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RESEARCH ARTICLE

Selenium for the mitigation of toxicity induced by lead in chicken testes through regulating mRNA expressions of HSPs and selenoproteins

He Huang[1] · Yan Wang[1] · Yang An[1] · Yaguang Tian[1] · Shu Li[2] · Xiaohua Teng[1]

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Abstract Lead (Pb) is a toxic element and environmental pollutant. Pb toxicity and antagonistic effect of selenium (Se) on Pb have been deeply studied in mammals. The testis is one of the target organs of Pb in birds. The aim of this study was to investigate the mitigating effect of Se on Pb toxicity in chicken testes by determining messenger RNA (mRNA) expressions of 5 heat shock proteins (HSPs) and 25 selenoproteins. Sixty male chickens (7-day-old) were randomly divided into the control group, the Se group, the Pb group, and the Pb + Se group, and were fed for 90 days. The feeding methods of chickens were as follows: The control group was fed drinking water and commercial diet (0.49 mg/kg Se). Lead acetate was added into the drinking water (350 mg/L Pb). Sodium selenite was added into the commercial diet (1 mg/kg Se). Multivariate correlation analysis and principal component analysis (PCA) were used to define the relationships among all the measured factors and the most important parameters that could be used as key factors, respectively. The results indicated that Se decreased the increase of mRNA expressions of all the HSPs and increased the decrease of mRNA expressions of all the selenoproteins induced by Pb in the chicken testes. HSP70 may be a biomarker of Pb poisoning in the chicken testes. Se alleviated Pb-induced toxicity in the chicken testes through regulating mRNA expressions of HSPs and selenoproteins.

Keywords Sodium selenite · Lead acetate · Bird · Testis · HSP70 · Selenoprotein

Introduction

Lead (Pb) is a toxic element. With the development of industry, Pb caused soil (Taheri et al. 2015), water (Ayrault et al. 2014), and food (Szczygłowska et al. 2014) pollution. A research found that Pb accumulated in the bones of feral pigeons in Seoul, Korea (Nam and Lee 2006). Pb had adverse effect on reproductive potential in wild mallard ducks (Tsuji and Karagatzides 2001). Excess Pb is harmful to humans and animals, such as workers (Chinde et al. 2014), rats (Wang et al. 2013; Mabrouk et al. 2016), and chickens (Jin et al. 2016; Zheng et al. 2016). Pb treatment caused reproductive toxicity in mice (Cao et al. 2016b).

Selenium (Se) is an essential trace element (Zhang et al. 2016b). Se alleviated Pb toxicity in the gills of crawfishes (White et al. 2012). Se reduced Pb-induced reproductive toxicity in male rats (Apaydin et al. 2015). Se enhanced the upward trend of selenoprotein expressions induced by Pb exposure in chicken neutrophils (Li et al. 2017). A recent study showed that Se alleviated the decrease of mRNA expressions of iodothyroninedeiodinases 1 (Dio1), iodothyroninedeiodinases 2 (Dio2), iodothyroninedeiodinases 3 (Dio3), glutathione peroxidase 1 (GPx1), glutathione peroxidase 2 (GPx2), glutathione peroxidase 3 (GPx3), glutathione peroxidase 4 (GPx4),

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✉ Shu Li
lishu@neau.edu.cn

✉ Xiaohua Teng
tengxiaohua@neau.edu.cn

[1] College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, People's Republic of China

[2] College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, People's Republic of China

selenoprotein H (SelH), selenoprotein I (SelI), selenoprotein K (SelK), selenoprotein M (SelM), selenoprotein O (SelO), selenoprotein pb (Selpb), selenoprotein S (SelS), selenoprotein T (SelT), selenoprotein U (SelU), selenoprotein W (SelW), 15-kDa selenoprotein (Sep15), selenoprotein N1 (SepN1), selenoprotein P (Sepp1), selenoprotein X1 (SepX1), selenophosphatesynthetase 2 (SPS2), thioredoxin reductase 1 (Txnrd1), thioredoxin reductase 2 (Txnrd2), and thioredoxin reductase 3 (Txnrd3) caused by Pb poisoning in chicken cartilages (Gao et al. 2016).

Heat shock proteins (HSPs) engage in response to a variety of stressors (Yamashita et al. 2010). Heavy metals increased the expressions of HSPs in *Tigriopus japonicus* (Kim et al. 2014**)**. HSP27 and HSP70 expressions increased in chromium-treated mice hepatocytes (Lee and Lim 2012). Copper and cadmium (Cd) increased HSP40 mRNA expression in clams (Li et al. 2011). Molybdenum and Cd induced high mRNA expressions of HSP60, HSP70, and HSP90 in duck livers (Cao et al. 2016a). Excess Cd increased the synthesis of HSP70 and damaged testicular cells in rats (Selim et al. 2012). Se alleviated the increase of HSP27, HSP40, HSP60, HSP70, and HSP90 mRNA expressions induced by Pb in the cartilages (Zheng et al. 2016) and livers (Wang et al. 2016) of chickens. However, alleviative effect of Se on Pb poisoning in chicken testes is still unclear. Therefore, we simulated a chicken model to mitigate the effect of Se on Pb poisoning and detected mRNA expressions of five HSPs (HSP27, HSP40, HSP60, HSP70, and HSP90) and 25 selenoproteins (Dio1, Dio2, Dio3, GPx1, GPx2, GPx3, GPx4, SelH, SelI, SelK, SelM, SelO, SelPb, SelS, SelT, SelU, SelW, Sep15, SepN1, Sepp1, SepX1, SPS2, Txnrd1, Txnrd2, and Txnrd3) in chicken testes.

Materials and methods

Animal model and tissue samples

One-day-old Hyline chickens were fed commercial diet (containing 0.49 mg/kg Se) and drinking water for 7 days. Sixty male chickens were randomly divided into four groups (15 chickens each group): the control group, the Se group, the Pb group, and the Pb + Se group. The control group was fed drinking water and the commercial diet. Sodium selenite (Analytical reagent grade, Tianjin, China) was provided in the commercial diet (1 mg/kg Se). Lead acetate (Analytical reagent grade, Tianjin, China) was provided in the drinking water (350 mg/L Pb), according to the median lethal dose (LD50) of Pb for chickens (Vengris and Mare 1974) and the need of the chicken experiment in toxicology (Klaassen and Watkins 2013). The chickens were maintained in Laboratory Animal Center, College of Veterinary Medicine, Northeast Agricultural University, China, and were fed feed and water ad libitum.

All chickens were euthanized on the 90th day of the experiment. Testes of chickens were quickly removed, washed with sterile deionized water, frozen in liquid nitrogen, and stored at −80 °C until required for subsequent experiments. All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University.

Relative mRNA expressions of HSPs and selenoproteins

Primer

Primer sequences of five HSPs and 25 selenoproteins published in GeneBank were listed in Table 1. The primers were synthesized by Invitrogen Biotechnology Co. Ltd. in Shanghai, China.

Total RNA and reverse transcription

Total RNA of each sample was isolated using TRIzol reagent according to the manufacturer's protocol (Invitrogen, China). Purity and concentration of RNA were determined with spectrophotometer (Healthcare Bio-Sciences AB, Sweden) at the wavelength of 260/280 nm. Total RNA was reversely transcribed into complementary DNA (cDNA) using PrimeScript™ RT reagent Kit (TaKaRa, Japan) in a final volume of 60 μL according to manufacturer's instructions. The cDNA product was stored at −20 °C until use.

Quantitative real-time reverse transcription PCR

The reaction mix for quantitative real-time reverse transcription PCR consisted of 1 μL diluted cDNA, 0.3 μL forward and reverse primer, 5 μL SYBR green PCR master mix (Roche, Switzerland), and 3.4 μL PCR-grade water. Real-time quantitative PCR was performed using LightCycler® 96 (Roche, Switzerland). The PCR conditions included heating the reaction mixture (52 °C for 2 min and 95 °C for 10 min), 40 cycles of amplification and quantification (95 °C for 15 s and 60 °C for 1 min), and melting curve analysis (95 °C for 15 s and 60 °C for 20 s). Melt curve analysis was performed to verify the specificity of primers. There were three duplications for each sample. The relative mRNA expression levels were calculated according to the $2^{-\Delta\Delta}$CT method (Livak and Schmittgen 2001) using

Table 1 Primers sequences

Gene	Serial number	Forward prime (5′→3′)	Reverse primer (5′→3′)
HSP27	NM_205134	ACACGAGGAGAAACAGGATGAG	ACTGGATGGCTGGCTTGG
HSP40	NM_204267	GGGCATTCAACAGCATAGA	TTCACATCCCCAAGTTTAGG
HSP60	NM_001194983	AGCCAAAGGGCAGAAATG	TACAGCAACAACCTGAAGACC
HSP70	NM_001167718	CGGGCAAGTTTGACCTAA	TTGGCTCCCACCCTATCTCT
HSP90	NM_001109785.1	TCCTGTCCTGGCTTTAGTTT	AGGTGGCATCTCCTCGGT
Dio1	NM_001030762.2	GCGCTATACCACAGGCAGTA	GGTCTTGCAAATGTCACCAC
Dio2	NM_001122691.1	ATTTGCTGATCACGCTTCAG	GCTCAGAAACAGCACCATGT
Dio3	NM_001122777.1	CTGTGCATTCGCAAGAAGAT	GCCGACTTGAAGAAGTCCAG
GPx1	NM_001277853.1	ACGGCGCATCTTCCAAAG	TGTTCCCCCAACCATTTCTC
GPx2	NM_001277854.1	ACGGCACCAACGAGGAGAT	TTCAGGTAGGCGAAGACGG
GPx3	NM_001163232.1	CCTGCAGTACCTCGAACTGA	CTTCAGTGCAGGGAG GATCT
GPx4	AF498316.2	CTTCGTCTGCATCATCACCAA	TCGACGAGCTGAGTGTAATTCAC
SelH	NM_001277865.1	CATCGAGCACTGCCGTAG	GACACCTCGAAGCTGTTCCT
SelI	NM_001031528.2	TGCCAGCCTCTGAACTGGAT	TGCAAACCCAGACATCACCAT
SelK	NM_001025441.2	GAAGAGGGCCTCCAGGAAAT	CAGCCATTGGTGGTGGACTAG
SelM	NM_001277859.1	AAGAAGGACCACCCAGACCT	GCTGTCCTGTCTCCCTCATC
SelO	NM_001115017.1	CCAGCGTTAACCGGAATGAT	ATGCGCCTCCTGGATTTCT
SelPb	XM_003641687.2	AGGCCAACAGTACCATGGAG	GTGGTGAGGATGGAGATGGT
SelS	NM_173120.2	GCCTGCGTCGCCATCTATCTCA	TTCTGCCTTCGCTTCTGTTCTTCAA
SelT	NM_001006557.3	AGGAGTACATGCGGGTCATCA	GACAGACAGG AAGGATGCTATGTG
SelU	NM_001193518.1	GATGCTTTCAGGCTTCTTCC	CTGTCTTCCTGCTCCAATCA
SelW	NM_001166327.1	TGGTGTGGGTCTGCTTTACG	CCAAAGCTGGAAGGTGCAA
Sep15	NM_001012926.2	ACTTGGCTTCTCCAGTAACTTGCT	GCCTACAGAATGGATCCAACTGA
SepN1	NM_001114972.1	CCAAGTGGTCAGCATTCACATC	ATGACGACCACCCTCACGAT
Sepp1	NM_001004357.1	CAGGATCCATGCTGAGTTCCA	GAGAGGACGATGTAACCCGTAAAC
SepX1	NM_001135558.2	TGGCAAGTGTGGCAATGG	GAATTTGAGCGAGCTGCTGAAT
SPS2	BM489698.1	CGTTGGGTATCGGAACTGAC	CGTCCACCAGAGGGTAGAAA
Txnrd1	NM_001122777.1	TACGCCTCTGGGAAATTCG	CTTGCAAGGCTTGTCCCAGTA
Txnrd2	NM_001097614.1	GCTCTTAAAGATGCCCAGCACTAC	GAACAGCTTGAGCCATCACAGA
Txnrd3	NM_204114.3	CCTGGCAAAACGCTAGTTGTG	CGCACCATTACTGTGACATCTAGAC
GADPH	NM_204305.1	AGAACATCATCCCAGCGT	AGCCTTCACTACCCTCTTG

glyceraldehyde-3-phosphate dehydrongenase (GADPH) as the internal reference gene.

Statistical analysis

All data were presented as the mean ± standard deviation (SD). One-way and two-way analyses of variance (ANOVA) were used for statistical analysis with SPSS (version 19, SPSS Inc., Chicago, IL, USA). Kruskal-Wallis ANOVA test and Mann-Whitney *U* test were used to verify the comparison of groups. Pearson's *r* was used to measure linear correlations among the determined factors. Principal component analysis (PCA) was used to define the most important parameters, which could be used as key factors for individual variations using Statistics 6.0 program (version 19; SPSS Inc., Chicago, IL, USA).

Results

Relative mRNA expressions of five HSPs

As shown in Fig. 1, there was no significant difference ($P > 0.05$) of HSP27, HSP40, HSP60, HSP70, and HSP90 mRNA expressions between the control group and the Se group. Relative mRNA expressions of five HSPs in the Pb group were significantly higher ($P < 0.05$) than those in the control, Se, and Pb + Se groups. They were 25.64, 23.92, 29.41, 40.13, 28.88 times as much in the control group, respectively. HSP70 was the highest level and significantly higher ($P < 0.05$) than HSP27, HSP40, HSP60, and HSP90 in the Pb group. Relative mRNA expressions of five HSPs in the Pb + Se group were significantly higher ($P < 0.05$) than those in the control and Se groups.

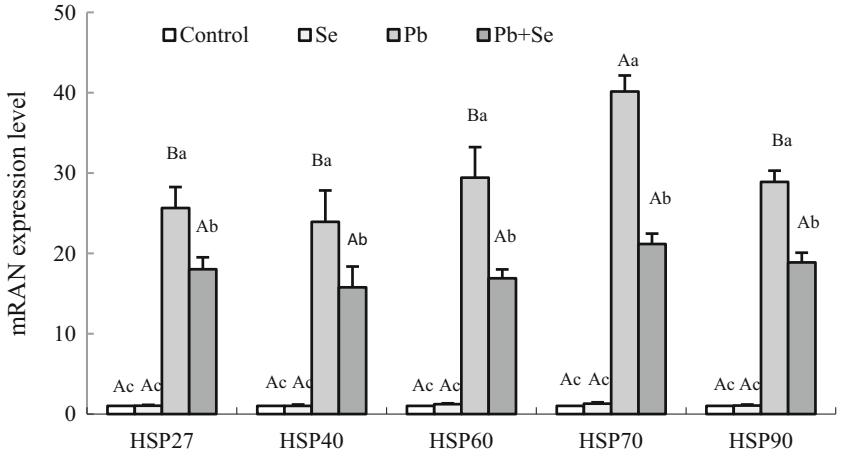

Fig. 1 Relative mRNA expressions of five HSPs on the 90th day in the chicken testes. Fifteen chickens consisted of three replicate pens, with each pen containing five chickens. *Bars* represent mean ± SD. Statistically significant differences: *bars with different uppercase letters* in the same group among different genes are significantly different ($P < 0.05$), and *bars with different lowercase letters* in the same gene among different groups are significantly different ($P < 0.05$)

Relative mRNA expressions of 25 selenoproteins

Relative mRNA levels of 25 selenoprotein genes (Dio1, Dio2, Dio3, GPx1, GPx2, GPx3, GPx4, SelH, SelI, SelK, SelM, SelO, SelPb, SelS, SelT, SelU, SelW, Sep15, SepN1, Sepp1, SepX1, SPS2, Txnrd1, Txnrd2, and Txnrd3) were detected in chicken testes (Fig. 2). Relative mRNA levels of 25 selenoprotein genes showed the highest expression in the Se group, followed by those in the control group, Pb, and Pb + Se groups. There were significant differences ($P < 0.05$) among the groups.

Multivariate correlation analysis

Pearson's correlation coefficient analysis was used for multivariate correlation analysis (Table 2). There were significant positive correlations among HSP27, HSP40, HSP60, HSP70, and HSP90 at the 0.01 level, and among Dio1, Dio2, Dio3, GPx1, GPx2, GPx3, GPx4, SelH, SelI, SelK, SelM, SelO, SelPb, SelS, SelT, SelU, SelW, Sep15, SepN1, Sepp1, SepX1, SPS2, Txnrd1, Txnrd2, and Txnrd3 at the 0.01 or 0.05 level, except between Dio3 and SelH, or SelK, or SelS, or SelT, or SelW. There were negative correlations between five HSPs and 25 selenoproteins.

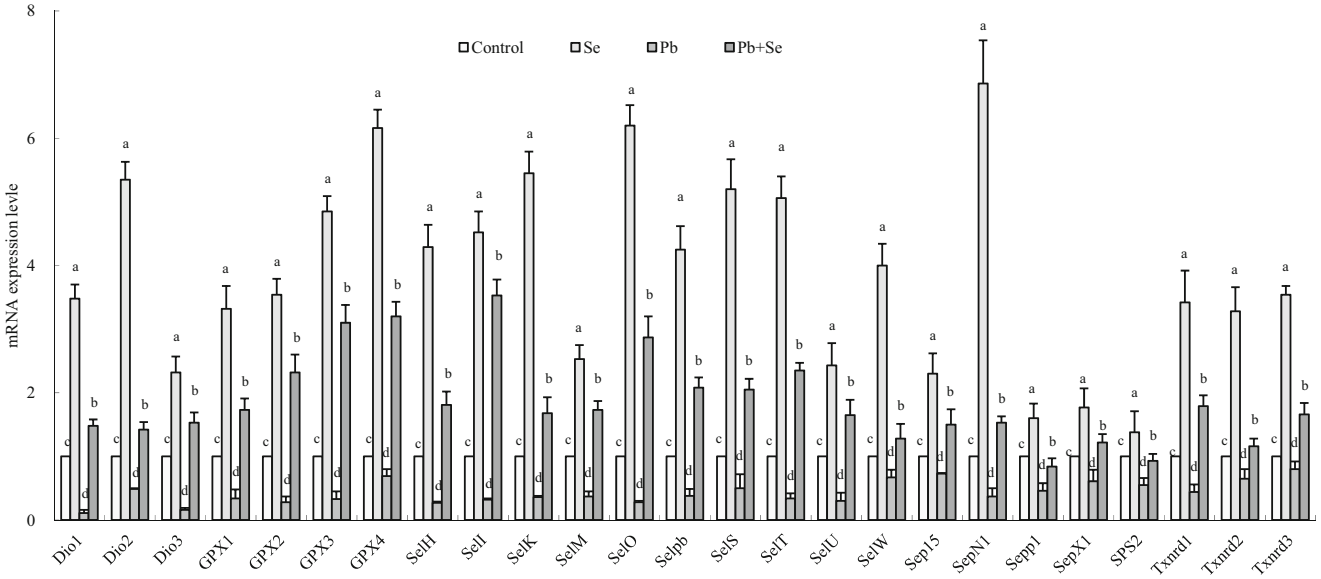

Fig. 2 Relative mRNA levels of 25 selenoproteins on the 90th day in the chicken testes. Fifteen chickens consisted of three replicate pens, with each pen containing five chickens. *Bars* represent mean ± SD. Statistically significant differences: *bars with different lowercase letters* in the same gene are significantly different ($P < 0.05$)

Table 2 Correlation matrix

Gene	HSP27	HSP40	HSP60	HSP70	HSP90	Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelH	SelI	SelK
HSP27	1.00**														
HSP40	.998**	1.00**													
HSP60	.991**	.994**	1.00**												
HSP70	.967**	.974**	.992**	1.00**											
HSP90	.990**	.993**	.999**	.993**	1.00**										
Dio1	−.624	−.634	−.605	−.607	−.642	1.00**									
Dio2	−.561	−.576	−.564	−.589	−.604	.984**	1.00**								
Dio3	−.316	−.340	−.363	−.433	−.406	.833**	.918**	1.00**							
GPx1	−.462	−.472	−.437	−.442	−.480	.981**	.972**	.848**	1.00**						
GPx2	−.600	−.609	−.578	−.580	−.616	.999**	.983**	.832**	.987**	1.00**					
GPx3	−.614	−.615	−.558	−.526	−.590	.959**	.895**	.655*	.947**	.962**	1.00**				
GPx4	−.574	−.574	−.511	−.473	−.544	.941**	.871**	.623*	.939**	.946**	.998**	1.00**			
SelH	−.719*	−.716	−.654	−.606	−.679	.911**	.823**	.531	.871**	.911**	.981**	.977**	1.00**		
SelI	−.592	−.593	−.535	−.504	−.569	.959**	.896**	.662*	.953**	.963**	.987**	.998**	.975**	1.00**	
SelK	−.963**	−.962**	−.933**	−.900**	−.942**	.787*	.711*	.435	.660*	.770*	.803**	.773*	.880**	.785*	1.00**
SelM	−.872**	−.878**	−.856**	−.848**	−.879**	.927**	.888**	.678*	.837**	.915**	.894**	.863**	.914**	.883**	.955**
SelO	−.529	−.528	−.463	−.424	−.497	.931**	.861**	.618*	.940**	.938**	.993**	.998**	.966**	.995**	.738*
Selpb	−.567	−.576	−.541	−.540	−.580	.996**	.977**	.825**	.992**	.999**	.967**	.955**	.912**	.969**	.747**
SelS	−.892**	−.893**	−.856**	−.826**	−.874**	.889**	.820**	.552	.795*	.877**	.904**	.881**	.949**	.892**	.980**
SelT	−.658	−.657	−.595	−.553	−.624	.935**	.857**	.588	.912**	.937**	.995**	.994**	.995**	.993**	.837**
SelU	−.868**	−.880**	−.882**	−.898**	−.904**	.889**	.884**	.743*	.787*	.873**	.799*	.756*	.808**	.787*	.914**
SelW	−.927**	−.927**	−.893**	−.861**	−.907**	.847**	.774*	.500	.739*	.834**	.865**	.839**	.924**	.850**	.994**
Sep15	−.587	−.588	−.529	−.497	−.563	.955**	.891**	.654*	.950**	.960**	.999**	.999**	.976**	.985**	.782*
SepN1	−.851**	−.853**	−.816**	−.791*	−.839**	.928**	.867**	.617*	.849**	.919**	.935**	.913**	.961**	.925**	.957**
Sepp1	−.700	−.711	−.691	−.699	−.725*	.992**	.981**	.834**	.950**	.987**	.933**	.907**	.898**	.930**	.834**
SepX1	−.451	−.469	−.469	−.512	−.512	.942**	.986**	.970**	.947**	.942**	.819**	.793*	.721*	.823**	.598
SPS2	−.565	−.571	−.526	−.514	−.564	.987**	.951**	.767*	.986**	.991**	.987**	.980**	.940**	.989**	.756*
Txnrd1	−.597	−.601	−.549	−.526	−.584	.976**	.924**	.710*	.968**	.979**	.997**	.992**	.966**	.998**	.786*
Txnrd2	−.548	−.557	−.523	−.525	−.563	.995**	.979**	.836**	.995**	.998**	.962**	.950**	.901**	.965**	.730*
Txnrd3	−.580	−.589	−.556	−.557	−.595	.998**	.980**	.830**	.990**	.994**	.965**	.951**	.911**	.966**	.756*

Gene	SelM	SelO	Selpb	SelS	SelT	SelU	SelW	Sep15	SepN1	Sepp1	SepX1	SPS2	Txnrd1	Txnrd2	Txnrd3
HSP27															
HSP40															
HSP60															
HSP70															
HSP90															
Dio1															

Table 2 (continued)

Gene	SelM	SelO	Selpb	SelS	SelT	SelU	SelW	Sep15	SepN1	Sepp1	SepX1	SPS2	Txnrd1	Txnrd2	Txnrd3
Dio2															
Dio3															
GPx1															
GPx2															
GPx3															
GPx4															
SelH															
SelI															
SelK															
SelM	1.00**														
SelO	.835**	1.00**													
Selpb	.897**	.949**	1.00**												
SelS	.985**	.854**	.862**	1.00**											
SelT	.900**	.987**	.941**	.925**	1.00**										
SelU	.977**	.723*	.848**	.930**	.795**	1.00**									
SelW	.976**	.808**	.815**	.996**	.891**	.926**	1.00**								
Sep15	.879**	.996**	.966**	.890**	.993**	.780*	.847**	1.00**							
SepN1	.989**	.889**	.907**	.996**	.947**	.935**	.983**	.922**	1.00**						
Sepp1	.959**	.891**	.978**	.915**	.913**	.940**	.883**	.925**	.947**	1.00**					
SepX1	.809**	.786*	.938**	.717*	.767*	.830**	.666*	.817**	.775*	.937**	1.00**				
SPS2	.889**	.976**	.995**	.872**	.967**	.820**	.825**	.987**	.913**	.962**	.899**	1.00**			
Txnrd1	.897**	.988**	.983**	.894**	.986**	.814**	.851**	.997**	.930**	.951**	.859**	.996**	1.00**		
Txnrd2	.887**	.945**	.986**	.849**	.934**	.839**	.800**	.962**	.895**	.975**	.944**	.993**	.980**	1.00**	
Txnrd3	.904**	.944**	.979**	.868**	.939**	.859**	.823**	.963**	.911**	.982**	.941**	.993**	.982**	.999**	1.00**

*The correlation is significant at 0.05 level

**The correlation is significant at 0.01 level

Table 3 Principle component analysis results of HSPs and selenoproteins

Component	Initial eigenvalues			Extraction sums of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	25.090	83.633	83.633	25.090	83.633	83.633
2	3.676	12.255	95.888	3.676	12.255	95.888
3	1.234	4.112	100.000			
4	9.441E-16	3.147E-15	100.000			
5	7.191E-16	2.397E-15	100.000			
6	5.958E-16	1.986E-15	100.000			
7	5.379E-16	1.793E-15	100.000			
8	4.261E-16	1.420E-15	100.000			
9	3.904E-16	1.301E-15	100.000			
10	3.139E-16	1.046E-15	100.000			
11	2.449E-16	8.162E-16	100.000			
12	1.969E-16	6.562E-16	100.000			
13	1.517E-16	5.056E-16	100.000			
14	9.210E-17	3.070E-16	100.000			
15	4.482E-17	1.494E-16	100.000			
16	1.800E-17	5.999E-17	100.000			
17	−3.865E-17	−1.288E-16	100.000			
18	−7.223E-17	−2.408E-16	100.000			
19	−1.237E-16	−4.124E-16	100.000			
20	−1.314E-16	−4.380E-16	100.000			
21	−2.170E-16	−7.232E-16	100.000			
22	−2.386E-16	−7.953E-16	100.000			
23	−2.808E-16	−9.361E-16	100.000			
24	−3.168E-16	−1.056E-15	100.000			
25	−4.035E-16	−1.345E-15	100.000			
26	−5.123E-16	−1.708E-15	100.000			
27	−5.754E-16	−1.918E-15	100.000			
28	−6.285E-16	−2.095E-15	100.000			
29	−8.001E-16	−2.667E-15	100.000			
30	−1.603E-15	−5.343E-15	100.000			

Principal component analysis

All the examined parameters were used for PCA (Table 3). The results showed that all the parameters focused on the first two principal components. The first two principal components reflected 95.888% original data information of this study. Principal component (PC)1 and PC2 accounted for 83.633 and 12.255% of total variance, respectively. Therefore, the first two principal components were extracted. Twenty-five selenoproteins corresponded to PC1, and five HSPs corresponded to PC2 (Table 4 and Fig. 3).

Discussion

Cells have response to a variety of stressors (such as heavy metals) through the synthesis of HSPs (Yamashita et al. 2010). Arsenic (As) and Cd increased HSP27, HSP60, and HSP70 mRNA expressions in immortalized human proximal tubule cells (Kim et al. 2001). Excess As increased mRNA expressions of HSP27, HSP60, HSP70, and HSP90 in chicken livers (Zhang et al. 2016a). Expression levels of HSP27, HSP40, HSP60, HSP70, and HSP90 increased in As-treated chicken immune organs (Guo et al. 2016) and Pb-treated chicken livers (Wang et al. 2016). Our experiment results were consistent with the above researches. In our study, Pb poisoning increased mRNA expressions of HSP27, HSP40, HSP60, HSP70, and HSP90 in the chicken testes. HSP40 mRNA expression was the lowest in the Pb group and was 23.92 times as much in the control group. Our findings indicated that Pb toxicity resulted in higher mRNA expressions of HSPs. Sassi et al. (2013) reported that HSP70 could serve as a sensitive biomarker for the diagnosis of Cd contamination in Osteichthyes. Ferencz et al. (2012) demonstrated that Cd treatment caused the induction of HSP70 in the skin of *Cyprinus carpio* by 20-fold and HSP70 may be a biomarker of Cd poisoning in *Cyprinus carpio*. HSP70 was one of biomarkers of heavy metals (Copper, Pb, zinc, Cd, manganese, and iron) in the gills and livers of milk fishes (Rajeshkumar et al. 2013). We also found that HSP70 mRNA level in the Pb group was 40.13 times as much in the control group and HSP70 expression was the highest level in the Pb group. Our results implied that HSP70 may be a biomarker of Pb poisoning in the chicken testes. A research reported that Se alleviated the increase of HSP70 mRNA level induced by As in rat livers (Xu et al. 2013). Se alleviated Pb toxicity through the decrease of mRNA expressions of HSPs in the immune organs (Yang et al. 2016) and livers (Wang et al. 2016) of chickens. In our experiment, Se alleviated the increase of HSP27, HSP40, HSP60, HSP70, and HSP90 mRNA expressions induced by Pb in the chicken testes. Our results suggested that Se antagonized Pb-induced toxicity in the chicken testes.

Table 4 Principle component

Factors	PC1	PC2
HSP27	−.760	.648
HSP40	−.766	.642
HSP60	−.733	.675
HSP70	−.721	.660
HSP90	−.763	.638
Dio1	.954	.028
Dio2	.957	.221
Dio3	.894	−.428
GPx1	.979	.180
GPx2	.939	.219
GPx3	.732	.334
GPx4	.926	.368
SelH	.958	.124
SelI	.869	.300
SelK	.968	.221
SelM	.963	−.238
SelO	.959	.275
Selpb	.969	.237
SelS	.964	.261
SelT	.965	.254
SelU	.937	−.323
SelW	.924	.284
Sep15	.932	−.262
SepN1	.954	.225
Sepp1	.984	−.152
SepX1	.986	.067
SPS2	.979	−.200
Txnrd1	.974	.212
Txnrd2	.962	.194
Txnrd3	.942	.234

Se is involved in a variety of physiological processes in the form of selenoproteins (Yao et al. 2013a, 2014). Se-supplemented diet increased mRNA expression of SelW in the livers (Sun et al. 2011) and pancreatic tissues (Wang et al. 2011) of chickens. Gao et al. (2016) reported that Se increased mRNA expressions of Dio1, Dio2, Dio3, GPx1, GPx2, GPx3, GPx4, SelH, SelI, SelK, SelM, SelO, SelPb, SelS, SelT, SelU, SelW, Sep15, SepN1, Sepp1, SepX1, SPS2, Txnrd1, Txnrd2, and Txnrd3 in chicken cartilages. In our study, we also found that Se increased relative mRNA expressions of the same 25 selenoproteins in the chicken testes. Our results demonstrated the reliability of our data. Se has a protective effect on male reproductive function through selenoproteins in animals. GPxs were essential for fertility of human sperms (Foresta et al. 2002). Sepp1 was necessary for Se level of male rat testes and fertility (Gary et al. 2007). SelM had a key role in reproductive regulation during the rapid gonad development of Chinese mitten crabs (Lu et al. 2012). SelS had a special role in the spermatogenesis of *Psammomys obesus* (Windmill et al. 2007). SelW protected chicken myoblasts against apoptosis (Yao et al. 2013b). Selenoproteins can alleviate Pb poisoning in Nile tilapias (Tanekhy 2015). GPx1 and GPx4 had important protective role against the reproductive toxicity of mercury chloride in male rats (Martinez et al. 2016). Gao et al. (2016) reported that Se alleviated the decrease of mRNA expressions of 25 selenoproteins caused by Pb in chicken cartilages. Our results also indicated that Se alleviated the decrease of mRNA expressions of the same 25 selenoproteins caused by Pb in the chicken testes. Our results meant that Se alleviated Pb poisoning through increasing selenoproteins in the chicken testes.

In addition, Pearson's correlation coefficient analysis of our experiment showed that there were positive correlations among five HSPs (HSP27, HSP40, HSP60, HSP70, and HSP90) and among 25 selenoproteins (Dio1, Dio2, Dio3, GPx1, GPx2, GPx3, GPx4, SelH, SelI, SelK, SelM, SelO, SelPb, SelS, SelT, SelU, SelW, Sep15, SepN1, Sepp1,

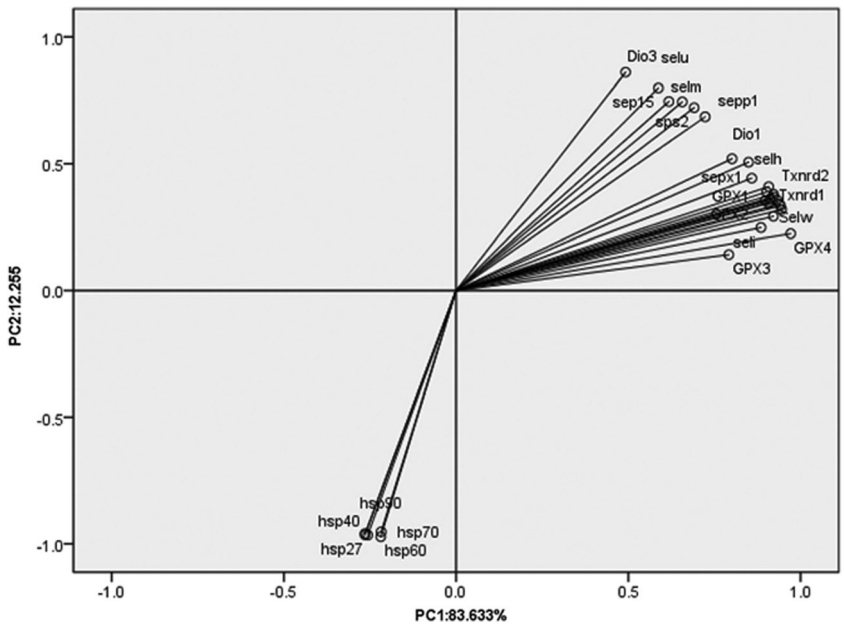

Fig. 3 Principle component analysis on the 90th day in the chicken testes

SepX1, SPS2, Txnrd1, Txnrd2, and Txnrd3). Song et al. (2014) also found that HSP40 was bound to HSP70 ATPase domain during assisting protein folding. There were negative correlations between the five HSPs and the 25 selenoproteins. Moreover, PCA showed that five HSPs belonged to PC2 and 25 selenoproteins belonged to PC1. Pearson's correlation analysis and PCA analysis indicated that five HSPs and 25 selenoproteins had the same feature, respectively, under alleviative effect of Se on Pb poisoning in the chicken testes. Our analysis further verified the reliability of our results.

Conclusion

Pb poisoning increased mRNA expressions of HSP27, HSP40, HSP60, HSP70, and HSP90 in the chicken testes. Pb poisoning decreased mRNA expressions of Dio1, Dio2, Dio3, GPx1, GPx2, GPx3, GPx4, SelH, SelI, SelK, SelM, SelO, SelPb, SelS, SelT, SelU, SelW, Sep15, SepN1, Sepp1, SepX1, SPS2, Txnrd1, Txnrd2, and Txnrd3 in the chicken testes. HSP70 may be a biomarker of Pb poisoning in the chicken testes. Se alleviated the changes of five HSPs and 25 selenoproteins caused by Pb in the chicken testes. Se alleviated Pb-induced toxicity in the chicken testes through regulating mRNA expressions of HSPs and selenoproteins.

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Compliance with ethical standards All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University.

Conflict of interest The authors declare that they have no conflicts of interest.

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