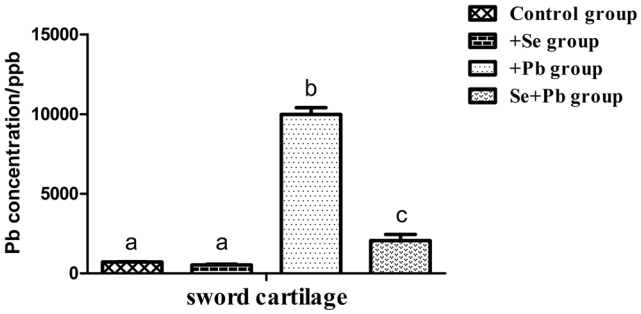

Fig. 4 Effects of dietary Se against Pb toxicity on the Pb content in the sword cartilage tissue of chicken. Values (mean ± SD) bearing *different letters in a row* differ significantly ($p < 0.05$). $n = 6$

depends on the production of monoselenophosphate (MSP), a Se donor compound from selenide and ATP, a reaction catalyzed by the enzyme, selenophosphate synthetase (SPS). Two isoforms of SPS were found in higher eukaryotes. In vitro experiments, SPS2 synthesized MSP from selenide and ATP [28]. Sepp1 is the major transport form of Se. Selh was recently found to reside in nucleoli and predicted to possess a thioredoxin-like fold [29]. SelN and SelW play a crucial role in muscle disorders [30]. Selm may function as thiol disulfide oxidoreductase that participates in the formation of disulfide bonds and can be implicated in calcium responses and redox regulation [31]. The functions for the proteins encoded by Selo, Sepx1, Sels, Selk, and Selu were less postulated in chicken.

Pb accumulates in the blood, soft tissues, and bones [32]. On the other hand, Pb depletes the antioxidant defenses, such as glutathione (GSH), and modifies activities and expression of antioxidant enzymes, such as superoxide dismutase or glutathione peroxidase (GPx) [33, 34]. Therefore, exposure to Pb results in the oxidative damage. In our current study, we found Pb exposure decreased the mRNA levels of Gpx1, Gpx2, Gpx3, Txnrd1, Txnrd2, Txnrd3, Dio1, Dio2, Dio3, Seli, Selh, Selu, SPS2, Sepx1, Selk, Sepp1, Selw, Selo, Selm, Sep15, Selpb, Sepn1, Sels, and Selt in the meniscus cartilage and decreased the mRNA levels of Gpx1, Gpx2, Gpx3, Gpx4, Txnrd1, Txnrd2, Txnrd3, Dio2, Dio3, Seli, Selh, Selu, SPS2, Sepx1, Selk, Sepp1, Selw, Selo, Selm, Sep15, Selpb, Sepn1, Sels, and Selt in the sword cartilage, which was consistent with the previous reports. Se is a non-metal element that has been linked to many health benefits in humans and other mammals such as decreasing the incidence of cancer, protecting against cardiovascular diseases, and treating certain muscle disorders. Dietary Se levels can regulate the expression of selenoproteins [35]. It has been demonstrated that selenoproteins, through their antioxidant properties, help to eliminate reactive oxygen species induced by metals [36]. In fact, appropriate Se added in feed possesses antioxidant properties [37]. In our current study, we found Se supplementation increased the mRNA levels of Gpx4, Txnrd1, Txnrd2,

Txnrd3, Dio1, Dio2, Dio3, Seli, Selh, SPS2, Sepx1, Selk, Sepp1, Selw, Selo, Selm, Sep15, Selpb, Sepn1, Sels, and Selt in tibial cartilage and increased the mRNA levels of all detective selenoprotein genes in the chest cartilage, which is similar to other reports. Su found a Se-supplemented diet could increase the expression of Selw and the mRNA levels of SecS in chicken liver [38]. Wang found that in the high-Se group, there was a significant increase ($p < 0.05$) in mRNA levels of Selw in chicken pancreatic tissue [39]. Thus, it can be seen that Se supplementation may be considered as a potential therapy for chronic Pb poisoning due to the antioxidant properties of Se [40]. In our study, Se alleviated the downtrend of the expression of Gpx1, Gpx2, Gpx4, Txnrd2, Txnrd3, Dio1, Dio2, Seli, Selu, Sepx1, Selk, Selw, Selo, Selm, Sep15, Sepnn1, Sels, and Selt induced by Pb exposure in the tibial cartilage and alleviated the downtrend of the expression of Gpx2, Gpx3, Gpx4, Txnrd1, Txnrd2, Dio2, Dio3, Seli, Selh, SPS2, Sepx1, Selk, Selw, Selo, Selm, Sep15, Selpb, Sepn1, and Selt induced by Pb exposure in the chest cartilage. The suggested antagonistic relationship between Pb and Se is that Se can alleviate the oxidative stress which is induced by Pb via the antioxidation of Selenoproteins.

In conclusion, with prolonged exposure to Se and Pb, the mRNA levels of selenoproteins genes were influenced in chicken cartilage tissue. Pb may act as an antagonist in Se metabolism and Se can help to alleviate the Pb toxicity. The present study provides some compensated data about the roles of Se and Pb in the regulation of selenoproteins.

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