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Original Research Article

Determination of optimal dietary selenium levels by full expression of selenoproteins in various tissues of broilers from 1 to 21 d of age

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ABSTRACT

The current NRC dietary selenium (Se) requirement (0.15 mg/kg) of broilers is primarily based on growth performance data reported in 1986. Our study aimed to determine optimal dietary Se levels of broilers fed a practical corn-soybean meal diet for the full expression of selenoproteins in various tissues. A total of 384 one-d-old male broilers ($n = 8$ replicates/diet) were fed a basal corn-soybean meal diet or the basal diet supplemented with 0.1, 0.2, 0.3, 0.4 or 0.5 mg Se/kg in the form of Na_2SeO_3 for 21 d. Regression analysis was conducted to evaluate the optimal dietary Se levels using broken-line, quadratic or asymptotic models. The activity of glutathione peroxidase (GPX) in the plasma, liver, kidney and pancreas, iodothyronine deiodinase (DIO) in the plasma, liver and pancreas, and thioredoxin reductase (Txnrd) in the liver and pancreas, the mRNA levels of *Gpx1*, *Gpx4*, *Dio1*, *selenoprotein* (*Seleno*) *h*, *Selenop* and *Selenou* in the liver, *Gpx4*, *Dio1*, *Txnrd1*, *Txnrd2*, *Selenoh*, *Selenop* and *Selenou* in the kidney, and *Gpx1*, *Gpx4*, *Selenoh* and *Selenou* in the pancreas, and the protein levels of GPX4 in the liver and kidney of broilers were influenced ($P < 0.05$) by added Se levels, and increased quadratically ($P < 0.05$) with the increase of added Se levels. The estimates of optimal dietary Se levels were 0.07 to 0.36 mg/kg based on the fitted broken-line, quadratic or asymptotic models ($P < 0.001$) of the aforementioned selenoprotein expression in the plasma, liver and kidney, and 0.09 to 0.46 mg/kg based on the fitted broken-line models ($P < 0.001$) of the aforementioned selenoprotein expression in the pancreas. The results indicate that the optimal dietary Se levels would be 0.36 mg/kg to support the full expression of selenoproteins in the plasma, liver and kidney, and 0.46 mg/kg to support the full expression of selenoproteins in the pancreas of broilers fed a practical corn-soybean meal diet from 1 to 21 d of age.

1. Introduction

Selenium (Se) is an essential trace element for humans and animals (Labunskyy et al., 2014). It mainly functions in the form of selenoproteins, which have various biological functions in redox homeostasis, immunity, reproduction, and thyroid hormone metabolism (Lei and Burk, 2018; Roman et al., 2014). Broiler chicks are very susceptible to dietary Se deficiency due to their fast growth rate (Sun et al., 2018). The current NRC dietary Se requirement for broilers is 0.15 mg/kg (NRC, 1994), which is primarily based on the body weight gain and feed intake of

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broilers fed corn-soybean and semi-purified diets reported in 1986 (Jensen et al., 1986). However, the growth performance may not reflect the metabolic and functional Se requirements for broilers. Furthermore, with constant improvements in genetic selection and nutrition, the growth performance of modern commercial broilers has been increased (Applegate and Angel, 2014). Therefore, it is necessary to evaluate Se status and requirements in modern commercial broiler strains using biochemical and molecular biomarkers.

Se functions in the body in the form of selenoproteins. There are 25 selenoproteins that are recognized in humans, 24 selenoproteins in rodents, and 24 confirmed selenoproteins in chickens, including glutathione peroxidase (GPX), iodothyronine deiodinase (DIO) and thioredoxin reductase (Txnrd) families, selenoprotein H (Selenoh), selenoprotein P (Selenop), selenoprotein U (Selenou), etc (Kryukov et al., 2003; Labunskyy et al., 2014; Li et al., 2018). Since Rotruck et al. (1973) reported that the GPX was a selenoenzyme in mammals, many studies have showed that supplemental Se could increase GPX activity in the plasma and tissues of chickens (Liu et al., 2019; Omaye and Tappel, 1974; Rao et al., 2013; Yuan et al., 2012). Nevertheless, few studies have been conducted to determine the Se status and requirements of broiler chickens fed diets with multiple different Se levels by using GPX activity and other selenoprotein expressions as biomarkers (Li and Sunde, 2016). A recent study showed that enzyme activity and transcript level of selenoproteins were sensitive indexes for evaluating Se status and Se requirements of chicks fed a semi-purified diet supplemented with graded levels of Se, and the optimal Se requirements were 0.15 to 0.20 mg/kg based on enzyme activity and transcript level of selenoproteins in different tissues of broilers from 1 to 29 d of age (Li and Sunde, 2016). However, this study might not be suitable for commercial diets due to lower levels of antinutritional factors in semi-purified diets and slower growth and feed intake of chicks, and thus it is still unknown whether the enzyme activity, mRNA and protein expressions of selenoproteins in different tissues could be used to assess dietary Se requirements of chicks fed practical diets. Therefore, we hypothesized that the enzyme activity, mRNA and protein expression levels of selenoproteins in targeted tissues might be sensitive indices to evaluate dietary Se requirements of broilers fed a practical corn-soybean meal diet, and the Se requirements for the full expression of selenoproteins in modern commercial broilers might be higher than the current NRC Se requirement for the optimum growth performance. This study aims to determine the effect of multiple graded levels of Se on enzyme activity as well as mRNA and protein expression levels of selenoproteins, and other commonly used indexes including growth performance and Se concentrations in different tissues, and to find sensitive indexes to reflect Se status and determine optimal dietary Se levels of broilers fed a practical corn-soybean meal diet.

2. Materials and methods

2.1. Experimental design and treatments

This experiment was a completely randomized design. The supplemental Se levels for the 6 treatments were 0 (control), 0.1, 0.2, 0.3, 0.4, or 0.5 mg Se/kg from Na_2SeO_3.

2.2. Birds and diets

All experimental procedures were approved by the animal experimental ethics committee of the Institute of Animal Science, Chinese Academy of Agricultural Sciences. The Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines for reporting animal research were followed in this experiment (Kilkenny et al., 2012). A total of 384 one-d-old Arbor Acres male broiler chicks (Huadu Broiler Breeding Corp., Beijing, China) were kept in a thermostatically controlled room and raised according to the Arbor Acres broilers management guidelines during the experiment. All broilers were allowed ad libitum access to feed and tap water. The 1-d-old birds with similar body weight were randomly allocated to 1 of 6 treatments with 8 replicate cages of 8 chicks in each cage. Dead chicks were recorded every day, and the chick weight and feed intake of each cage were measured on d 21. The basal corn-soybean meal diet was formulated according to NRC (1994) nutrient requirements except for Se (Table 1). The analyzed dietary Se concentrations were 0.028, 0.129, 0.231, 0.329, 0.430, and 0.528 mg/kg for the 6 groups on an as-fed basis, respectively. The diets were fed to birds in mash form.

2.3. Sample collections and preparations

At 21 d of age, 4 birds from each replicate cage were chosen based on the average body weight. Blood samples were obtained through heart puncture and then centrifuged at 3,000 × *g* for 10 min to collect plasma, which was frozen at −20 °C for analyses of plasma Se content and GPX, DIO and Txnrd activity. Then the selected birds were sacrificed by cervical dislocation for collecting liver, kidney and pancreas samples. For all samples, one part of each sample was stored at −20 °C for analyses of Se contents and GPX, DIO and Txnrd activity, and another part of each sample was stored in liquid nitrogen and then frozen at −80 °C for determinations of *Gpx1*, *Gpx4*, *Dio1*, *Txnrd1*, *Txnrd2*, *Selenoh*, *Selenop* and *Selenou* mRNA levels as well as GPX1 and GPX4 protein levels. Samples of 4 chicks in each replicate cage were mixed into 1 sample in the same proportion before testing.

2.4. Se concentrations and enzyme activity

The fluorescence method was used to determine Se concentration by using a Hitachi 850 fluorescence spectrophotometer (Whetter and Ullrey, 1978). The standard reference material bovine liver powder (GBW [E] 080,193, National Institute of Standards and Technology, Beijing, China) was used for validation of the Se analysis. GPX and Txnrd activity (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) as well as DIO activity (Shanghai Jianglaibio Company Ltd, Shanghai, China) were determined using corresponding commercial assay kits through the colorimetric method. All these procedures were conducted in accordance with the manufacturers' instructions.

2.5. mRNA levels

Total RNA in the liver, kidney and pancreas was extracted by using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacture's protocol. The content and purity of RNA were determined using the NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and the quality was checked by denatured RNA electrophoresis. One microgram of RNA was used for reverse transcription by using the PrimeScript RT reagent kit with gDNA Eraser (Takara, Otsu, Japan). Primer sequences for *Gpx1*, *Gpx4*, *Dio1*, *Txnrd1*, *Txnrd2*, *Selenoh*, *Selenop* and *Selenou*, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and β-actin (Table 2) were used for amplification

Table 1
Composition of the basal diet for broilers during 1 to 21 d of age (as-fed basis, %).

Item	Content
Ingredients	
Corn	52.77
Soybean meal	38.36
Soybean oil	4.64
$CaHPO_4$[1]	2.05
$CaCO_3$[1]	1.02
Sodium chloride[1]	0.30
DL-Met[1]	0.32
Micronutrients[2]	0.34
Corn starch + Se additive[3]	0.20
Nutrient composition	
Metabolizable energy, kcal/kg	3000
Crude protein[4]	21.53
Lysine	1.14
Methionine	0.61
Methionine + cysteine	0.90
Calcium[4]	1.04
Nonphytate P	0.45
Se[4], mg/kg	0.028

[1] Reagent grade.
[2] Supplied per kilogram of diet: 15,000 IU vitamin A (all trans-retinol acetate); 4,500 IU cholecalciferol; 24 IU vitamin E (all-rac-α-tocopherol acetate); 3 mg vitamin K (menadione sodium bisulfate); 3 mg thiamin (thiamin mononitrate); 9.6 mg riboflavin; 3 mg vitamin B_6; 0.018 mg vitamin B_{12}; 15 mg calcium pantothenate; 39 mg niacin; 1.5 mg folic acid; 0.15 mg biotin; 700 mg choline (choline chloride); 8 mg Cu ($CuSO_4$ $5H_2O$); 60 mg ($FeSO_4 \cdot 7H_2O$); 110 mg Mn ($MnSO_4 \cdot H_2O$); 60 mg Zn ($ZnSO_4 \cdot 7H_2O$); 0.35 mg I (KI).
[3] Se additives, sodium selenite was added in place of the equivalent weight of corn starch.
[4] Analyzed values based on triplicate determinations, and the others were calculated values.

reactions according to their gene sequences published in GenBank, respectively. SYBR-Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) was used to conduct real-time PCR reactions using an ABI 7500 real-time PCR system. The reactions lasted for 10 min at 95 °C, and then followed 40 cycles. The cycle conditions were 94 °C for 15 s and 60 °C for 1 min. The $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to calculate the mRNA level of the target gene and was normalized by the geometric mean of β-actin and *GAPDH*.

2.6. Protein levels

The GPX1 and GPX4 protein levels in the liver, kidney and pancreas were measured by Western blot method. Frozen liver, pancreas and kidney samples were homogenized in ice-cold RIPA lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) added with protease inhibitor (Biotool, Houston, TX, USA). The homogenate was centrifuged at 10,000 × *g* for 10 min at 4 °C to collect supernatant, and then the total protein was determined by the BCA protein assay kit (Pierce, Rockford, IL, USA). The extracted protein was quantified by western blotting using the primary antibodies of GPX1 and GPX4 (Abcam, Cambridge, MA, USA) and GAPDH (Huaxingbio, Beijing, China) and the second antibodies of rabbit anti-goat horseradish peroxidase-conjugated antibody (Huaxingbio, Beijing, China) and goat anti-mouse horseradish peroxidase-conjugated antibody (Huaxingbio, Beijing, China). The ratios of GPX1 or GPX4 protein band intensity to internal reference GAPDH protein band intensity was used to express the protein expression level.

Table 2
Primer sequences for real-time PCR amplification.

Gene	GenBank identity	Primer sequences
β-actin	NM_205518.1	Forward:5′-ACCTGAGCGCAAGTACTCTGTCT-3′ Reverse:5′-CATCGTACTCCTGCTTGCTGAT-3′
GAPDH	NM_204305.1	Forward:5′-CTTTGGCATTGTGGAGGGTC-3′ Reverse:5′-ACGCTGGGATGTGTTCTGG-3′
Gpx1	NM_001277853.2	Forward:5′-ACGGCGCATCTTCCAAAG-3′ Reverse:5′-TGTTCCCCCAACCATTTCTC-3′
Gpx4	NM_001346448.1	Forward:5′-CTTCGTCTGCATCATCACCAA-3′ Reverse:5′-TCGACGAGCTGAGTGTAATTCAC-3′
Dio1	NM_001097614.1	Forward:5′-GCGCTATACCACAGGCAGTA-3′ Reverse:5′-GGTCTTGCAAATGTCACCAC-3′
Txnrd1	NM_001352023.1	Forward:5′-TACGCCTCTGGGAAATTCGT-3′ Reverse:5′-CTTGCAAGGCTTGTCCCAGTA-3′
Txnrd2	NM_001122691.2	Forward:5′-GCTCTTAAAGATGCCCAGCACTAC-3′ Reverse:5′-GAACAGCTTGAGCCATCACAGA-3′
Selenoh	NM_001277865.1	Forward:5′-CATCGAGCACTGCCGTAG-3′ Reverse:5′-GACACCTCGAAGCTGTTCCT-3′
Selenop	NM_001031609.2	Forward:5′-CCAAGTGGTCAGCATTCACATC-3′ Reverse:5′-ATGACGACCACCCTCACGAT-3′
Selenou	NM_001193519.2	Forward:5′-GATGCTTTCAGGCTTCTTCC-3′ Reverse:5′-CTGTCTTCCTGCTCCAATCA-3′

GAPDH = glyceraldehyde-3-phosphate dehydrogenase; *Gpx1* = glutathione peroxidase 1; *Gpx4* = glutathione peroxidase 4; *Dio1* = iodothyronine deiodinase 1; *Txnrd1* = thioredoxin reductase 1; *Txnrd2* = thioredoxin reductase 2; *Selenoh* = Selenoprotein H; *Selenop* = selenoprotein P; *Selenou* = Selenoprotein U.

Table 3
Effect of Se inclusion level on growth performance of chicks during 1 to 21 d of age[1].

Item	Weight gain, g/d	Feed intake, g/d	Feed efficiency, g gain/g feed	Mortality[2], %
Added Se, mg/kg				
0.0	34.9[b]	52.9[b]	0.66	0.00
0.1	35.7[a]	53.0[b]	0.68	0.00
0.2	36.5[a]	54.8[ab]	0.67	3.10
0.3	36.5[a]	55.4[a]	0.66	0.00
0.4	36.4[a]	55.1[a]	0.66	0.00
0.5	36.5[a]	54.9[a]	0.66	0.00
Pooled SE	0.27	0.65	0.01	0.02
P-value				
Se concentration	0.0002	0.021	0.67	0.06
Linear	<0.0001	0.003	–	–
Quadratic	0.007	0.10	–	–

[a,b] Means with different superscripts within a column differ ($P < 0.05$).
[1] Data represented the means of 8 replicates.
[2] Percentage data for mortality of birds were transformed to arcsine for analysis.

Table 4
Effect of Se inclusion level on Se concentrations in plasma and tissues of chicks on d 21[1].

Item	Plasma Se, μg/mL	Liver Se[2], μg/g	Kidney Se[2], μg/g	Pancreas Se[2], μg/g
Added Se, mg/kg				
0.0	0.009[e]	0.093[d]	0.141[d]	0.090[e]
0.1	0.105[d]	0.403[c]	0.466[c]	0.174[d]
0.2	0.167[c]	0.507[b]	0.615[b]	0.235[c]
0.3	0.172[bc]	0.578[a]	0.665[b]	0.260[b]
0.4	0.187[ab]	0.573[a]	0.655[b]	0.269[b]
0.5	0.205[a]	0.613[a]	0.728[a]	0.305[a]
Pooled SE	0.007	0.015	0.020	0.006
P-value				
Se concentration	<0.0001	<0.0001	<0.0001	<0.0001
Linear	<0.0001	<0.0001	<0.0001	<0.0001
Quadratic	<0.0001	<0.0001	<0.0001	0.0004

[a-e] Means with different superscripts within a column differ ($P < 0.05$).
[1] Data represented the means of 8 replicates.
[2] Fresh basis.

Table 5
Effect of Se inclusion level on selenoenzyme activity in plasma and tissues of chicks on d 21[1].

Item	Plasma			Liver			Kidney			Pancreas		
	GPX, U/mL	Txnrd, U/mL	DIO, U/L	GPX, U/mg protein	Txnrd, U/mg protein	DIO, U/g protein	GPX, U/mg protein	Txnrd, U/mg protein	DIO, U/g protein	GPX, U/mg protein	Txnrd, U/mg protein	DIO, U/g protein
Added Se, mg/kg												
0.0	49.5[d]	1.91	6.6[b]	9.59[d]	0.80[b]	0.553[c]	12.4[c]	1.59[d]	0.889	7.31[e]	0.83[b]	0.575[b]
0.1	1814[c]	2.04	10.9[a]	33.9[c]	1.06[a]	0.592[b]	22.5[b]	2.22[c]	0.933	13.3[d]	1.03[a]	0.657[a]
0.2	2171[b]	2.10	11.1[a]	36.4[bc]	1.05[a]	0.645[a]	27.2[a]	2.39[bc]	0.968	17.4[bc]	1.10[a]	0.651[a]
0.3	2318[ab]	2.26	11.4[a]	36.7[bc]	1.02[a]	0.637[a]	27.7[a]	2.45[ab]	0.927	16.2[c]	1.09[a]	0.669[a]
0.4	2423[a]	2.08	11.2[a]	38.7[ab]	1.03[a]	0.629[a]	28.8[a]	2.61[a]	0.953	19.1[b]	1.03[a]	0.664[a]
0.5	2509[a]	2.19	10.9[a]	40.5[a]	1.00[a]	0.647[a]	29.6[a]	2.44[ab]	0.978	22.0[a]	1.05[a]	0.669[a]
Pooled SE	69.6	0.10	0.27	1.16	0.04	0.01	1.30	0.07	0.03	0.92	0.03	0.01
P-value												
Se concentration	<0.0001	0.18	<0.0001	<0.0001	0.0003	<0.0001	<0.0001	<0.0001	0.25	<0.0001	<0.0001	<0.0001
Linear	<0.0001	–	<0.0001	<0.0001	0.009	<0.0001	<0.0001	<0.0001	–	<0.0001	<0.0001	<0.0001
Quadratic	<0.0001	–	<0.0001	<0.0001	0.01	<0.0001	0.031	0.18	–	0.004	0.008	<0.0001

GPX = glutathione peroxidase; Txnrd = thioredoxin reductase; DIO = iodothyronine deiodinase.
[a–e] Means with different superscripts within a column differ ($P < 0.05$).
[1] Data represented the means of 8 replicates.

Table 6
Effect of Se inclusion level on mRNA levels of selenoproteins in the liver of chicks on d 21[1].

Item	*Gpx1*, RQ	*Gpx4*, RQ	*Txnrd1*, RQ	*Txnrd2*, RQ	*Selenop*, RQ	*Selenou*, RQ	*Selenoh*, RQ	*Dio1*, RQ
Added Se, mg/kg								
0.0	0.86[b]	0.75[b]	0.41[ab]	0.87	1.00[b]	0.87[c]	0.11[c]	0.55[b]
0.1	1.82[a]	1.37[a]	0.45[a]	1.00	2.20[a]	1.93[b]	0.70[a]	0.99[a]
0.2	1.68[a]	1.35[a]	0.36[abc]	0.91	2.30[a]	2.27[ab]	0.49[ab]	1.00[a]
0.3	1.66[a]	1.49[a]	0.39[ab]	0.99	2.28[a]	2.42[a]	0.63[ab]	1.03[a]
0.4	2.00[a]	1.49[a]	0.34[bc]	1.02	2.27[a]	2.52[a]	0.48[ab]	0.98[a]
0.5	1.70[a]	1.24[a]	0.27[c]	0.92	2.34[a]	2.16[ab]	0.40[b]	0.89[a]
Pooled SE	0.16	0.09	0.03	0.05	0.14	0.14	0.08	0.07
P-value								
Se concentration	0.0004	<0.0001	0.007	0.19	<0.0001	<0.0001	0.0003	0.0003
Linear	0.001	0.0007	0.0007	–	<0.0001	<0.0001	0.16	0.007
Quadratic	0.005	<0.0001	0.20	–	<0.0001	<0.0001	0.0001	<0.0001

Gpx1 = glutathione peroxidase 1; *Gpx4* = glutathione peroxidase 4; *Txnrd1* = thioredoxin reductase 1; *Txnrd2* = thioredoxin reductase 2; *Selenop* = selenoprotein P; *Selenou* = Selenoprotein U; *Selenoh* = Selenoprotein H; *Dio1* = iodothyronine deiodinase 1.
[a–c] Means with different superscripts within a column differ ($P < 0.05$).
[1] Data represented the means of 8 replicates. The mRNA expression was calculated as the relative quantities (RQ) of the target gene mRNA to the geometric mean of β-actin and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA, RQ = $2^{-\Delta\Delta Ct}$.

2.7. Statistical analyses

One-way ANOVA was used to test the effect of added Se treatment with the general linear model procedure of SAS (version 9.4; SAS Inst. Inc.). Percentage data for mortality of broilers were converted to arcsine before analysis. The least significant difference method was used to test the differences among means. The replicate cage served as the experimental unit. The linear and quadratic

Table 7
Effect of Se inclusion level on mRNA levels of selenoproteins in the kidney of chicks on d 21[1].

Item	*Gpx1*, RQ	*Gpx4*, RQ	*Txnrd1*, RQ	*Txnrd2*, RQ	*Selenop*, RQ	*Selenou*, RQ	*Selenoh*, RQ	*Dio1*, RQ
Added Se, mg/kg								
0.0	0.97^{b}	1.00^{b}	1.00^{b}	1.00^{bc}	1.04^{c}	1.00^{d}	0.85^{b}	1.00^{b}
0.1	1.55^{a}	1.65^{a}	0.94^{b}	1.16^{a}	1.89^{b}	1.62^{c}	2.28^{a}	1.56^{a}
0.2	1.61^{a}	1.86^{a}	0.97^{b}	1.13^{ab}	2.01^{ab}	1.84^{bc}	3.18^{a}	1.78^{a}
0.3	1.65^{a}	1.77^{a}	1.13^{a}	1.15^{a}	2.10^{ab}	1.91^{abc}	2.54^{a}	1.81^{a}
0.4	1.85^{a}	1.87^{a}	0.89^{bc}	1.05^{abc}	2.22^{a}	2.18^{a}	2.96^{a}	1.81^{a}
0.5	1.90^{a}	1.81^{a}	0.80^{c}	0.95^{c}	2.25^{a}	2.02^{ab}	2.54^{a}	1.84^{a}
Pooled SE	0.14	0.08	0.04	0.04	0.09	0.10	0.32	0.09
P-value								
Se concentration	0.0007	<0.0001	<0.0001	0.005	<0.0001	<0.0001	0.0004	<0.0001
Linear	<0.0001	<0.0001	0.004	0.11	<0.0001	<0.0001	0.001	<0.0001
Quadratic	0.13	<0.0001	0.001	0.0003	<0.0001	0.0003	0.0009	0.0001

Gpx1 = glutathione peroxidase 1; *Gpx4* = glutathione peroxidase 4; *Txnrd1* = thioredoxin reductase 1; *Txnrd2* = thioredoxin reductase 2; *Selenop* = selenoprotein P; *Selenou* = Selenoprotein U; *Selenoh* = Selenoprotein H; *Dio1* = iodothyronine deiodinase 1.
[a–d] Means with different superscripts within a column differ ($P < 0.05$).
[1] Data represented the means of 8 replicates. The mRNA expression was calculated as the relative quantities (RQ) of the target gene mRNA to the geometric mean of β-actin and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA, RQ = $2^{-\Delta\Delta Ct}$.

Table 8
Effect of Se inclusion level on mRNA levels of selenoproteins in the pancreas of chicks on d 21[1].

Item	*Gpx1*, RQ	*Gpx4*, RQ	*Txnrd1*, RQ	*Txnrd2*, RQ	*Selenop*, RQ	*Selenou*, RQ	*Selenoh*, RQ	*Dio1*, RQ
Added Se, mg/kg								
0.0	0.87^{c}	1.00^{c}	1.00	0.92	1.00^{b}	1.00^{c}	1.00^{d}	0.90
0.1	1.10^{c}	1.04^{c}	1.07	0.93	1.05^{b}	1.08^{c}	1.51^{cd}	0.73
0.2	1.46^{b}	1.40^{b}	1.32	0.96	1.35^{ab}	1.50^{b}	2.31^{ab}	1.05
0.3	1.85^{a}	1.75^{a}	1.11	1.18	1.62^{a}	2.02^{a}	2.09^{bc}	0.98
0.4	2.05^{a}	2.00^{a}	1.15	1.12	1.75^{a}	2.07^{a}	2.91^{a}	1.03
0.5	1.88^{a}	1.68^{ab}	1.31	1.03	1.57^{a}	1.82^{ab}	2.39^{ab}	1.04
Pooled SE	0.11	0.11	0.10	0.08	0.13	0.12	0.24	0.09
P-value								
Se concentration	<0.0001	<0.0001	0.19	0.20	0.0005	<0.0001	<0.0001	0.13
Linear	<0.0001	<0.0001	–	–	<0.0001	<0.0001	<0.0001	–
Quadratic	0.012	0.026	–	–	0.14	0.0071	0.03	–

Gpx1 = glutathione peroxidase 1; *Gpx4* = glutathione peroxidase 4; *Txnrd1* = thioredoxin reductase 1; *Txnrd2* = thioredoxin reductase 2; *Selenop* = selenoprotein P; *Selenou* = Selenoprotein U; *Selenoh* = Selenoprotein H; *Dio1* = iodothyronine deiodinase 1.
[a–d] Means with different superscripts within a column differ ($P < 0.05$).
[1] Data represented the means of 8 replicates. The mRNA expression was calculated as the relative quantities (RQ) of the target gene mRNA to the geometric mean of β-actin and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA, RQ = $2^{-\Delta\Delta Ct}$.

Table 9
Effect of Se inclusion level on glutathione peroxidase protein levels in the liver, kidney and pancreas of chicks on d 21[1].

Item	Liver, arbitrary unit		Kidney, arbitrary unit		Pancreas, arbitrary unit	
	GPX1	GPX4	GPX1	GPX4	GPX1	GPX4
Added Se, mg/kg						
0.0	0.75	0.08^{b}	1.65	0.33^{b}	0.53	0.26^{d}
0.1	0.99	1.73^{a}	1.60	2.37^{a}	0.71	0.37^{cd}
0.2	1.00	1.99^{a}	1.69	2.59^{a}	0.61	0.45^{bc}
0.3	1.07	2.09^{a}	1.58	2.73^{a}	0.60	0.60^{a}
0.4	0.98	2.07^{a}	1.40	2.84^{a}	0.56	0.57^{ab}
0.5	0.74	1.68^{a}	1.49	2.81^{a}	0.66	0.69^{a}
Pooled SE	0.16	0.25	0.25	0.26	0.07	0.05
P-value						
Se concentration	0.62	<0.0001	0.97	<0.0001	0.58	<0.0001
Linear	–	<0.0001	–	<0.0001	–	<0.0001
Quadratic	–	<0.0001	–	0.0001	–	0.37

GPX1 = glutathione peroxidase 1; GPX4 = glutathione peroxidase 4.
[a–d] Means with different superscripts within a column differ ($P < 0.05$).
[1] Data represented the means of 8 replicates. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein was used to normalize the levels of the GPX proteins.

responses of responsive indexes to added Se level were tested using orthogonal comparisons. The optimal added Se levels were obtained from the best-fit broken-line, quadratic or asymptotic model between responsive criteria and added Se level (Corzo et al., 2006; Ma et al., 2016; Robbins et al., 2007). $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Growth performance

Supplemental Se level affected ($P < 0.05$) weight gain and feed intake while it did not affect ($P > 0.05$) feed efficiency and

Table 10
The optimal dietary Se levels of chicks during 1 to 21 d of age as estimated based on fitted broken-line, quadratic or asymptotic models.

Dependent variable	Regression equation[1]	Coefficient of determination (R^2)	*P*-value	Optimal added Se level	Optimal dietary Se level[2] (mg/kg)
Plasma Se	$Y = 0.0093 + 0.9580X$ ($0 \le X \le 0.1546$); $Y = 0.1371 + 0.1312X$ ($0.1546 < X \le 0.5$)	0.94	<0.0001	0.15	0.18
Liver Se	$Y = 0.6048\text{-}0.5103*e^{-8.892X}$	0.95	<0.0001	0.32	0.35
Kidney Se	$Y = 0.1408 + 3.254X$ ($0 \le X \le 0.14$); $Y = 0.5502 + 0.3294X$ ($0.14 < X \le 0.5$)	0.93	<0.0001	0.14	0.17
Pancreas Se	$Y = 0.09 + 0.8417X$ ($0 \le X \le 0.1623$); $Y = 0.1911 + 0.2185X$ ($0.1623 < X \le 0.5$)	0.95	< 0.0001	0.16	0.19
Plasma GPX activity	$Y = 49.51 + 17649X$ ($0 \le X \le 0.1158$); $Y = 1964 + 1118X$ ($0.1158 < X \le 0.5$)	0.96	<0.0001	0.11	0.14
Liver GPX activity	$Y = 9.612 + 243.1X$ ($0 \le X \le 0.1025$); $Y = 33.06 + 14.25X$ ($0.1025 < X \le 0.5$)	0.92	<0.0001	0.10	0.13
Kidney GPX activity	$Y = 12.40 + 100.6X$ ($0 \le X \le 0.1409$); $Y = 25.40 + 8.321X$ ($0.1409 < X \le 0.5$)	0.75	<0.0001	0.14	0.17
Pancreas GPX activity	$Y = 7.309 + 59.71X$ ($0 \le X \le 0.1284$); $Y = 12.84 + 16.67X$ ($0.1284 < X \le 0.5$)	0.75	<0.0001	0.13	0.16
Plasma DIO activity	$Y = 6.561 + 43.16X$ ($0 \le X \le 0.1089$); $Y = 11.32\text{-}0.53X$ ($0.1089 < X \le 0.5$)	0.85	<0.0001	0.11	0.14
Liver DIO activity	$Y = 0.5518 + 0.4355X$ ($0 \le X \le 0.2$); $Y = 0.6388 + 0.0007X$ ($0.2 < X \le 0.5$)	0.75	<0.0001	0.20	0.23
Pancreas DIO activity	$Y = 0.5746 + 1.223X$ ($0 \le X \le 0.064$); $Y = 0.6505 + 0.0374X$ ($0.064 < X \le 0.5$)	0.73	<0.0001	0.06	0.09
Liver Txnrd activity	$Y = 0.7949 + 2.618X$ ($0 \le X \le 0.1001$); $Y = 1.071\text{-}0.1398X$ ($0.1001 < X \le 0.5$)	0.42	<0.0001	0.10	0.13
Pancreas Txnrd activity	$Y = 0.8264 + 2.031X$ ($0 \le X \le 0.1432$); $Y = 1.151\text{-}0.2346X$ ($0.1432 < X \le 0.5$)	0.57	<0.0001	0.14	0.17
Liver *Gpx1* mRNA	$Y = 0.8629 + 19.36X$ ($0 \le X \le 0.0458$); $Y = 1.745 + 0.0931X$ ($0.0458 < X \le 0.5$)	0.37	0.0002	0.04	0.07
Liver *Gpx4* mRNA	$Y = 0.7532 + 6.157X$ ($0 \le X \le 0.1191$); $Y = 1.534\text{-}0.3943X$ ($0.1191 < X \le 0.5$)	0.45	<0.0001	0.12	0.15
Liver *Selenop* mRNA	$Y = 0.9997 + 12.01X$ ($0 \le X \le 0.1052$); $Y = 2.248 + 0.1380X$ ($0.1052 < X \le 0.5$)	0.62	<0.0001	0.10	0.13
Liver *Selenou* mRNA	$Y = 0.9484 + 9.546X\text{-}14.29X^2$	0.65	<0.0001	0.33	0.36
Liver *Selenoh* mRNA	$Y = 0.1086 + 6.212X$ ($0 \le X \le 0.0893$); $Y = 0.7166\text{-}0.5966X$ ($0.0893 < X \le 0.5$)	0.41	0.0001	0.09	0.12
Liver *Dio1* mRNA	$Y = 0.5509 + 4.407X$ ($0 \le X \le 0.1153$); $Y = 1.101\text{-}0.3603X$ ($0.1153 < X \le 0.5$)	0.41	<0.0001	0.11	0.14
Kidney *Gpx4* mRNA	$Y = 1.003 + 6.523X$ ($0 \le X \le 0.1276$); $Y = 1.841\text{-}0.0446X$ ($0.1276 < X \le 0.5$)	0.69	<0.0001	0.13	0.16
Kidney *Txnrd1* mRNA	$Y = 0.9463 + 0.38X$ ($0 \le X \le 0.3$); $Y = 1.476\text{-}1.386X$ ($0.3 < X \le 0.5$)	0.39	<0.0001	0.30	0.33
Kidney *Txnrd2* mRNA	$Y = 1.022 + 1.235X\text{-}2.813X^2$	0.29	0.0004	0.22	0.25
Kidney *Selenop* mRNA	$Y = 1.038 + 8.482X$ ($0 \le X \le 0.1061$); $Y = 1.848 + 0.8525X$ ($0.1061 < X \le 0.5$)	0.76	<0.0001	0.10	0.13
Kidney *Selenou* mRNA	$Y = 2.115\text{-}1.107*e^{-7.321X}$	0.68	<0.0001	0.32	0.35
Kidney *Selenoh* mRNA	$Y = 0.8478 + 14.28X$ ($0 \le X \le 0.1571$); $Y = 3.323\text{-}1.478X$ ($0.1571 < X \le 0.5$)	0.40	<0.0001	0.16	0.19
Kidney *Dio1* mRNA	$Y = 1 + 5.594X$ ($0 \le X \le 0.1385$); $Y = 1.751 + 0.1748X$ ($0.1385 < X \le 0.5$)	0.61	<0.0001	0.14	0.17
Pancreas *Gpx1* mRNA	$Y = 0.828 + 3.299X$ ($0 \le X \le 0.3807$); $Y = 2.743\text{-}1.732X$ ($0.3807 < X \le 0.5$)	0.70	<0.0001	0.38	0.41
Pancreas *Gpx4* mRNA	$Y = 0.9022 + 2.683X$ ($0 \le X \le 0.4197$); $Y = 3.865\text{-}4.375X$ ($0.4197 < X \le 0.5$)	0.62	<0.0001	0.42	0.45
Pancreas *Selenou* mRNA	$Y = 0.8872 + 3.462X$ ($0 \le X \le 0.3664$); $Y = 3.065\text{-}2.483X$ ($0.3664 < X \le 0.5$)	0.63	<0.0001	0.36	0.39
Pancreas *Selenoh* mRNA	$Y = 1.075 + 4.390X$ ($0 \le X \le 0.4272$); $Y = 6.276\text{-}7.784X$ ($0.4272 < X \le 0.5$)	0.45	<0.0001	0.43	0.46
Liver GPX4 protein	$Y = 0.0826 + 16.52X$ ($0 \le X \le 0.1259$); $Y = 2.276\text{-}0.903X$ ($0.1259 < X \le 0.5$)	0.63	<0.0001	0.12	0.15
Kidney GPX4 protein	$Y = 0.3306 + 20.36X$ ($0 \le X \le 0.1088$); $Y = 2.458 + 0.8101X$ ($0.1088 < X \le 0.5$)	0.63	<0.0001	0.11	0.14

GPX = glutathione peroxidase; DIO = iodothyronine deiodinase; *Txnrd* = thioredoxin reductase; *Selenoh* = selenoprotein H; *Selenop* = selenoprotein P; *Selenou* = selenoprotein U.

[1] Regression equations based on added Se level (mg/kg).

[2] Optimal dietary Se level = optimal added Se level + Se in the basal diet (0.028 mg/kg).

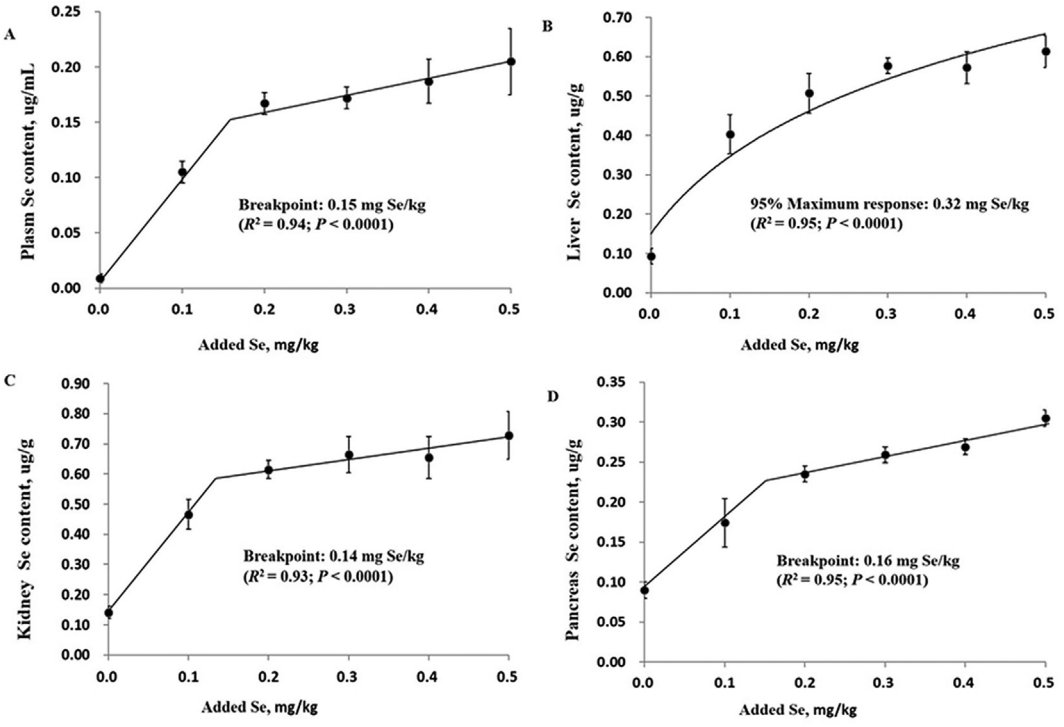

Fig. 1. Relations between added Se and Se concentrations in the plasma (A) or liver (B) or kidney (C) or pancreas (D) of broilers at 21 d of age. Values are means ± SE, $n = 8$.

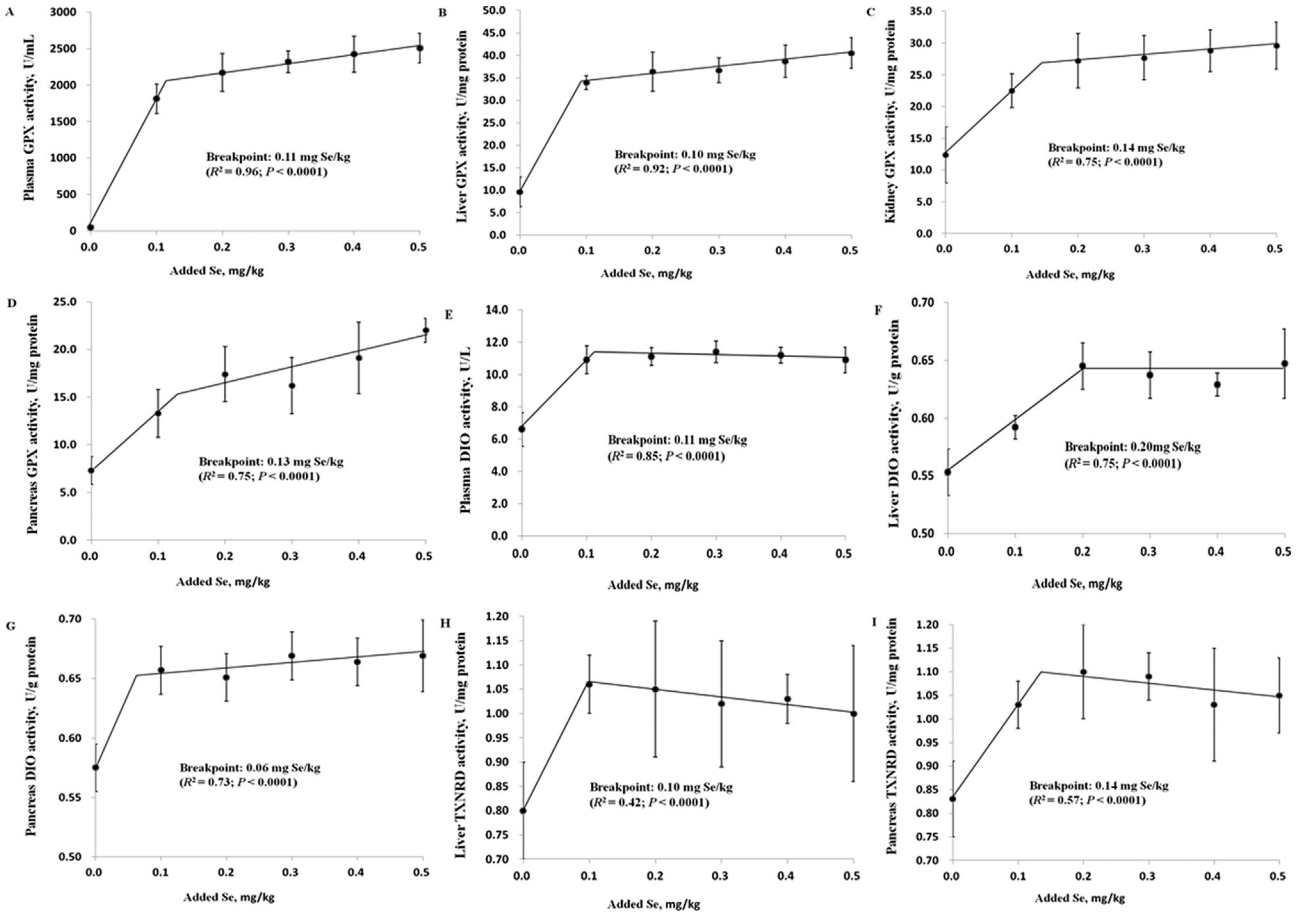

Fig. 2. Relations between added Se and GPX activity in plasma (A) or liver (B) or kidney (C) or pancreas (D), DIO activity in plasma (E) or liver (F) or pancreas (G), and Txnrd activity in the liver (H) or pancreas (I) of broilers at 21 d of age. GPX = glutathione peroxidase; DIO = iodothyronine deiodinase; Txnrd = thioredoxin reductase. Values are means ± SE, n = 8.

mortality of broilers (Table 3). The weight gain increased linearly and quadratically (P < 0.05), as well as feed intake which increased linearly (P < 0.05) with the increase of added Se level.

3.2. Se concentrations

The Se concentrations in the plasma, liver, kidney and pancreas were affected (P < 0.001) by added Se level (Table 4). The linear and quadratic responses were significant (P < 0.001)for the Se concentrations in the plasma and tissues. The Se concentration in the liver reached a plateau at supplemental levels of 0.3 to 0.5 mg Se/kg, while the Se concentrations in the plasma, kidney and pancreas reached the highest point at supplemental level of 0.5 mg Se/kg.

3.3. Enzyme activity

Supplemental Se level did not influence (P > 0.05) plasma Txnrd and kidney DIO activity (Table 5), but it affected (P < 0.001) GPX activity in the plasma, liver, kidney and pancreas, Txnrd activity in the liver, kidney and pancreas as well as DIO activity in the plasma, liver and pancreas; furthermore, the activity increased linearly and quadratically (P < 0.05) as supplemental Se level increased, except for kidney Txnrd activity which only increased linearly (P < 0.001). The DIO activity in the plasma and pancreas as well as Txnrd activity in the liver and pancreas reached plateaus at supplemental levels of about 0.1 to 0.5 mg Se/kg; the DIO activity in the liver and GPX activity in the kidney reached plateaus at supplemental levels of 0.2 to 0.5 mg Se/kg; the GPX activity in the plasma and Txnrd activity in the kidney reached plateaus at supplemental levels of about 0.3 to 0.5 mg Se/kg; and the GPX activity in the liver and pancreas reached the highest point at supplemental level of 0.5 mg Se/kg.

3.4. mRNA levels

Supplemental Se level did not affect liver *Txnrd2*, pancreas *Dio1*, *Txnrd1* and *Txnrd2* mRNA levels (P > 0.05), but did affect *Gpx1*, *Gpx4*, *Selenoh*, *Selenop* and *Selenou* mRNA levels in the liver, kidney and pancreas, *Dio1* and *Txnrd1* mRNA levels in the liver and kidney, as well as *Txnrd2* mRNA level in the kidney (P < 0.05) (Tables 6–8). As the level of supplemental Se increased, the mRNA levels of *Txnrd1* in the liver, *Gpx1* in the kidney and *Selenop* in the pancreas increased linearly (P < 0.001), and the mRNA levels of *Selenoh* in the liver and *Txnrd2* in the kidney increased quadratically (P < 0.001), and the other indexes increased linearly and quadratically (P < 0.05). The mRNA levels of *Gpx1* and *Gpx4* in the liver and kidney, *Dio1* and *Selenop* in the liver, and *Dio1* and *Selenoh* in the kidney reached plateaus at supplemental levels of 0.1 to 0.5 mg Se/kg; and the mRNA levels of *Txnrd1* and *Selenoh* in the liver as well as *Txnrd2* in the kidney reached the highest point at approximate supplemental level of 0.1 mg Se/kg; the mRNA levels of *Selenou* in the liver, *Selenop* and *Selenou* in the kidney, and *Gpx1*, *Gpx4*, *Selenop* and *Selenou* in the pancreas reached plateaus at about supplemental levels of 0.3 to 0.5 mg Se/kg.

3.5. Protein levels

Supplemental Se level did not affect GPX1 protein level ($P > 0.05$) while it did affect GPX4 protein level ($P < 0.001$) in the liver, kidney and pancreas (Table 9). The GPX4 protein level in the liver and kidney increased linearly and quadratically ($P < 0.001$) with the increase of added Se level and maintained a stabilized level at supplemental 0.1 mg Se/kg. The GPX4 protein level in the pancreas increased linearly ($P < 0.001$) with the increase of added Se level.

3.6. Optimal dietary Se levels

The optimal dietary Se level of chicks during 1 to 21 d of age as estimated based on fitted models are showed in Table 10 and Figs. 1–6. The fitted models for weight gain were not significant ($P > 0.05$) though the quadratic response was significant for this index. Based on fitted broken-line, quadratic-curve or asymptotic-line models ($P < 0.001$) of the Se concentrations in the plasma, liver and kidney, GPX activity in the plasma, liver and kidney, DIO activity in the plasma and liver, Txnrd activity in the liver, the mRNA levels of *Gpx1*, *Gpx4*, *Dio1*, *Selenoh*, *Selenop* and *Selenou* in the liver, *Gpx4*, *Dio1*, *Txnrd1*, *Txnrd2*, *Selenoh*, *Selenop* and *Selenou* in the kidney, and the protein levels of GPX4 in the liver and kidney, optimal dietary Se levels were estimated to be between 0.07 and 0.36 mg/kg; and based on fitted broken-line models ($P < 0.001$) of the Se concentration, GPX, DIO and Txnrd activity, *Gpx1*, *Gpx4*, *Selenoh* and *Selenou* mRNA levels in the pancreas, optimal dietary Se levels were estimated to be between 0.09 and 0.46 mg/kg for chicks fed a practical corn-soybean meal diet during 1 to 21 d of age.

4. Discussion

The key findings of the present study demonstrate that the activity of GPX in the plasma, liver, kidney and pancreas, DIO in the

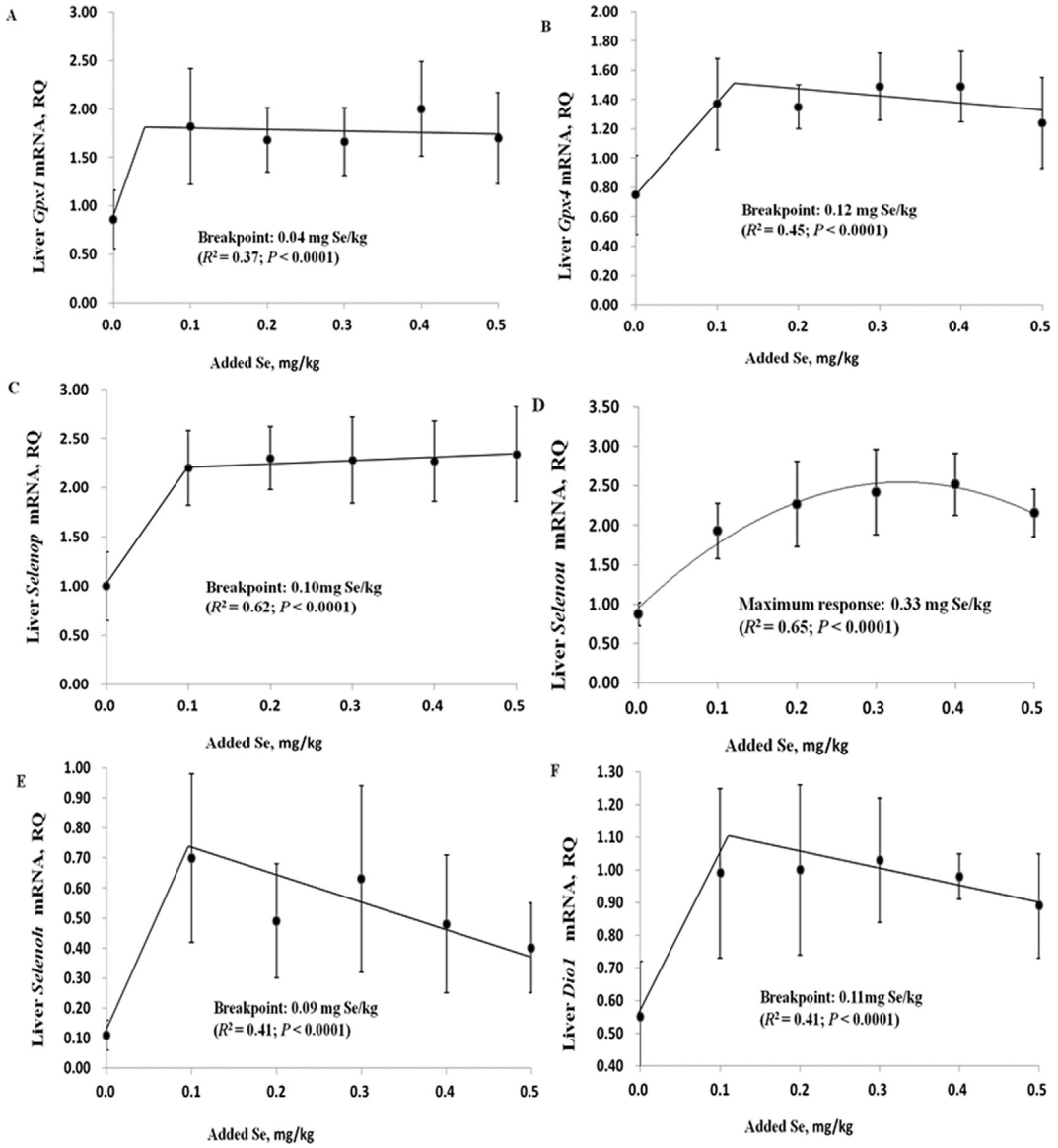

Fig. 3. Relations between added Se and mRNA levels of liver *Gpx1* (A) or *Gpx4* (B) or *Selenop* (C) or *Selenou* (D) or *Selenoh* (E) or *Dio1* (F) in broilers at 21 d of age. *Gpx1* = glutathione peroxidase 1; *Gpx4* = glutathione peroxidase 4; *Selenop* = selenoprotein P; *Selenou* = Selenoprotein U; *Selenoh* = Selenoprotein H; *Dio1* = iodothyronine deiodinase 1. Values are means ± SE, $n = 8$.

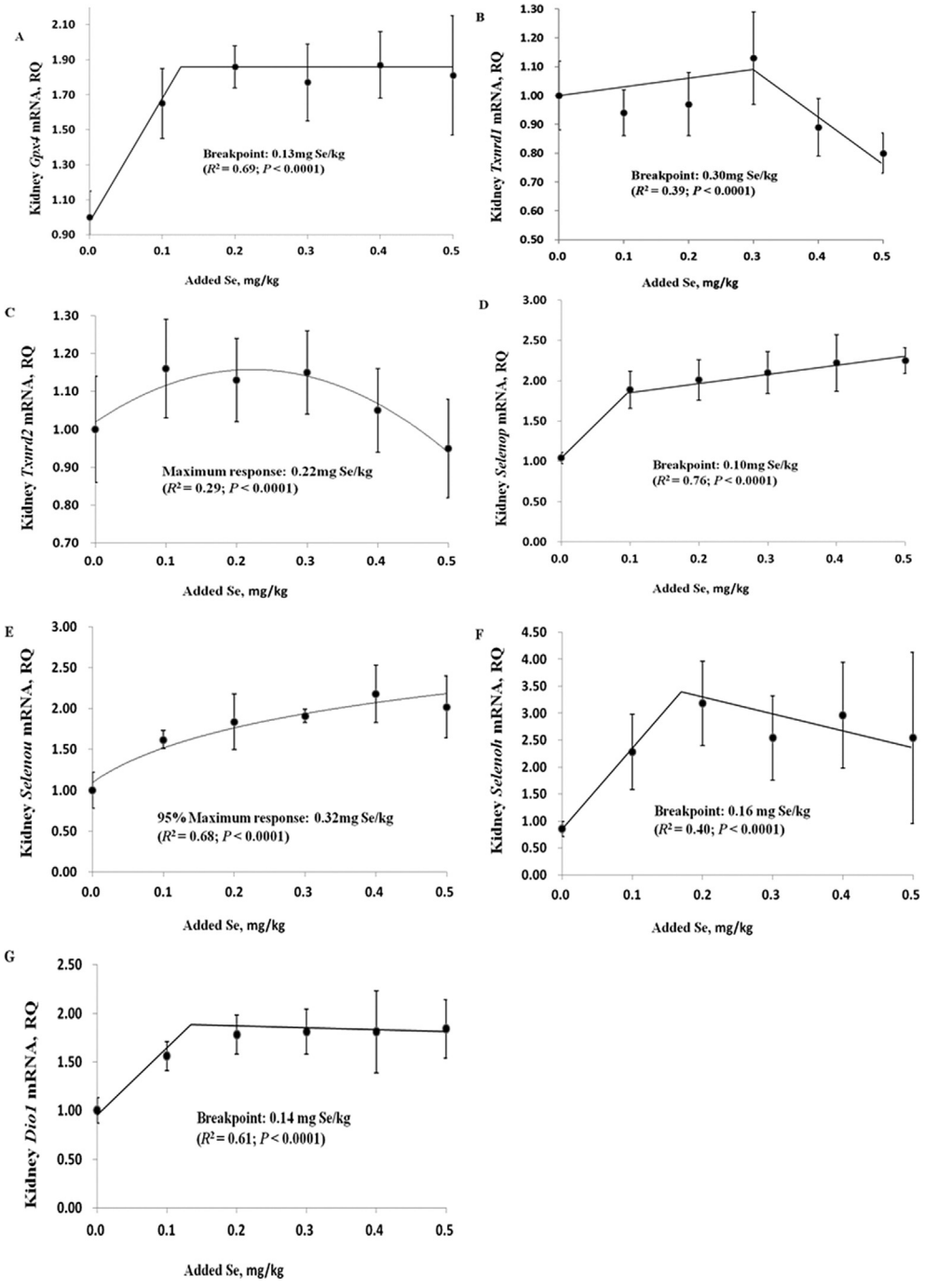

Fig. 4. Relations between added Se and mRNA levels of kidney *Gpx4* (A) or *Txnrd1* (B) or *Txnrd2* (C) or *Selenop* (D) or *Selenou* (E) or *Selenoh* (F) or *Dio1* (G) in broilers at 21 d of age. *Gpx4* = glutathione peroxidase 4; *Txnrd1* = thioredoxin reductase 1; *Txnrd2* = thioredoxin reductase 2; *Selenop* = selenoprotein P; *Selenou* = Selenoprotein U; *Selenoh* = Selenoprotein H; *Dio1* = iodothyronine deiodinase 1. Values are means ± SE, n = 8.

plasma, liver and pancreas, and Txnrd in the liver and pancreas, the mRNA levels of *Gpx1*, *Gpx4*, *Dio1*, *Selenoh*, *Selenop* and *Selenou* in the liver, *Gpx4*, *Dio1*, *Txnrd1*, *Txnrd2*, *Selenoh*, *Selenop* and *Selenou* in the kidney as well as *Gpx1*, *Gpx4*, *Selenoh* and *Selenou* in the pancreas, and the protein levels of GPX4 in the liver and kidney of broilers are sensitive criteria for estimating Se requirements of chicks fed a corn-soybean meal diet. Moreover, the optimal dietary Se levels are 0.36 mg/kg to support the full expression of selenoproteins in the plasma, liver and kidney, and 0.46 mg/kg to support the full expression of selenoproteins in the pancreas of broilers. These levels are higher than the NRC Se requirement (0.15 mg/kg). Therefore, our hypotheses that the enzyme activity, mRNA and protein expression levels of selenoproteins in targeted tissues might be sensitive indices to evaluate dietary Se requirements of broilers fed a practical corn-soybean meal diet, and the Se requirements for the full expression of selenoproteins in modern commercial broilers might be higher than the current NRC Se requirement for the optimum growth performance have been supported by the above findings from our current study. It is of great significance to find sensitive indices to evaluate Se requirements of broiler chicks, so as to better

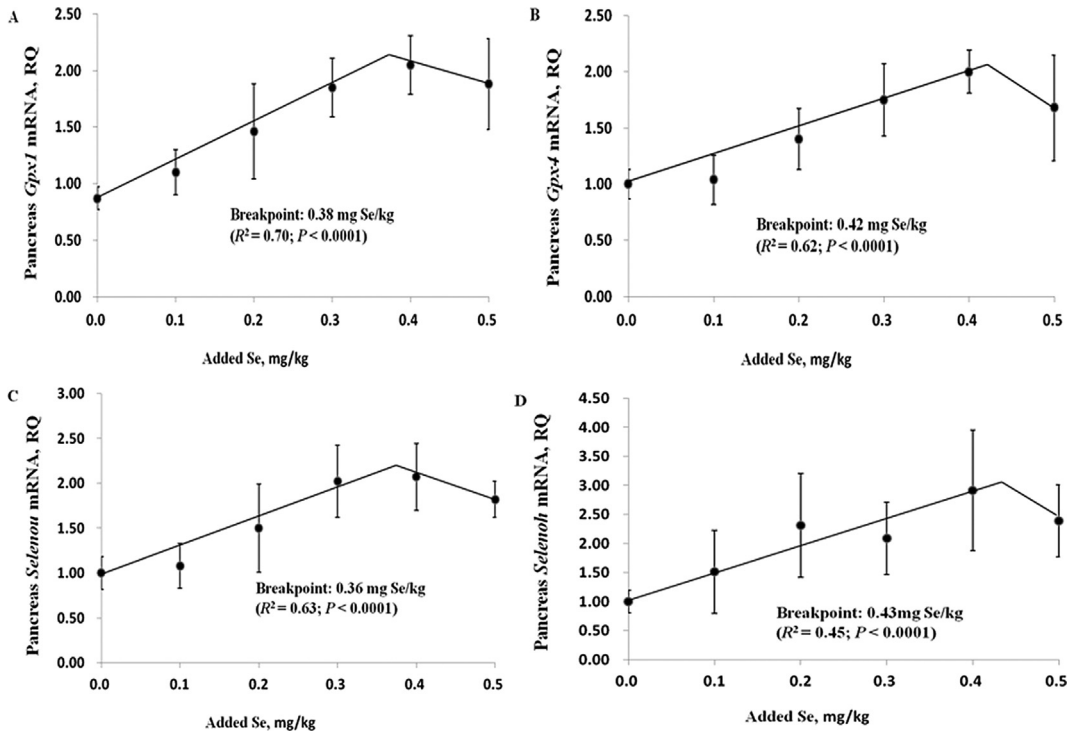

Fig. 5. Relations between added Se and mRNA levels of pancreas *Gpx1* (A) or *Gpx4* (B) or *Selenou* (C) or *Selenoh* (D) in broilers at 21 d of age. *Gpx1* = glutathione peroxidase 1; *Gpx4* = glutathione peroxidase 4; *Selenou* = Selenoprotein U; *Selenoh* = Selenoprotein H. Values are means ± SE, n = 8.

characterize Se requirements and meet all metabolic functions of Se as either selenoenzymes or other selenoproteins.

Growth performance and Se concentration are the early and commonly used indexes for reflecting Se deficiency or evaluating Se requirements in animals. Our results indicate that supplemental 0.1 to 0.2 mg Se/kg as Na_2SeO_3 is necessary to maintain normal growth and feed intake for broiler chicks up to 21 d of age fed the Se-deficient, corn-soybean meal diet. These results are in agreement with the findings from an early study in 1986 (Jensen et al., 1986), which demonstrated that the analyzed dietary Se concentrations of 0.17 and 0.18 mg/kg were required for optimum body weight and feed intake for broilers fed semi-purified and corn-soybean meal diet, respectively. Nevertheless, another two studies showed that the minimum Se requirements were 0.025 or 0.05 mg/kg for growth in broiler chicks or turkeys fed a semi-purified diet, respectively (Li and Sunde, 2016; Taylor and Sunde, 2016). The above different results may be attributed to different type of birds, basal diets, growth period, or different supplemental graded levels of Se. In addition, our data showed that all the Se contents in the plasma, liver, kidney and pancreas of broilers increased both linearly and quadratically, and could be used to estimate the broiler Se requirements. Moreover, we found that the liver Se concentration reached a plateau at supplemental 0.3 to 0.5 mg Se/kg. Sunde et al. (2016) reported that liver Se concentration in mice could reach a plateau, but it did not reach the plateau in the same manner as the increase of Se concentration in other animals. Therefore, the tissue Se concentrations as Se biomarkers might have different changing patterns among different species.

Many studies have demonstrated that GPX activity, mRNA or protein levels are sensitive biochemical and molecular biomarkers to evaluate Se requirements in humans and animals. Omaye and Tappel (1974) reported that dietary Se level was 0.12 mg/kg for the optimum plasma GPX activity in chicks. Lei et al. (1998) reported that dietary supplemental 0.2 mg Se/kg could support the full expression of GPX1, GPX3 and GPX4 in the plasma or tissues of piglets. Furthermore, researchers have expanded these biomarkers to include other selenoenzyme activity and selenoprotein transcript level (Barnes et al., 2009; Sunde et al., 2005; Weiss et al.,

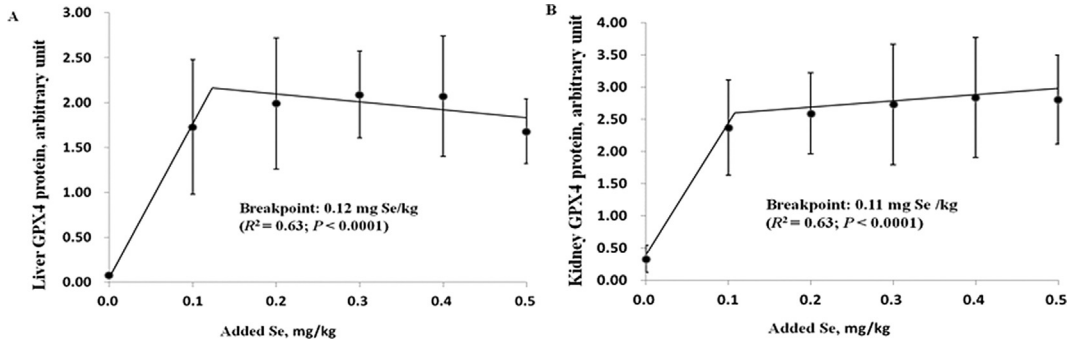

Fig. 6. Relations between added Se and GPX4 protein levels of liver (A) or kidney (B) in broilers at 21 d of age. GPX4 = glutathione peroxidase 4. Values are means ± SE, n = 8.

1997; Weiss and Sunde, 2001; Xia et al., 2005). Xia et al. (2005) found that SELENOP full expression requires a greater Se intake than does plasma GPX full expression in humans. Li and Sunde (2016) found that Txnrd activity in the liver, along with mRNA levels of 25 selenoprotein transcripts (*Dio1*, *Selenoh*, *Selenom*, *Selenop*, *Selenou*, *Selenow*, etc.) in the liver, gizzard and pancreas, were sensitive biomarkers for assessing Se requirements of chicks fed a semi-purified diet. The present study also showed that DIO and Txnrd activity in the plasma or liver and pancreas, the mRNA levels of *Dio1*, *Txnrd1*, *Txnrd2*, *Selenoh*, *Selenop* and *Selenou* in the liver, kidney and pancreas were sensitive biomarkers for evaluating Se requirements of chicks fed a practical corn-soybean meal diet. Moreover, the results from the current study indicate that the protein levels of GPX4 in the liver and kidney are new and sensitive indexes for estimating Se requirements of chicks, which has been not reported before. A complex situation in the present study is that the mRNA level of the selenoprotein is not completely consistent with its protein expression. For example, Se supplementation increased hepatic *Gpx1* and *Gpx4* transcripts, but increased only hepatic GPX4 protein expression. One possible explanation is that different selenoproteins, or even different isoforms of the same selonoprotein, might take different times to be translated. The GPX1 might take a longer time to be translated, and thus, its protein expression was not changed. The exact reasons are not clear and need a further study in the future. Therefore, the above studies collectively demonstrated that selenoenzyme activity, mRNA and protein expression levels of selenoproteins including but not limited to GPX, could be used as sensitive biomarkers for estimating dietary Se requirements of chickens.

The current study showed that the optimal dietary Se levels are 0.13 to 0.19 mg/kg based on the best-fit models for most Se concentrations and GPX activity in the plasma or tissues of broilers as estimated, which is in line with the current NRC Se requirement. But the optimal dietary Se level is 0.35 mg/kg based on the liver Se concentration, which is higher than other indexes and the current NRC Se requirement, possibly due to the high Se retention and selenoprotein synthesis in the liver (Burk and Hill, 2015). In addition, the maximum response (0.36 mg/kg) of *Selenou* mRNA level in the liver was observed in the present study, but the breakpoints (0.39 to 0.46 mg/kg) of selenoprotein mRNA levels in the pancreas were higher than those in other tissues, indicating that the pancreas could be the targeted sensitive tissue for Se. This was also reported by Li and Sunde (2016), who found that the plateau breakpoint for selenoenzyme activity in the pancreas was higher than in the liver and gizzard and suggested that the NRC Se requirement should be increased to 0.2 mg/kg to provide additional protection for the pancreas of chicks fed a semi-purified diet. Thus, the differences in biomarker–response curves for expression of selenoproteins in different tissues might help us better understand Se metabolism and Se deficiency in tissues, thus to better characterize Se requirements for broiler chickens (Sunde et al., 2016). Furthermore, the level of gene expression of selenoproteins might be influenced by the dynamics of the organ investigated, which affects Se requirements. The organs with lower Se requirements may have more rapid protein turnover and are more responsive. This might partially explain the greater differences in Se requirements as estimated based on expression of selenoproteins in different tissues. However, in the current study, the feed formulations are mainly based on NRC (1994), which might have little relevance to modern broiler chickens and in many countries, since micronutrients are no longer formulated based on these outdated values. Therefore, in hindsight, diet formulation for the micronutrients could have been preferably based on more updated specifications. Thus, future validation studies are recommended.

5. Conclusions

The results from the current study indicate that the optimal dietary Se levels would be 0.36 mg/kg to support the full expression of selenoproteins in the plasma, liver and kidney, and 0.46 mg/kg to support the full expression of selenoproteins in the pancreas of broilers fed a practical corn-soybean meal diet from 1 to 21 d of age, which are higher than the current NRC Se requirement (0.15 mg/kg).

Author contributions

Xiudong Liao: data curation, writing - original draft preparation. **Guoqing Liu**: investigation, data curation. **Guangming Sun**: investigation. **Xiaoming Sun**: investigation. **Tao Liu**: investigation. **Lin Lu**: methodology. **Liyang Zhang**: investigation. **Minhong Zhang**: methodology. **Yanli Guo**: methodology. **Xugang Luo**: supervision, writing - review & editing.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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