

Comparison of different forms of dietary selenium supplementation on gene expression of cytoplasmic thioredoxin reductase, selenoprotein P, and selenoprotein W in broilers

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ABSTRACT: Effects of different forms of dietary selenium (Se) supplementation on gene expression of cytoplasmic thioredoxin reductase (*TrxR1*), selenoprotein P (*SelP*), and selenoprotein W (*SelW*) in broilers were investigated. A total of six hundred Ross 308 broilers (1-day-old) with similar body weight were randomly divided into three groups, each of which included 5 replicates of 40 birds. These three treatments received the same basal diet with only background Se level of 0.04 mg Se/kg, supplemented with 0.15 mg Se/kg as sodium selenite (SS) or L-selenomethionine (L-Se-Met) or D-selenomethionine (D-Se-Met) for 42 days. The SS supplemented diet increased *TrxR1* activity in liver ($P < 0.01$) and kidney ($P < 0.01$) as well as *SelP* concentration in serum ($P < 0.05$) and liver ($P < 0.01$) more than the D-Se-Met supplemented diet. The addition of SS also highly increased liver ($P < 0.01$) and kidney ($P < 0.01$) *TrxR1* activities of broilers in comparison with broilers fed L-Se-Met diet. In addition, liver *TrxR1* activity in L-Se-Met group was higher than that in D-Se-Met group ($P < 0.05$). Liver and kidney mRNA levels of *TrxR1* and *SelP* as well as breast muscle *SelW* mRNA level were significantly increased by L- and D-Se-Met supplementation in comparison with SS supplementation ($P < 0.01$), while the D-Se-Met group showed more effective ($P < 0.01$) than the L-Se-Met group in increasing the mRNA levels of *TrxR1* and *SelP* in liver and kidney. Therefore, dietary L-Se-Met and D-Se-Met supplementation could improve mRNA levels of different selenoproteins studied and reduce amounts of *TrxR1* and *SelP* in broilers compared with SS. Besides, L-Se-Met is more effective than D-Se-Met in raising *TrxR1* activity and decreasing mRNA abundance of *TrxR1* and *SelP* in broilers.

Keywords: sodium selenite; L-selenomethionine; D-selenomethionine; mRNA abundance

INTRODUCTION

Selenium (Se) is a nutritionally essential trace element for a wide range of species, including birds. It is known primarily for its functions as an antioxidant, anti-inflammatory and chemopreventive, and its roles in immune system, fertility, reproduction, and viral inhibition (Rayman 2000; Hoffmann and Berry 2008). Selenium is fed to

animals either as inorganic sodium selenite (SS) or as organic selenized yeast (SY). The use of SY generally results in better absorption, higher antioxidant properties, more effective utilization, and larger body deposits of Se than SS (Mahmoud and Edens 2003; Qin et al. 2007; Rider et al. 2010). More importantly, organic Se is usually found to be less toxic and more environmental friendly than inorganic forms of the element (Kim and

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Mahan 2001; Tiwary et al. 2006). However, the chemical composition of independent batches of SY was thought to be variable (Lippman et al. 2005, 2009), so the development of new organic Se sources becomes inevitable.

Many previous studies attributed the high biological effect of SY to L-selenomethionine (L-Se-Met), a Se analog of L-methionine (L-Met), which was the most abundant Se chemical form in SY (Kelly and Power 1995; Cobanova et al. 2011; Slowinska et al. 2011). Besides, L-Se-Met is also the main Se species in cereals, forage crops, soybeans, and grassland legumes (Whanger 2002). Numerous studies have since established that L-Se-Met and SY are suitable for nutritional Se supplementation (Schrauzer 1998; Li et al. 2011; Slowinska et al. 2011; Wang 2011b).

In nature, selenomethionine (Se-Met) is found almost exclusively in the L-form, the other stereoisomer is the D-form (Cukierski et al. 1989). DL-selenomethionine is a racemic mixture of Se-Met in which the D and L stereoisomers are present in equal amounts when Se-Met is manufactured in the laboratory (Maier et al. 1993). Ideally, Se should be supplemented in the form in which it occurs naturally in foods. However, there are many problems in the chiral resolution technology during production. Therefore, it is necessary to study the biological effects of D-Se-Met on animals. In a previous study from our laboratory, we found that dietary L-Se-Met and D-Se-Met supplementation could improve antioxidant capability in tissues of broilers compared with SS; while L-Se-Met is more effective than D-Se-Met in improving antioxidant status in broilers (Wang et al. 2011b), but their mechanism of action is not fully understood.

The biological functions of Se are primarily implemented through its presence in a family of selenoproteins in which Se is present as selenocysteine (Sec) incorporated in the peptide backbone (Pappas et al. 2008; Davis et al. 2012). Among them, selenoprotein P (*SelP*) is involved in maintaining Se homeostasis, antioxidant protection, and has been identified as a Se transport protein (Burk and Hill 2005). Selenoprotein W (*SelW*), which is the smallest identified selenoprotein, seems to be implicated in antioxidant defense of cardiac and skeletal muscle as well as in cell cycle progression (Whanger 2000; Jeong et al. 2002; Hawkes et al. 2009). The thioredoxin reductase (*TrxR*) family has at least three members: cytoplasmic *TrxR* (*TrxR1*), mitochondrial *TrxR*, and testis-specific

TrxR; which is considered to be involved in various physiological functions, including antioxidant defense, regulation of other antioxidant enzymes and apoptosis, modulation of several transcription factors and protein phosphorylation (Surai 2006), while *TrxR1* is the most abundant isoenzyme and is found in the cytosol (Lu et al. 2009).

Since mechanistic studies of different forms of Se at the molecular level of selenoproteins are scarce, we investigated the effects of SS, L-Se-Met, and D-Se-Met on several parameters in the broilers, including *SelP* concentration in serum, liver, and kidney, *TrxR1* activity in liver and kidney, *SelP* and *TrxR1* mRNA levels in liver and kidney, and muscle *SelW* mRNA level. Our findings might provide a theoretical basis for the industrialization and scientific application of DL-selenomethionine.

MATERIAL AND METHODS

Chickens, diets, and experimental design. All procedures used in the present study were approved by the Animal Care and Use Committee of Zhejiang University, which has adopted Animal Care and Use Guidelines governing all animal use in experimental procedures.

Six hundred Ross 308 broilers (1-day-old) with an average body weight (BW) of 44.30 ± 0.49 g were randomly assigned to 3 treatments, each of which was replicated 5 times with 40 birds per replicate (20 males and 20 females). The chickens were offered the same basal diet supplemented with Se through the addition of 0.15 mg Se/kg as SS (Sigma-Aldrich Co., St. Louis, USA) or L-Se-Met (Sigma-Aldrich Co.) or D-Se-Met (Hangzhou King Techina Technology Co., Ltd, Hangzhou, P.R. China) at the expense of maize, respectively. All groups were fed the experimental diet for 6 weeks. The basal diet was formulated to meet or exceed requirements of broilers according to the National Research Council (1994) except for Se (Table 1), which contained 0.046 mg Se/kg. The starting phase (1–21 days of age) diets were provided as crumbles and the growing phase (22–42 days of age) diets were provided as pellets.

Chicks were housed in a tunnel-ventilated building with 15 concrete floor pens (2.2×2.2 m) bedded with 10 cm rice hulls. Water and feed were for *ad libitum* consumption. Infrared brooding lamps per pen were used for the first week. The lighting program was 24 h of light. Daily observations were made to record mortality and temperature.

Table 1. Ingredients and nutrient content of the basal diets¹ (g/kg) of broilers ($n = 5$)

Items	Starter (days 1–21)	Grower (days 22–42)
Ingredients		
Maize	591.9	638.6
Soybean meal	360.0	310.0
Soy oil	10.0	14.0
Monocalcium phosphate	15.0	12.0
Limestone	12.0	15.0
Salt	3.0	3.0
L-Lysine HCl	1.3	1.0
D,L-Methionine	1.8	1.4
Vitamin-mineral premix ²	5.0	5.0
Composition		
Metabolizable energy (MJ/kg)	11.88	12.18
Crude protein	205.1	186.6
Lysine	10.4	9.2
Methionine	4.8	3.4
Methionine + cysteine	8.2	6.5
Calcium	9.3	9.1
Total phosphorus	6.5	5.8

¹sodium selenite (SS) (Sigma-Aldrich Co., St. Louis, USA), L-selenomethionine (L-Se-Met) (Sigma-Aldrich Co.), and D-selenomethionine (D-Se-Met) (Hangzhou King Techina Technology Co., Ltd, Hangzhou, P.R. China) were premixed in maize and added to the diets at 0.15 mg/kg of Se to achieve the appropriate treatment levels. The Se assay for the diet samples was performed by hydride generation atomic fluorescence spectrometry (Gamiz-Gracia and Luque de Castro 1999). The analyzed Se concentration (mg/kg) in the diets was as follows: basal diet (starter) 0.046, SS supplemented diet (starter) 0.199, L-Se-Met supplemented diet (starter) 0.203, D-Se-Met supplemented diet (starter) 0.201, basal diet (grower) 0.047, SS supplemented diet (grower) 0.203, L-Se-Met supplemented diet (grower) 0.198, D-Se-Met supplemented diet (grower) 0.203

results are presented as means

²contents per kg of diet: retinyl acetate 3440 µg, cholecalciferol 100 µg, DL- α -tocopheryl acetate 10 mg, menadione 3 mg, thiamine 1.5 mg, riboflavin 3.5 mg, pyridoxine 3 mg, cobalamin 15 µg, niacin 30 mg, folic acid 0.5 mg, pantothenic acid 10 mg, biotin 150 µg, iron 80 mg, copper 8 mg, manganese 60 mg, zinc 40 mg, iodine 0.33 mg, ethoxyquin 100 mg

Sample collection and preparation. At the end of the study, 10 female broilers from each treatment (2 birds per replicate) were randomly selected, fasted for 12 h, and anesthetized with sodium pentobarbital. Blood samples were collected from the neck vein and allowed to coagulate at room temperature for 1 h. Serum was separated by centrifugation at 1000 g and 4°C for 20 min and transferred into 1.0 ml microcentrifuge tubes. After blood collection, the birds were killed by cervical dislocation. Samples of liver, kidney, and pectoral muscle were quickly collected from each of the individual birds on an ice-cold surface. The tissues were blotted and rinsed with ice-cold phosphate buffer saline (pH 7.2). Then, the samples were divided into aliquots and snap-frozen in liquid nitrogen. Frozen tissues and serum samples were stored at –80°C prior to analysis.

SelP concentration and TrxR1 activity assay. 1 g of liver and kidney in 9 ml of homogenization buffer (10mM Tris-HCl, pH 7.4, 0.1mM EDTA-2Na, 10M sucrose, and 0.1% peroxide-free Triton X-100) was homogenized on ice with an Ultra-Turrax T8 (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 8000 rpm for 10 s. Homogenates were centrifuged at 3500 g and 4°C for 15 min and the supernatant was used for analysis of SelP concentration. The serum, liver, and kidney SelP concentrations were measured using ELISA, as described previously (Saito et al. 2001). The assays were conducted with the commercial kits (Life Sciences Advanced Technologies, Saint Petersburg, USA). In addition, the homogenates were centrifuged at 12 000 g and 4°C for 15 min to provide a supernatant fraction for the analysis of the TrxR1 activity. The activity of TrxR1 was assayed using the method described in our previous study (Yuan et al. 2012), based on thioredoxin as substrate and oxidized insulin as the final electron acceptor. All samples were measured in duplicate. The protein content of the tissue was determined with a Coomassie blue dye-binding assay (Bradford 1976). Bovine serum albumin was used as a protein standard.

RNA extraction and reverse transcription. Total cellular RNA was extracted from the liver, kidney, and muscle samples (100 mg tissue) with Trizol reagent as recommended by the supplier (Life Technologies, Carlsbad, USA). Then, the dried RNA pellets were resuspended in 50 µl of diethylpyrocarbonate-treated water. RNA concentration and purity were determined by measurement of the absorbance at 260 and 280 nm using a ND-1000 spectrophotometer (Nanodrop Tech-

Table 2. Gene accession numbers and primer sequences

Target gene ¹	GenBank accession No.	Primer sequence (5'–3')	PCR product size (bp)
<i>SelW</i>	GQ919055	F: CGCTCACCGAATGCTGCTCCT R: GGCAGCCCAAAGTTCCCATGACT	99
<i>SelP</i>	NM_001031609	F: GAGGGACTGGTCAACATCTCATAACG R: GGAAGACCCAGGTGGTACTACT	216
<i>TrxR1</i>	NM_001030762	F: GCAGGACAGGCTGGAACCTACA R: CGAGAAGTGCAGGTGAACG	152
<i>GAPDH</i>	AF036934	F: GCAGGAACATCCCGAAGAAGC R: CCCGGAAGTGACCATGAGTAG	191

¹*SelW* = selenoprotein W, *SelP* = selenoprotein P, *TrxR1* = cytoplasmic thioredoxin reductase, *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase

nologies, Wilmington, USA), while the ratios of absorption (260 : 280 nm) of all samples were 1.8–2.0. First-strand cDNA was synthesized with 2 µg of total RNA using Oligo dT primers and SuperScript[®] II reverse transcriptase (Life Technologies) according to the manufacturer's protocol. Synthesized cDNA was stored at –20°C before use.

Quantitative real-time PCR analysis of selenoprotein mRNA levels. Primers for *TrxR1*, *SelP*, and *SelW* were designed using Primer Premier Software (Version 5.0, 1999) based on known chicken sequences (Table 2).

Quantitative real-time PCR was performed on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, USA) with 25 µl reaction system, which comprised of 12.5 µl of 2 × SYBR[®] Green I PCR Master Mix (TaKaRa Biotechnology Co. Ltd., Tokyo, Japan), 2.0 µl of cDNA, 1.0 µl of each primer (10 µM), and 8.5 µl of doubled-distilled water. The PCR procedure for *TrxR1*, *SelP*, *SelW*, and *GAPDH* consisted of denaturation at 95°C for 1 min, followed by 45 cycles of 95°C for 5 s, and of 60°C for 25 s. The melting curve analysis showed only one peak for each PCR product. Results (fold changes) were expressed as $2^{-\Delta\Delta Ct}$ as follows:

$$\Delta\Delta Ct = (Ct_{TrxR1/SelP/SelW} - Ct_{GAPDH})t - (Ct_{TrxR1/SelP/SelW} - Ct_{GAPDH})c$$

where:

$Ct_{TrxR1/SelP/SelW}$, Ct_{GAPDH} = cycle thresholds for chicken *TrxR1*, *SelP*, *SelW*, and *GAPDH* genes in the treated groups, respectively

t = treatment group

c = control group

The target mRNA level was normalized to the mean expression of *GAPDH*. All samples were measured in triplicate.

Statistical analysis. Data were analyzed using SPSS statistical software for MS Windows (Version 16.0, 2008). Comparison between groups was made by One-Way ANOVA followed by the Duncan's Multiple-Range Test. A level of $P < 0.05$ was considered to be statistically significant unless indicated otherwise. Replicate was considered as the experimental unit. The analytical data were presented as means ± SE.

RESULTS AND DISCUSSION

Selenomethionine treatments vs sodium selenite. There is evidence from *in vitro* and *in vivo* studies that expression of *TrxR1*, *SelP*, and *SelW* mRNA is highly up-regulated by SS supplementation in comparison with Se deficiency (Hill et al. 1996; Hadley and Sunde 2001; Pagmantidis et al. 2005; Gao et al. 2012). However, the regulation of *TrxR1*, *SelP*, and *SelW* mRNA by different Se forms in avian liver, kidney, and muscle is not known. Therefore, the quantitative real-time PCR was employed by the present study to explore whether differences observed in antioxidant status between birds supplemented with inorganic and organic Se from our previous study (Wang et al. 2011b) could be explained by differences in expression of *TrxR1*, *SelP*, and *SelW* mRNA. Results from the present study indicated a difference in expression of *TrxR1*, *SelP*, and *SelW* that was dependent on Se source. There was a significant increase of *TrxR1*, *SelP*, and *SelW* mRNA levels in the Se-Met groups when compared with the SS group ($P < 0.01$) (Table 5). It seems that our results were consistent with previous studies of Yuan et al. (2012), which reported that liver and kidney *TrxR1* mRNA levels in broiler breeders were significantly higher in SY and Se-Met groups than those in SS group. The likely possible explanation for this finding is that

Se supplied via organic forms has a higher bioavailability and thus enhances the Se level (Wang et al. 2011b), leading to the induction of *TrxR1*, *SelP*, and *SelW* gene expression. However, the detailed mechanisms involved, by which different forms of Se regulate the expression of *TrxR1*, *SelP*, and *SelW* mRNA, remain unclear.

The beneficial effects of Se are thought to be mediated through the function of selenoproteins. Our particular interest is the potential regulation of *TrxR1*, *SelP*, and *SelW* in broilers by Se supplementing due to their potential relevance to antioxidant defense mechanisms. It was reported that organic Se was more effective in increasing antioxidant property than inorganic Se (Mahan and Parrett 1996; Payne and Southern 2005; Jiang et al. 2009; Wang et al. 2011a, c). However, data from the present study indicated that the SS supplemented diet increased more serum ($P < 0.05$) and liver ($P < 0.01$) *SelP* concentrations as well as liver ($P < 0.01$) and kidney ($P < 0.01$) *TrxR1* activities than the D-Se-Met supplemented diet; while the activity of liver and kidney *TrxR1* was significantly ($P < 0.01$) lower in the L-Se-Met-supplemented group than in the SS-supplemented group (Tables 3 and 4). These results suggested that SS had higher bioactivity than Se-Met in increasing *TrxR1* activity and *SelP* concentration in broilers. This can be attributed to three possibilities. First, Se must be transformed to Sec before it can be incorporated into the selenoprotein regardless of source (Sunde and Evenson 1987; Allmang et al. 2009; Stoytcheva and Berry 2009). It has been demonstrated that SS was metabolized into Sec more efficiently than Se-Met (Sunde and Hoekstra 1980; Henry and Ammerman 1995). Second, some proportion of ingested Se-Met escapes the Se metabolism and is non-specifically incorporated into the general body proteins in place of Met (Schrauzer 2000). In a work with mammalian cells in culture, ^{75}Se from Se-Met was found to be initially incorporated into a wide spectrum of cellular proteins and only later incorporated into selenoprotein, whereas Se from SS was rapidly incorporated into selenoprotein (White and Hoekstra 1979). Thus competition with Met for incorporation in non-Se-requiring proteins may affect the availability of Se from Se-Met for synthesis of selenoproteins. Third, the post-transcriptional stabilization of *TrxR1*, *SelP*, and *SelW* mRNA in Se-Met groups may be lower than that in the SS group, but we cannot be sure of this because the *TrxR1*, *SelP*, and *SelW* mRNA half-life were not

Table 3. Effects of different forms of dietary selenium on selenoprotein P (SelP) concentration in serum, liver, and kidney of broilers ($n = 5$)

	SS	L-Se-Met	D-Se-Met
Serum ($\mu\text{g/ml}$)	66.27 \pm 3.87 ^a	61.32 \pm 5.11 ^{ab}	49.75 \pm 4.40 ^b
Liver ($\mu\text{g/mg protein}$)	17.54 \pm 0.90 ^A	15.78 \pm 0.58 ^{AB}	13.81 \pm 0.80 ^B
Kidney ($\mu\text{g/mg protein}$)	20.18 \pm 1.40	19.72 \pm 1.64	18.77 \pm 1.70

SS = sodium selenite, L-Se-Met = L-selenomethionine, D-Se-Met = D-selenomethionine

^{A,B}different letters within a row indicate significant differences ($P < 0.01$)

^{a,b}different letters within a row indicate significant differences ($P < 0.05$)

values are means \pm SE

measured. While our previous study showed that L-Se-Met and D-Se-Met were more effective for enhancing the antioxidant status of broilers than SS (Wang et al. 2011b), results from this study indicated that *TrxR1* activity and *SelP* concentration may not be conclusive indexes of bioavailability or ability of Se to influence antioxidant capability in broilers. The muscle *SelW* concentration was not measured in the present study because of the lack of appropriate antibodies, further studies should focus on characterizing *SelW* parameters.

D-Selenomethionine vs L-selenomethionine.

D-Methionine (D-Met) was the amino acid most effectively used by animals in place of L-Met (Man and Bada 1987), while the bioavailability of exogenous D-Met depends on whether it can be ef-

Table 4. Effects of different forms of dietary selenium on cytoplasmic thioredoxin reductase (*TrxR1*) activity in liver and kidney of broilers ($n = 5$)

	SS	L-Se-Met	D-Se-Met
Liver (U/g protein)	23.85 \pm 0.37 ^{Aa}	20.35 \pm 0.69 ^{Bb}	18.34 \pm 0.46 ^{Bc}
Kidney (U/g protein)	27.79 \pm 0.95 ^A	23.02 \pm 0.43 ^B	20.79 \pm 1.16 ^B

SS = sodium selenite, L-Se-Met = L-selenomethionine, D-Se-Met = D-selenomethionine

^{A,B}different letters within a row indicate significant differences ($P < 0.01$)

^{a-c}different letters within a row indicate significant differences ($P < 0.05$)

values are means \pm SE

ficiently converted to L-Met. It has been shown that almost all D-Met can be converted into L-Met in rats (Sugiyama and Muramatsu 1987; Hasegawa et al. 2005) and broilers (Katz and Baker 1975). According to the findings reported by Schrauzer (2000), the metabolism of L-Se-Met in animals is analogous to that of L-Met and without involving L-Se-Met specific enzymes. So we hypothesized that D-Se-Met metabolism in animals may be analogous to that of D-Met and its effects on animals also depended on the ability of animals to convert D-Se-Met to L-Se-Met. In the present study, the L-Se-Met group exhibited a higher ($P < 0.05$) liver *TrxR1* activity and also had a trend to increase the kidney *TrxR1* activity as well as serum, liver, and kidney *SelP* concentrations than the D-Se-Met group (Tables 3 and 4). Results from this study support previous findings from our lab which indicated that L-Se-Met was more effective in increasing the antioxidant status of broilers than D-Se-Met (Wang et al. 2011b). These results further support the hypothesis from our previous studies (Wang et al. 2011b) that a part of D-Se-Met may be converted into L-Se-Met, but we cannot be certain of this because many metabolic key enzymes and products were not determined. On the other hand, with respect to *TrxR1* and *SelP* mRNA levels, the chickens that were fed the D-Se-Met supplemented diet had higher mRNA abundance compared with the chickens that were fed L-Se-Met supplemented diet ($P < 0.01$) (Table 5) and we cannot fully explain this phenomenon, because no differences in the Se retention existed between L-Se-Met and D-Se-Met

Table 5. Effects of different forms of dietary selenium on relative mRNA levels of selenoprotein P (*SelP*), cytoplasmic thioredoxin reductase (*TrxR1*), and selenoprotein W (*SelW*) in tissues of broilers ($n = 5$)

	SS	L-Se-Met	D-Se-Met
Liver <i>SelP</i>	1.00 ± 0.10 ^C	2.53 ± 0.10 ^B	3.59 ± 0.16 ^A
Kidney <i>SelP</i>	1.00 ± 0.01 ^C	1.64 ± 0.01 ^B	2.52 ± 0.11 ^A
Liver <i>TrxR1</i>	1.00 ± 0.03 ^C	1.70 ± 0.09 ^B	2.98 ± 0.25 ^A
Kidney <i>TrxR1</i>	1.00 ± 0.04 ^C	1.43 ± 0.02 ^B	2.12 ± 0.06 ^A
Muscle <i>SelW</i>	1.00 ± 0.09 ^B	1.72 ± 0.14 ^A	2.07 ± 0.15 ^A

SS = sodium selenite, L-Se-Met = L-selenomethionine, D-Se-Met = D-selenomethionine

^{A-C}different letters within a row indicate significant differences ($P < 0.01$)

values are means ± SE

treatments (Wang et al. 2011b). The underlying mechanisms for regulation of *TrxR1*, *SelP*, and *SelW* gene expression by different forms of Se-Met should be investigated by further research.

CONCLUSION

The present study indicates that *TrxR1* activity, *SelP* concentration, and mRNA levels of *TrxR1*, *SelP*, and *SelW* in broilers are regulated differently by different dietary forms of Se, namely SS, D-Se-Met, and L-Se-Met. Compared with SS, L- and D-Se-Met seem to be more effective in decreasing the *TrxR1* activity and *SelP* concentration as well as increasing the mRNA levels of *TrxR1*, *SelP*, and *SelW*. Besides, D-Se-Met was more effective than L-Se-Met in improving the levels of *TrxR1* and *SelP* mRNA and decreasing the *TrxR1* activity and *SelP* concentration. To our knowledge, this is the first report to study *TrxR1*, *SelP*, and *SelW* gene expression caused by dietary D-Se-Met supplementation in broilers. These findings may be helpful in understanding the association of the dietary Se forms and expression of *TrxR1*, *SelP*, and *SelW* genes in antioxidant function. Besides, these findings also establish a platform for future research on the characteristics of *TrxR1*, *SelP*, and *SelW* in the antioxidant system. Subsequent studies are necessary to verify the detailed mechanism by which different forms of Se regulate *TrxR1*, *SelP*, and *SelW* gene expression in chickens.

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