Relative bioavailability of ultrafine sodium selenite for broilers fed a conventional corn–soybean meal diet¹

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ABSTRACT: The particle size of selenium (Se) sources could affect Se absorption and utilization, and thus it is hypothesized that the Se bioavailability might be higher in ultrafine sodium selenite (USSe) than in sodium selenite (SSe) for broilers because of USSe's smaller particle size. An experiment was conducted to investigate the relative bioavailability of Se as USSe relative to SSe for broiler chicks fed a conventional corn–soybean meal diet. A total of 504 one-d-old Arbor Acres commercial male broilers were randomly allotted to 1 of 7 treatments with 6 replicates per treatment in a completely randomized design involving in a 2 (Se sources) × 3 (added Se levels) factorial arrangement of treatments plus a Se-unsupplemented control diet containing 0.05 mg Se/kg by analysis for 21 d. The 2 Se sources were USSe and SSe, and the 3 added Se levels were 0.15, 0.30, or 0.45 mg Se/kg. The Se concentrations, glutathione peroxidase (GSH-Px)

activities, and mRNA relative abundances in plasma, liver, or pancreas of broilers on day 14 and 21 were determined. The results showed that Se concentrations, GSH-Px activities in plasma, liver, and pancreas, and mRNA relative abundances in the liver and pancreas of broilers on day 14 and 21 increased linearly ($P < 0.05$) as the added Se-level increased. Furthermore, a difference ($P < 0.05$) between USSe and SSe was detected for GSH-Px mRNA relative abundance in the pancreas of broilers on day 14. On the basis of the slope ratios from the multiple linear regression of the pancreatic GSH-Px mRNA relative abundance of broilers at 14 d of age on daily dietary analyzed Se intake, the Se bioavailability of USSe relative to SSe (100%) was 158% ($P < 0.05$). The results from this study indicated that the Se from USSe was more available to broilers than the Se from SSe in enhancing the pancreatic GSH-Px mRNA expression.

Key words: ultrafine sodium selenite, bioavailability, broilers, glutathione peroxidase expression

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INTRODUCTION

Selenium (Se) is an essential trace element, and it plays important roles in reproduction, metabolism, immune functions, chemoprevention, redox reactions, and many other aspects of health in human or other species (Naziroglu et al., 2004; Loflin et al., 2006; Yu et al., 2011; Zhang et al., 2011; Naziroglu and Yurekli, 2013). The biologically active form of Se was recognized in 1973, when glutathione peroxidase (GSH-Px) was identified as a very potent antioxidant for protecting

the body from oxidative damages due to free radicals (Rotruck et al., 1973). Selenium source added to the animal diets is categorized primarily as inorganic sodium selenite (SSe) produced by chemical synthesis, or organic Se forms, such as Se-enriched yeast produced by fermentation. Many studies have shown that Se bioavailability in organic forms was higher than that in inorganic forms (Surai, 2002; Wang and Xu, 2008; Surai and Fisinin, 2014). Organic Se is absorbed more effectively than inorganic Se in broilers (Mahmoud and Ednes, 2003; Payne and Southern, 2005). However, organic Se forms are more expensive than inorganic Se forms, and the cost of feed usually is increased when organic Se is used. The SSe, a form of inorganic Se, is often added to feed for its cheap cost. Recently, Se in ultrafine form (ultrafine sodium selenite [USSe], the ultrafinely ground SSe with much smaller particles) has been produced and used in animal feeds, but the Se bioavailability has not been evaluated in such ultrafine form. Many studies have demonstrated that Se concentrations, GSH-Px activity, or mRNA relative abundances in tissues of animals could be affected by dietary Se source or Se level (Liao et al., 2012; Pilarczyk et al., 2012; Liang et al., 2014), which might be sensitive indices to assess the Se bioavailability of different Se sources in broilers. The particle size of Se sources could affect Se absorption and utilization, and thus it is hypothesized that the Se bioavailability might be higher in USSe than in SSe for broilers because of USSe's smaller particle size. Therefore, the objective of this study was to estimate the Se bioavailability of USSe relative to SSe based on Se concentrations, GSH-Px activities, and mRNA relative abundances in plasma, liver, or pancreas of broilers fed a conventional corn–soybean meal diet in order to test the above hypothesis.

MATERIALS AND METHODS

Experimental Design and Treatments

A completely randomized design involving in a 2 (Se sources) $\times$ 3 (added Se levels) factorial arrangement of treatments plus a Se-unsupplemented control was used in this study. The 2 Se sources were SSe and USSe, and 3 supplemental Se concentrations were 0.15, 0.30, or 0.45 mg Se/kg diet, respectively. The feed-grade SSe (more than 95% passed through a 80-mesh sieve [about 100–180 μ m], but only less than 10% SSe passed through 80-mesh sieve also passed through 325-mesh sieve [about 0.5–45 μ m]) and USSe (more than 95% passed

through a 325-mesh sieve) were provided by a commercial company (Anshan Xinda Weiye Minerral Premix Ltd., Anshan, China). The 2 Se sources shared 1 Se-unsupplemented basal diet control, and there were a total of 7 different treatments.

Animals and Diets

All experimental procedures were approved by the Animal Management Committee (in charge of animal welfare issue) of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (IAS-CAAS, Beijing, China) and performed in accordance with the guidelines. A total of 504 one-day-old Arbor Acres commercial male chicks were purchased from a commercial company (Huadu Broiler Breeding Corp., Beijing, China), and randomly allotted to 1 of 7 treatments with 6 replicate cages of 12 birds each based on birthweight (BW). Broilers were housed in an electrically heated, thermostatically controlled room equipped with fiberglass feeders and stainless steel cages coated with plastics. The room was maintained on a 24 h constant light schedule for 21 d. The lighting and feeding managements were following the Arbor Acres commercial broiler management guidelines. All broilers were allowed ad libitum access to tap water containing no detectable Se. Dead chicks were recorded daily, and chick weight and feed intake per cage were measured weekly to calculate the ADG, ADFI, G:F, and mortality.

The basal corn–soybean meal diet (Table 1, containing 0.05 mg Se/kg by analysis) was formulated to meet or exceed the nutrient requirements (NRC, 1994) of starter broilers (day 1 to 21) except for Se. The SSe and USSe were diluted with talcum powder into premix containing Se 1,086 and 1,125 mg/kg, respectively. The Se sources were added to the basal diet according to the above-mentioned experimental treatments, and the analyzed Se concentrations of diets were listed in Table 2.

Sample Collections and Preparations

Samples of the feed ingredients and diets from all the treatments were taken and submitted for CP, Ca, and Se analyses prior to the initiation of the trial to confirm CP, Ca, and Se contents in diets. The tap water was collected for analysis of Se content. At 14 and 21 d of age, 4 or 3 chicks were selected from each replicate cage according to the average body weight after a 12 h fast, respectively. The blood samples were taken from the selected birds via heart puncture with stainless steel needles that

Table 1. Composition of the basal diets for broilers (as-fed basis)

Item	Content
Ingredients, %	
Ground yellow corn	53.5
Soybean meal	38.1
Soybean oil	4.55
CaHPO ₄ ¹	1.80
CaCO ₃ ¹	0.93
NaCl ¹	0.30
DL-Methionine ¹	0.33
Micronutriments ^{1,2}	0.34
Talc powder+Se additive ³	0.20
Nutriment composition, %	
ME (MJ/kg)	12.6
CP ⁴	21.5
Lysine	1.14
Methionine	0.62
Methionine+ cysteine	0.91
Ca ⁴	1.05
Nonphytate P	0.45
Se ⁴ , mg/kg	0.05

¹Reagent grade.

²Provided per kilogram of diet: 15,000 IU vitamin A (all *trans*-retinol acetate); 4,500 IU cholecalciferol; 24 IU vitamin E (as all-rac- α -tocopherol acetate); 3 mg vitamin K (as menadione sodium bisulfate); 3 mg thiamin (as thiamin mononitrate); 9.6 mg riboflavin; 3 mg vitamin B₆; 0.018 mg vitamin B₁₂; 15 mg calcium pantothenate; 39 mg niacin; 1.5 mg folic acid; 0.15 mg biotin; 700 mg choline (choline chloride); 60 mg Zn (ZnSO₄ · 7H₂O); 8 mg Cu (CuSO₄ · 5H₂O); 110 mg Mn (MnSO₄ · H₂O); 40 mg Fe (FeSO₄ · 7H₂O); 0.35 mg I (KI).

³Se additives were added to diets by replacing an equivalent weights of talc powder.

⁴Determined by analysis. Each value was based on triplicate determinations.

Table 2. Analyzed Se concentrations in diets for broilers (as-fed basis)

Se source	Added Se, mg/kg	Analyzed Se, ¹ mg/kg
Control	0	0.05
SSe ²	0.15	0.20
	0.30	0.35
	0.45	0.46
	0.45	0.46
USSe ³	0.15	0.21
	0.30	0.34
	0.45	0.48

¹Values are based on triplicate determinations.²Sse = sodium selenite through a 80-mesh sieve.³USSe = ultrafine sodium selenite through a 325-mesh sieve.

were equipped with heparinized blood-collection vacationers. The blood samples were centrifuged at 3,000 × *g* for 10 min at 4 °C to harvest plasma samples, and the plasma were stored at −20 °C for the analyses of Se concentration and GSH-Px activity. The birds were subsequently killed, and their liver and pancreas were collected. Subsamples were

taken from the collected liver and pancreas, which were frozen at −20 °C for analyses of Se contents and GSH-Px activities and in liquid nitrogen for *GSH-Px* mRNA relative abundance analysis. All samples from 4 or 3 chicks in each cage were pooled into one sample in equal ratios before analysis.

Sample Analysis

Measurement of Se, Ca, and CP contents. The Se concentrations in the feed ingredients, water, diets, plasma, and tissues were measured using the fluorescence method with a Hitachi 850 fluorescence spectrophotometer (Liao et al., 2012). The Ca concentrations in feed ingredients and diet samples were measured using an inductively coupled plasma emission spectroscope (model IRIS Intrepid II, Thermal Jarrell Ash, Waltham, MA) after wet digestions with HNO₃ and HClO₄ (Zhu et al., 2015a; Zhang et al., 2018). The concentrations of CP in feed ingredient and diet samples were determined using the methods of Association of Official Analytical Chemists (1990).

Determination of antioxidant indices in tissues.

The GSH-Px activities in plasma, liver, and pancreas were analyzed using a commercial assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), and the results were expressed as units per milligram of protein. Total protein concentrations in the supernatants of tissue homogenates were measured using a BCA Protein Assay kit (Thermo Scientific, Rockford, IL). All the procedures were carried out according to the manufacturers' instructions.

RNA extraction, reverse transcription, and real-time quantitative PCR.

Total RNA in the liver and pancreas were isolated using TRIZOL reagent (Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. The concentration of each isolated RNA sample was determined using a NanoDrop Spectrophotometer (ND-2000; Gene Company Ltd.), and the integrity of the RNA was checked using denatured RNA electrophoresis. A total of 1 µg of RNA was used to obtain complementary DNA (cDNA) by reverse transcription using the Super Script First-Strand Synthesis System (Qiagen, Chatsworth, CA). Real-time quantitative PCRs were performed on an ABI 7500 real-time quantitative PCR system using SYBR-Green PCR Master Mix (Life Technologies, Carlsbad, CA). The primer sequences for *GSH-Px*, glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*),

and β -actin were synthesized by Invitrogen Biotechnology Co., Ltd. in Shanghai, China, which are listed in Table 3. The PCR protocol was as follows: denaturation at 95 °C for 2 min followed by 40 cycles of 95 °C for 60 s, 60 °C for 30 s, and 72 °C for 30 s (Zhu et al., 2015b). The $2^{-\Delta\Delta CT}$ was used to calculate the mRNA level of target gene (Livak and Schmittgen, 2001). The geometric mean of internal reference genes, β -actin and GAPDH, was used to normalize the expression relative abundance of the targeted gene (Zhang et al., 2016). The run was performed in triplicate.

Statistical Analysis

To evaluate the effect of Se supplementation, the data of growth performance and mortality, Se concentrations, GSH-Px activities and GSH-Px mRNA levels were analyzed using single degree of freedom contrast to compare all supplemental Se treatments with the control (Liao et al., 2012; Suo et al., 2015). Data excluding the control were further analyzed as a 2×3 (sources $\times$ levels) factorial arrangement of treatments by two-way ANOVA using the GLM procedure of the SAS 9.2 (SAS Institute Inc., Cary, NC). The model included the main effects of Se source, added Se level, and their interaction. Cage was the experimental unit. Orthogonal polynomials were used to assess linear and quadratic responses to the added Se level. Relative bioavailability values of Se as USSe were estimated using SSe as the standard source by slope ratio comparison from multiple linear regressions (Li et al., 2004; Huang et al., 2009, 2013; Ma et al., 2014; Zhang et al., 2016). The regressions were calculated using daily dietary Se intake (based on dietary Se content by analysis) as the independent variable rather than added Se level (Wedekind et al., 1994; Boling et al., 1998; Yu et al., 2000). Percentage data for mortality of broilers were transformed to arcsine value prior to the statistical analyses. Slope ratios and their SE were estimated using the method

of error propagation as described by Zhang et al. (2016). Differences between the Se sources were determined by differences in their respective regression coefficients (Suo et al., 2015; Zhang et al., 2016). Differences in means among treatments were tested using the LSD method, and significant differences were set at $P < 0.05$.

RESULTS

Growth Performance and Mortality

No differences ($P > 0.05$) were observed in the BW, ADG, ADFI, G:F, and mortality between the birds fed the control diet and the Se-supplemented diets during each experimental period (Table 4). The Se source and added Se level in diets had no effect ($P > 0.05$) on BW, ADG, ADFI, G:F, and mortality of broilers during each experimental period. However, the interaction between Se source and added Se level affected ($P < 0.05$) BW of broilers on day 7 and ADG during 1 to 7 d of age, while no interaction ($P > 0.05$) was observed in other indices. No differences ($P > 0.05$) were observed in both BW on day 7 and ADG from 1 to 7 d of age among the chicks fed the diets supplemented with the SSe at 0.15, 0.30, or 0.45 mg Se/kg. However, the chicks fed the diet supplemented with the USSe at 0.30 mg Se/kg had higher ($P < 0.05$) BW on day 7 and ADG from 1 to 7 d of age than those fed the diet supplemented with the USSe at 0.15 mg Se/kg.

Se Concentrations in Plasma, Liver, and Pancreas

Compared with the control chicks, chicks fed Se-supplemented diets had higher ($P < 0.05$) Se concentrations in plasma, liver, and pancreas at both 14 and 21 d of age (Table 5). The Se source affected ($P < 0.05$) Se concentration in the pancreas of broilers at 14 d of age, but it did not affect ($P > 0.05$) Se concentrations in plasma and liver on day 14 and 21 and pancreas on day 21. Added

Table 3. Primer sequences for real-time PCR amplification

Genes ¹	Gene bank ID ²	Primer sequences ³
β -actin	NM205518.1	F: 5'-ACCTGAGCGCAAGTACTCTGTCT-3' R: 5'-CATCGTACTCCTGCTTGCTGAT-3'
GAPDH	NM204305.1	F: 5'-CTTTGGCATTGTGGAGGGTC-3' R: 5'-ACGCTGGGATGATGTTCTGG-3'
GSH-Px	BX935086.2	F: 5'-ACGGCGCATCTTCCAAAG-3' R: 5'-TGTTCCTCCCAACCATTTCTC-3'

¹GAPDH = glyceraldehyde-3-phosphate dehydrogenase; GSH-Px = glutathione peroxidase.

²ID = identity.

³F = forward; R = reverse.

Table 4. Effects of dietary Se source and level on the growth performance and mortality of broilers

Item	Control		SSe		USSe		Se source ²		Added Se level, ³ mg/kg				P-value						
	Added Se level, mg/kg		Added Se level, mg/kg		Added Se level, mg/kg								Added Se level						
	0 ¹		0.15 ¹	0.30 ¹	0.45 ¹	0.15 ¹	0.30 ¹	0.45 ¹	SEM	SSe ⁵	USSe ⁶	SEM	0.15	0.30	0.45	SEM	Se source	Source × level	
D 1 to 7																			
BW, g	161	163 ^{ab}	159 ^b	162 ^b	167 ^a	160 ^b	167 ^a	163 ^{ab}	1.6	162	163	0.9	162	163	162	1.1	0.18	0.67	0.006
ADG, g/d	16.3	16.7 ^{ab}	16.1 ^b	16.4 ^b	17.2 ^a	16.2 ^b	17.2 ^a	16.6 ^{ab}	0.2	16.4	16.6	0.1	16.4	16.6	16.5	0.2	0.21	0.66	0.006
ADFI, g/d	21.5	21.1	20.9	21.4	21.9	21.2	21.9	21.9	0.9	21.2	21.7	0.5	21.2	21.4	21.7	0.6	0.49	0.86	0.90
G:F, g/g	0.76	0.79	0.77	0.77	0.78	0.76	0.78	0.76	0.03	0.78	0.77	0.01	0.78	0.78	0.76	0.02	0.78	0.87	0.83
Mortality ⁴ , %	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.00	0.03	0.00	0.03	0.02	0.02	0.02	0.00	0.02	0.17	0.61	0.61
D 8 to 14																			
BW, g	447	453	444	440	442	442	451	452	4.7	445	448	2.7	447	447	446	3.3	0.48	0.96	0.053
ADG, g/d	40.9	41.3	40.6	39.8	40.3	40.3	40.6	41.3	0.6	40.6	40.7	0.4	40.8	40.6	40.6	0.4	0.78	0.92	0.14
ADFI, g/d	54.5	53.9	53.1	53.1	53.6	53.6	54.8	53.8	0.7	53.4	54.1	0.4	53.8	54	53.4	0.5	0.19	0.74	0.33
G:F, g/g	0.75	0.76	0.76	0.75	0.75	0.75	0.74	0.76	0.01	0.76	0.75	0.01	0.76	0.75	0.76	0.01	0.25	0.75	0.06
Mortality ⁴ , %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.02	0.00	0.02	0.01	0.00	0.00	0.03	0.01	0.33	0.38	0.38
D 15 to 21																			
BW, g	870	894	867	863	879	879	891	886	17	875	885	10	887	879	874	12	0.44	0.77	0.42
ADG, g/d	87.5	90.1	86.6	87.5	86.3	86.3	91.6	85.9	2.1	88.1	87.9	1.2	88.2	89.1	86.7	1.5	0.94	0.51	0.11
ADFI, g/d	60.4	63.1	60.5	60.3	62.4	62.4	62.8	62.1	2.1	61.3	62.4	1.2	62.8	61.7	61.2	1.5	0.51	0.74	0.76
G:F, g/g	0.69	0.70	0.70	0.69	0.71	0.71	0.68	0.72	0.03	0.69	0.70	0.01	0.71	0.69	0.70	0.02	0.45	0.55	0.37
Mortality ⁴ , %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	—	—	—
D 1 to 21																			
ADG, g/d	39.2	40.4	39.1	38.9	39.6	39.6	40.2	40.0	0.8	39.4	39.9	0.5	40.0	39.6	39.4	0.6	0.44	0.77	0.42
ADFI, g/d	54.5	55.0	53.5	54.0	53.7	53.7	56.1	53.9	0.9	54.2	54.6	0.5	54.4	54.8	53.9	0.6	0.59	0.61	0.10
G:F, g/g	0.72	0.73	0.73	0.72	0.74	0.74	0.71	0.74	0.02	0.72	0.73	0.01	0.74	0.72	0.73	0.02	0.69	0.56	0.31
Mortality ⁴ , %	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.05	0.03	0.00	0.05	0.02	0.02	0.02	0.02	0.02	0.09	1.00	1.00

Means within a column lacking a common superscript alphabet differ ($P < 0.007$).¹Data represent the means of 6 replicate cages ($n = 6$).²Data represent the means of 18 replicate cages ($n = 18$).³Data represent the means of 12 replicate cages ($n = 12$).⁴Percentage data for mortality of chicks were transformed to arcsine for analysis.⁵SSe = sodium selenite through a 80-mesh sieve.⁶USSe = ultrafine sodium selenite through a 325-mesh sieve.

Table 5. Effects of dietary Se source and level on Se concentrations in plasma, liver, and pancreas of broilers on day 14 and 21

Item	Control		SSe			USSe			Se source ²			Added Se level, ³ mg/kg			P-value				
	0 ¹	Added Se level, mg/kg	Added Se level, mg/kg			Added Se level, mg/kg			SEM	SSe ⁶	USSe ⁷	SEM	Added Se level, ³ mg/kg			Se source	Added Se level	Source × level	Linear effect ⁴
			0.15 ¹	0.30 ¹	0.45 ¹	0.15 ¹	0.30 ¹	0.45 ¹					0.15	0.30	0.45				
D 14																			
Plasma, µg/mL	0.039*	0.157	0.166	0.173	0.158	0.165	0.174	0.003	0.165	0.165	0.002	0.158 ^c	0.165 ^b	0.173 ^a	1.00	0.0004	0.90	<0.0001	
Liver ⁵ , µg/g	0.191*	0.622	0.638	0.692	0.631	0.649	0.675	0.014	0.651	0.652	0.008	0.627 ^b	0.644 ^b	0.684 ^a	0.93	0.001	0.54	<0.0001	
Pancreas ⁵ , µg/g	0.153*	0.246	0.277	0.312	0.246	0.297	0.325	0.005	0.279 ^b	0.289 ^A	0.003	0.246 ^c	0.287 ^b	0.319 ^a	0.02	<0.0001	0.19	<0.0001	
D 21																			
Plasma, µg/mL	0.037*	0.152	0.162	0.179	0.151	0.157	0.184	0.004	0.164	0.164	0.002	0.151 ^c	0.159 ^b	0.181 ^a	0.91	<0.0001	0.33	<0.0001	
Liver ⁵ , µg/g	0.211*	0.635	0.642	0.700	0.628	0.651	0.697	0.010	0.66	0.659	0.006	0.632 ^b	0.647 ^b	0.698 ^a	0.86	<0.0001	0.73	<0.0001	
Pancreas ⁵ , µg/g	0.109*	0.169	0.197	0.210	0.171	0.207	0.216	0.008	0.193	0.198	0.005	0.170 ^b	0.202 ^a	0.213 ^a	0.51	<0.0001	0.90	<0.0001	

*Different ($P < 0.0001$) from all Se supplemental groups.
^{a,b,c}Means within a row lacking a common superscript differ ($P < 0.002$).
^{A,B}Means within a row lacking a common superscript differ ($P < 0.03$).
¹Data represent the means of 6 replicate cages ($n = 6$).
²Data represent the means of 18 replicate cages ($n = 18$).
³Data represent the means of 12 replicate cages ($n = 12$).
⁴Linear effect of added Se levels.
⁵Fresh tissue basis.
⁶Sse = sodium selenite through a 80-mesh sieve.
⁷USSe = ultrafine sodium selenite through a 325-mesh sieve.

Se level affected ($P < 0.05$) Se concentrations in plasma, liver, and pancreas of broilers on both day 14 and 21, but the interaction between Se source and added Se level did not affect ($P > 0.05$) Se concentrations in plasma, liver, and pancreas on both day 14 and 21. Compared with the chicks fed the diets supplemented with SSe, the chicks fed the diet supplemented with USSe had higher ($P < 0.05$) Se concentration in pancreas on day 14. As added Se level increased, the Se concentrations in plasma, liver, and pancreas increased linearly ($P < 0.05$) on both day 14 and 21.

GSH-Px Activities in Plasma, Liver, and Pancreas

Compared with the control broilers, broilers fed Se-supplemented diets had higher ($P < 0.05$) GSH-Px activities in plasma, liver, and pancreas on both day 14 and 21 (Table 6). Added Se level affected ($P < 0.05$) GSH-Px activities in plasma and pancreas on day 14 as well as in plasma, pancreas, and liver on day 21, but had no effect ($P > 0.05$) on GSH-Px activity in liver on day 14. However, Se source or the interaction between Se source and added Se level had no effect ($P > 0.05$) on GSH-Px activities in plasma, liver, and pancreas at either 14 or 21 d of age. The GSH-Px activities in plasma and pancreas on day 14 as well as in plasma, liver, and pancreas on day 21 increased linearly ($P < 0.05$) as added Se level increased.

GSH-Px mRNA Relative Abundance in the Liver and Pancreas

Compared with the control chicks, chicks fed Se-supplemented diets had higher ($P < 0.05$) GSH-Px mRNA relative abundances in the liver and pancreas at both 14 and 21 d of age (Table 7). The Se source affected ($P < 0.05$) GSH-Px mRNA relative abundance in the pancreas on day 14, but it did not affect ($P > 0.05$) GSH-Px mRNA relative abundances in the liver on day 14 and 21 and pancreas on day 21. Added Se level affected ($P < 0.05$) GSH-Px mRNA relative abundances in the liver and pancreas on day 14, but it did not affect ($P > 0.05$) GSH-Px mRNA relative abundances in the liver and pancreas on day 21. The interaction between Se source and Se level did not affect ($P > 0.05$) GSH-Px mRNA relative abundances in the liver and pancreas on both day 14 and 21. Compared with the chicks fed the diet supplemented with SSe, chicks fed the diet supplemented with USSe had higher ($P < 0.05$) GSH-Px mRNA relative abundance in pancreas on day 14. The GSH-Px mRNA

relative abundances in plasma, liver, and pancreas on both day 14 and 21 increased linearly ($P < 0.05$) as added Se level increased.

Relative Bioavailability Estimate of USSe

Regressions were calculated based on daily dietary analyzed Se intake by age (Table 8). Significant ($P < 0.05$) multiple linear regression relationships were observed in Se concentrations and GSH-Px activities in plasma, liver, and pancreas as well as GSH-Px mRNA relative abundances in the liver and pancreas of broilers at both 14 and 21 d of age. The relative bioavailability values were estimated based on daily dietary analyzed Se intake (Table 9). The differences ($P < 0.05$) in slopes between SSe and USSe were observed only in pancreatic GSH-Px mRNA relative abundance of broilers at 14 d of age. No differences ($P > 0.05$) in slopes between SSe and USSe were observed in other indices. When the response to Se in SSe was set at 100%, the estimated relative bioavailability value of Se in USSe was 158% ($P < 0.05$) for GSH-Px mRNA relative abundance in the pancreas of broilers at 14 d of age.

DISCUSSION

The bioavailability of trace minerals is defined as the degree to which an ingested element in a particular source is absorbed, transported to its site of action, and converted to a physiologically active form that can be used in metabolism by the animals (Ammerman et al., 1995; Li et al., 2015). Therefore, the sensitive criteria should be selected for estimating the relative bioavailability of Se in USSe. The current study showed that Se source and added Se level did not affect growth performance of broilers. Other studies also found that dietary supplementation of Se-enriched yeast, Se-enriched alga, nano-Se, or Se protein did not have better effect in improving growth performance of broilers compared with supplementation of SSe (Wang et al., 2008; Heindl et al., 2010; Liao et al., 2012; Briens et al., 2014; Boostani et al., 2015). Thus, both the current study and the above reports indicate that growth performance might not be sensitive index for assessing Se bioavailabilities of different Se sources in broilers, which might be affected by many factors, such as different basal diets, type of chicks, growth phases, or different supplementation.

Tissue accumulations of minerals were considered to be sensitive indices for mineral utilization in broilers (Liao et al., 2012; Zhang et al., 2016). Wang (2009) observed that the Se concentrations

Table 6. Effects of dietary Se source and level on glutathione peroxidase (GSH-Px) activities in plasma, liver, and pancreas of broilers on day 14 and 21

Item	Control			SSe			USSe			Se source ²			Added Se level, ³ mg/kg			P-value		
	Added Se level, mg/kg			Added Se level, mg/kg			Added Se level, mg/kg											
	0 ¹			0.15 ¹			0.30 ¹			0.45 ¹			0.15 ¹			0.30 ¹		
	SEM	USSe ⁶	SEM	SSe ⁵	USSe ⁶	SEM	SSe ⁵	USSe ⁶	SEM	SSe ⁵	USSe ⁶	SEM	SSe ⁵	USSe ⁶	SEM	SSe ⁵	USSe ⁶	SEM
D 14																		
Plasma, U/mL	236*	1533	1566	1605	1492	1587	1688	52	1568	1589	30	1512 ^b	1576 ^{ab}	1647 ^a	37	0.61	0.048	0.50
Liver, U/mg protein	5.43*	17.6	18.6	19.7	18.7	19.7	19.9	0.7	18.6	19.4	0.4	18.2	19.2	19.8	0.5	0.19	0.082	0.75
Pancreas, U/mg protein	2.11*	3.12	3.68	3.96	3.27	3.54	4.34	0.20	3.59	3.71	0.10	3.19 ^b	3.61 ^b	4.15 ^a	0.20	0.49	0.0009	0.52
D 21																		
Plasma, U/mL	388*	1816	1987	2101	2014	2007	2186	74	1968	2069	43	1915 ^b	1997 ^{ab}	2144 ^a	52	0.11	0.014	0.48
Liver, U/mg protein	7.1*	27.9	28.4	29.3	26.4	28.3	28.8	0.7	28.5	27.8	0.4	27.1 ^b	28.4 ^{ab}	29.0 ^a	0.5	0.25	0.029	0.56
Pancreas, U/mg protein	2.72*	4.08	4.88	5.55	4.56	4.85	5.76	0.30	4.79	5.06	0.20	4.32 ^b	4.86 ^b	5.66 ^a	0.20	0.31	0.0007	0.71

*Different ($P < 0.0001$) from all Se supplemental groups.^{a,b}Means within a row lacking a common superscript differ ($P < 0.05$).¹Data represent the means of 6 replicate cages ($n = 6$).²Data represent the means of 18 replicate cages ($n = 18$).³Data represent the means of 12 replicate cages ($n = 12$).⁴Linear effect of added Se levels.⁵SSe = sodium selenite through a 80-mesh sieve.⁶USSe = ultrafine sodium selenite through a 325-mesh sieve.

Item	Control Added Se level, mg/kg	SSe		USSe		Se source ²			Added Se level, ³ mg/kg			P-value							
		Added Se level, mg/kg		Added Se level, mg/kg		SEM	SSe ⁵	USSe ⁶	SEM	0.15	0.30	0.45	SEM	Se source level	Added Se level	Source × level	Linear effect ⁷		
		0.15 ⁴	0.30 ⁴	0.15 ⁴	0.30 ⁴														
D 14																			
Liver	0.47*	0.84	0.89	0.94	0.84	0.91	1.11	0.05	0.89	0.95	0.03	0.84 ^b	0.90 ^b	1.03 ^a	0.04	0.17	0.006	0.24	<0.0001
Pancreas	0.46*	0.57	0.70	0.83	0.73	0.81	1.22	0.06	0.71 ^B	0.92 ^A	0.04	0.65 ^b	0.75 ^b	1.02 ^a	0.05	0.001	<0.0001	0.10	<0.0001
D 21																			
Liver	7.1*	27.9	28.4	29.3	26.4	28.3	28.8	0.7	1.01	1.07	0.06	1.04	1.01	1.07	0.07	0.47	0.82	0.87	0.005
Pancreas	2.72*	4.08	4.88	5.55	4.56	4.85	5.76	0.30	1.05	1.05	0.05	0.99	1.09	1.08	0.05	0.93	0.40	0.13	0.028

Table 8. Multiple linear regressions of Se concentrations and glutathione peroxidase (GSH-Px) activities and *GSH-Px* mRNA levels in plasma, liver, or pancreas on daily dietary analyzed Se intake by age¹

Age, d	Dependent variables	Intercepts	Regression coefficients		<i>R</i> ²	<i>P</i> -value
			SSe ²	USSe ³		
14	Plasma Se	0.1445	0.00167	0.00161	0.97	<0.0001
	Liver Se	0.5813	0.00568	0.00529	0.97	<0.0001
	Pancreas Se	0.1920	0.00690	0.00747	0.94	<0.0001
	Plasma GSH-Px activity	1417	11.59	13.61	0.95	<0.0001
	Liver GSH-Px activity	17.00	0.1393	0.1789	0.91	<0.0001
	Pancreas GSH-Px activity	2.468	0.0893	0.0955	0.63	<0.0001
	Liver <i>GSH-Px</i> mRNA	0.6686	0.0171	0.0226	0.72	<0.0001
	Pancreas <i>GSH-Px</i> mRNA	0.3522	0.0275	0.0435	0.65	<0.0001
21	Plasma Se	0.1261	0.00208	0.00204	0.96	<0.0001
	Liver Se	0.5741	0.00461	0.00453	0.97	<0.0001
	Pancreas Se	0.1401	0.00286	0.00307	0.79	<0.0001
	Plasma GSH-Px activity	1726	13.96	17.64	0.93	<0.0001
	Liver GSH-Px activity	26.35	0.1112	0.0843	0.95	<0.0001
	Pancreas GSH-Px activity	3.268	0.0880	0.0940	0.65	<0.0001
	Liver <i>GSH-Px</i> mRNA	0.9777	0.00210	0.00266	0.30	0.034
	Pancreas <i>GSH-Px</i> mRNA	0.9286	0.00630	0.00682	0.24	0.028

¹Regression analyses of Se concentrations and GSH-Px activities in plasma, liver, and pancreas, and *GSH-Px* mRNA levels in liver and pancreas were based on cage averages with 18 cages per Se source at 14 and 21 d of age; daily dietary analyzed Se intake = ADFI during each period times the dietary analyzed Se content for each respective Se source.

²SSe = sodium selenite through a 80-mesh sieve.

³USSe = ultrafine sodium selenite through a 325-mesh sieve.

Table 9. Relative bioavailability values (RBV) of USSe based on slope ratios from multiple linear regressions of Se concentrations and glutathione peroxidase (GSH-Px) activities in plasma, liver and pancreas, and *GSH-Px* mRNA levels in the liver and pancreas on daily dietary analyzed Se intake by age¹

Dependent variables	Age, d	Regression coefficients, slope (SE)		RBV, % (SE)		<i>P</i> -value ⁴
		SSe ²	USSe ³	SSe	USSe	
sPlasma Se	14	0.00167(0.00033)	0.00161(0.00032)	100	96(11.2)	0.54
	21	0.00208(0.00028)	0.00204(0.00027)	100	98(7.65)	0.79
Liver Se	14	0.00568(0.00138)	0.00529(0.00132)	100	93(13.6)	0.36
	21	0.00461(0.00081)	0.00453(0.00079)	100	98(9.76)	0.87
Pancreas Se	14	0.00690(0.00061)	0.00747(0.00059)	100	108(5.41)	0.11
	21	0.00286(0.00060)	0.00307(0.00060)	100	108(12.2)	0.48
Plasma GSH-Px activity	14	11.59(5.05)	13.61(4.83)	100	117(28.6)	0.66
	21	13.96(5.20)	17.64(5.03)	100	126(26.2)	0.23
Liver GSH-Px activity	14	0.139(0.070)	0.179(0.066)	100	128(36.3)	0.42
	21	0.111(0.050)	0.084(0.048)	100	76(24.3)	0.39
Pancreas GSH-Px activity	14	0.0893(0.0226)	0.0955(0.0216)	100	107(15.3)	0.66
	21	0.0880(0.0221)	0.0940(0.0206)	100	107(14.8)	0.69
Liver <i>GSH-Px</i> mRNA	14	0.0171(0.0052)	0.0226(0.0050)	100	132(22.9)	0.08
	21	0.00210(0.00723)	0.00266(0.00693)	100	126(24.2)	0.54
Pancreas <i>GSH-Px</i> mRNA	14	0.0275(0.0074) ^b	0.0435(0.0073) ^a	100	158(25.4)	0.001
	21	0.00630(0.00608)	0.00682(0.00586)	100	108(56)	0.89

^{a,b}Means within a row with different superscripts differ (*P* < 0.002).

¹Regression analyses of Se concentrations and GSH-Px activities in plasma, liver, and pancreas, and *GSH-Px* mRNA levels in liver and pancreas were based on cage averages with 18 cages per Se source at 14 and 21 d of age.

²SSe = sodium selenite through a 80-mesh sieve.

³USSe = ultrafine sodium selenite through a 325-mesh sieve.

⁴*P*-value for the difference in slopes among Se sources.

in the liver and muscle of broilers increased with dietary Se level increased, which is similar with our present study. Besides, Briens et al. (2014) reported

that broilers fed diet supplemented with Se-enriched yeast improved muscle Se concentration. Hu et al. (2012) found that the Se concentrations in the liver

and muscle were higher from broilers fed diet supplemented with nano-Se than those fed diet supplemented with SSe, which was similar to our current study. Our study showed that the chicks fed the diet supplemented with USSe had higher Se concentration in the pancreas on day 14 compared with the chicks fed the diet supplemented with SSe. The particle size of nano-Se ranged from 10 to 45 nm (0.010–0.045 μm) (Surai et al., 2017), which is much smaller than that of USSe (0.5–45 μm). Both the nano-Se and USSe have much smaller particle sizes than SSe (100–180 μm), thus they could be absorbed and utilized better in tissues of animals (Hu et al., 2012; Surai et al., 2017). However, to our knowledge, the exact Se absorption mode remains unknown, and it needs to be further studied in the future experiments. Additionally, in the present study, the Se concentrations in tissues also failed to reflect the differences of relative bioavailability between the two Se sources although they could be used for assessing Se bioavailability.

Selenium has a large number of biological functions in organism, and one of the most important functions is its antioxidant activity as the active core of the GSH-Px enzyme (Levander and Burk, 1994). Because the active form of GSH-Px is Se-dependent, the activity of GSH-Px is closely related to dietary supplemental Se level (Pilarczyk et al., 2012). In the present study, the activities of GSH-Px in plasma, liver, and pancreas of broilers on day 14 or 21 increased as added Se-level increased. Similarly, the increased GSH-Px activities in plasma and liver were also observed in broiler chickens fed diets supplemented with Se (Wang et al., 2008). Moreover, the GSH-Px activity in plasma was greater from the chickens fed diet supplemented with Se yeast than SSe (Wang et al., 2008). Chen et al. (2014) found that broilers fed diet supplemented with Se-enriched yeast had higher GSH-Px activity in plasma than those fed diet supplemented with SSe. In contrast, Boostani et al. (2015) reported that broilers fed organic Se, SSe, or nano-Se had no effects on GSH-Px activity in plasma, which is in consistent with our current results. Therefore, the GSH-Px activities in plasma or tissues of broilers were not sensitive enough to detect the differences between SSe and USSe when broilers were fed a practical corn–soybean meal diet. The previous studies also found that the enzyme activities were usually insensitive in detecting the differences among different forms of trace elements (Wang et al., 2012; Zhang et al., 2016).

The physiological functions of Se are considered to be mediated through selenium-containing

enzymes or proteins, and their mRNA relative abundances have been used as sensitive index for estimating Se relative bioavailability (Stadtman, 2000). The present study found that the expression of *GSH-Px* mRNA in the pancreas was higher in broilers fed diet supplemented with USSe than SSe at 14 d of age. As a selenium-containing enzyme, the relative abundances of *GSH-Px* mRNA in the liver and pancreas of broilers were sensitive to the added Se level at 14 or 21 d of age, which was agreed with the previous reports (Li et al., 2012; Yao et al., 2013; Yang et al., 2014). Thus, the relative abundance of *GSH-Px* mRNA could be used as a sensitive index for assessing the Se bioavailability of USSe relative to SSe. The maximal abundance of mRNA might occur a few hours before the increased amounts of the corresponding protein or activity, which might explain that the *GSH-Px* mRNA relative abundance was more sensitive than its activity in the current study (Blalock et al., 1988; Zhang et al., 2016).

In our laboratory, the multiple linear regression slope ratio method has been successfully used for estimating Fe, Mn, and Zn bioavailabilities of Fe, Mn, and Zn sources for broilers under conventional corn–soybean meal diets (Li et al., 2015; Suo et al., 2015; Zhang et al., 2016). Similarly, it is reported in other studies that Se-enriched yeast were more efficient in maintaining the GSH-Px activity level than SSe (Payne and Southern, 2005). Additionally, Briens et al. (2014) demonstrated that when the response to Se in Se-enriched yeast was set at 100%, the relative bioavailability value of Se in Se protein was 148% for muscle Se concentration of broilers using linear regression slope ratio method. In this study, when the response to Se in SSe was set at 100%, the estimated relative bioavailability value of Se in USSe was 158% for *GSH-Px* mRNA relative abundance in pancreas of broilers at 14 d of age, indicating that the Se from USSe was more available to broilers fed a conventional corn–soybean meal diet than the Se from SSe in enhancing the pancreatic *GSH-Px* mRNA expression. It is assumed that the increased relative bioavailability of Se as USSe might be caused by its increased intestinal Se absorption because of its smaller particle size and larger surface area (Hu et al., 2012). Therefore, the absorption traits of Se from USSe relative to SSe in the intestine of broilers need to be further studied in future experiments.

In conclusion, the results from the present study indicated that the Se from USSe was more available to broilers fed a conventional corn–soybean meal diet than the Se from SSe in enhancing the pancreatic *GSH-Px* mRNA expression.

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