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Dietary Selenium Affects Selenoprotein W Gene Expression in the Liver of Chicken

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Abstract As selenium in the form of "Selenoprotein W (SelW)" is essential for the maintenance of normal liver function, the expression of SelW liver depends on the level of selenium supplied with the diet. Whereas this is well known to be the case in mammals, relatively little is known about the effect of dietary Se on the expression SelW in the livers of avian species. To investigate the effects of dietary Se levels on the SelW mRNA expression in the liver of bird, 1-day-old male chickens were fed either a commercial diet or a Se-supplemented diet containing 1.0, 2.0, 3.0, and 5.0 mg/kg sodium selenite (Na_2SeO_3) for 90 days. The livers were collected and examined for Se content and mRNA levels of SelW, Selenophosphate synthetase-1, and selenocysteine-synthase (SecS). The data indicate that, within a certain range, a Se-supplemented diet can increase the expression of SelW and the mRNA levels of SecS, and also, that the transcription of SelW is very sensitive to dietary Se.

Keywords Chicken liver · Selenoprotein W · Selenium · mRNA expression · Selenophosphate synthetase-1 · Selenocysteine synthase

Introduction

The biological functions of selenium (Se) are primarily implemented through its presence in a family of Se-containing proteins [1]. Se is incorporated into a select group of proteins, selenoproteins, in the form of the amino acid, selenocysteine (Sec). Sec is the 21st amino acid in the genetic code [2, 3] and, unlike other amino acids, the biosynthesis of selenocysteine occurs on its tRNA [4, 5].

Selenoprotein W (SelW) was originally purified in cytosol from the liver of lambs, and has since been shown to prevent white muscle disease in domestic animals [6], suggesting that it functions as a catalyst of biological redox reactions. SelW exhibited an immediate

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response with exposure to hydrogen peroxide in proliferating myoblasts and it is apparently important in the development of the fetus [7]. Although the biological characteristics of SelW, including its amino acid sequence, distribution, and the regulatory effect of Se on its expression, are already known in rodents, but the characteristics of SelW in avian remain unknown, except for its amino acid sequence. Low intake of Se has been implicated with the increased incidence of disease and Se supplementation lowered both cancer incidence and mortality from cancer [8]. SelW is ubiquitously expressed in tissues, and its expression is regulated by Se status and intake. Se deficiency was shown to reduce the expression of SelW [9].

The level of Se in the feed affects the levels of selenoproteins as well as the expression of the enzymes affecting their biosynthesis. Selenophosphate synthetase-1 (SPS-1) was originally thought to have a role in selenophosphate synthesis [10–13]. SPS-1 was also have a role in recycling selenocysteine by a selenium salvage system [14] and has been reported to interact with selenocysteine-synthase (SecS) in vitro and in vivo [15]. SecS was initially established in *Escherichia coli* in the early 1990s. SecS invariably takes place on tRNA[Ser]Sec [16, 17] and play a important role in the complex machinery of insertion of Sec.

The liver plays a central role in nutrient homeostasis by regulating protein, carbohydrate, and fat metabolism. In addition to the turnover of macronutrients, hepatocytes are also essential for the metabolism, storage, and distribution of most vitamins and trace elements, including Se [18]. Therefore, the aim of this study was to determine the effects of different levels of supplemental on Se concentrations, mRNA levels of SelW, SecS, and of SPS-1, in the livers of 90-days-old male chickens.

Materials and Methods

Animal Care and Experimental Design

All experimental procedures were conducted with the approval of the Institutional Animal Care and Use Committee of Northeast Agricultural University. China. Thirty male chickens (1 day old; Weiwei Co. Ltd., Harbin, China) were divided into five groups (six chickens per group) and fed either the commercial diet or the Se-supplemented diet containing 1.0, 2.0, 3.0, or 5.0 mg/kg sodium selenite (Na_2SeO_3) for 90 days. The basal commercial diet was shown by analysis to contain 0.145 mg/kg Se. Food and water were provided ad libitum. All procedures, as well as the care, housing, and handling of the animals were conducted according to accepted commercial management practices. At the end of the experiment all chickens were anesthetized with sodium Pentobarbital and slaughtered; the livers were collected, immediately frozen in liquid nitrogen and subsequently stored at −80°C for determination of Se concentration, SelW, SecS, and SPS-1 mRNA expression levels.

Determination of Se Concentration in Livers

Se content in the livers was estimated by the method described by Hasunuma et al. [19]. The assay is based on the principle that Se contained in samples is converted to selenous acid in response to acid digestion. The reaction between selenous acid and aromatic-o-diamines, such as 2, 3-diamino-naphthalene, leads to the formation of 4, 5-benzopiazselenol, which displays a brilliant lime-green fluorescence when excited at 366 nm in cyclohexane. The fluorescence emission in extracted cyclohexane was measured

by a fluorescence spectrophotometer with an excitation and emission wavelengths of 366 nm and 520 nm, respectively. The Se content was calculated by reference to a standard curve.

Primer Design

To design primers, we used the chicken SelW, SecS, and SPS-1 mRNA GenBank sequence with accession number of GQ919055, NM_001031158.1, NM_001164084.1. Chicken GAPDH (glyceraldehydes phosphate dehydrogenase, GenBank accession number K01458) as a housekeeping gene was used as an internal reference. Primers (Table 1) were designed using the Oligo 6.0 Software (Molecular Biology Insights, Cascade, CO) and General PCRs were first performed to confirm the specificity of the primers. The PCR products were electrophoresed on 2% agarose gels, extracted, cloned into the pMD18-T vector (TaKaRa, China) and sequenced and the primers were synthesized by Invitrogen Biotechnology Co. Ltd. in Shanghai, China.

Total RNA Isolation and Reverse Transcription

Total RNA was isolated from the tissue samples (50-mg tissue; n=3/diet group) using Trizol reagent according to the manufacturer's instructions (Invitrogen, China). The dried RNA pellets were resuspended in 50 μl of diethyl-pyrocarbonate-treated water. The concentration and purity of the total RNA were determined spectrophotometrically at 260/280 nm. First-strand cDNA was synthesized from 5 μg of total RNA using oligo dT primers and Superscript II reverse transcriptase according to the manufacturer's instructions (Invitrogen, China). Synthesized cDNA was diluted five times with sterile water and stored at −80°C before use.

Real-Time Quantitative Reverse Transcription PCR

Real-time quantitative reverse transcription PCR was used to detect the expression of SelW, SecS, and SPS-1 gene in chicken's liver. Reaction mixtures were incubated in the ABI PRISM 7500 real-time PCR system (Applied Biosystems,USA). Reactions were consisted of the following: 10 μl of 2× SYBR Green I PCR Master Mix (TaKaRa, China), 2 μl of either diluted cDNA, 0.4 μl of each primer (10 μM), 0.4 μl of 50× ROX reference Dye II

Table 1 Primers used for quantitative real-time PCR

Target gene	GenBank accession no	Primer	Sequence (5′–3′)	PCR fragment length (bp)
Chicken				
SelW	GQ919055	Forward	5′-CTCCGCGTCACCGTGCTC-3′	150
		Reverse	5′-CACCGTCACCTCGAACCATCCC-3′	
Secs	NM_001031158.1	Forward	5′-CATGAACTTGCCATAATGGAC-3′	112
		Reverse	5′-GGATCAACCTATAGTGCCTT-3′	
SPS-1	NM_001164084.1	Forward	5′-CTGCTGGACTTATGCACAC-3′	108
		Reverse	5′-ACACCTCATTTCGCTGCT-3′	
GADPH	K01458	Forward	5′-AGAACATCATCCCAGCGT-3′	182
		Reverse	5′-AGCCTTCACTACCCTCTTG-3′	

and 6.8 μl of PCR-grade water. The PCR program for SelW and GADPH was 1 cycle at 95°C for 30 s, 40 cycles at 95°C for 15 s, and at 60°C for 30 s. The melting curve analysis showed only one peak for each PCR product. Electrophoresis was performed with the PCR products to verify primer specificity and product purity. A dissociation curve was run for each plate to confirm the production of a single product. The amplification efficiency for each gene was determined using the DART-PCR program [20]. The mRNA relative abundance was calculated according to the method of Pfaffl [21], accounting for gene-specific efficiencies and was normalized to the mean expression of GADPH, and expressed as the ratio of Se dietary content.

Statistical Analysis

Statistical analysis of Se concentration and mRNA levels were performed using SPSS statistical software for Windows (version 13; SPSS Inc., Chicago, IL, USA). The effect on mRNA levels in chickens was assessed by one-way ANOVA. Data are expressed as mean± standard deviation. Differences were considered to be significant at $P<0.05$.

Results

Se Content in Liver Tissues

The effects of the different concentrations of dietary sodium selenite on Se content in livers are shown in Fig 1. Chickens fed the basal diet had significantly lower ($P<0.05$) Se content in livers compared with those of chickens fed Se-supplemented diets. A significant increase of Se concentration was observed for chickens fed diets containing 1–5 mg/kg sodium selenite for 90 days. When chickens were fed the diets containing 1–3 mg/kg sodium selenite, the Se content in livers dose dependently increased with increasing dietary Se content (Fig 1).

Effect of Se-Supplemented Diet on the mRNA Levels of SelW in Liver Tissues of Chicken

The SelW mRNA abundance measured by quantitative RT-PCR is shown in Fig 2. When compared with the control group, a significant increase in the SelW mRNA levels was observed in Se-supplemented group. The greatest increases in SelW mRNA expression were observed in chickens fed the diet containing 3 mg/kg sodium selenite ($P<0.05$).

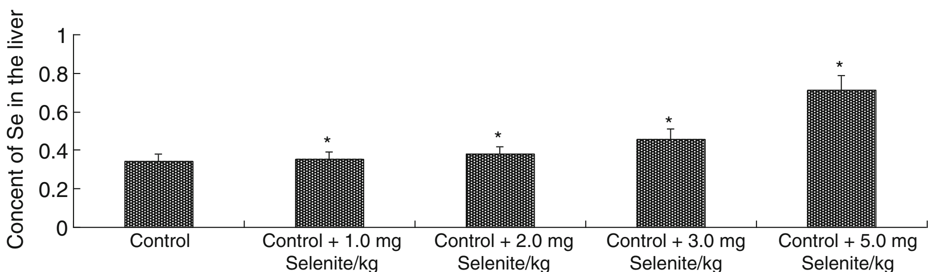

Fig. 1 Liver Se content in chickens fed diets containing various concentrations of Se. *Bars* represent mean± standard deviation (n=3/group). *Bars with asterisk* are statistically significantly different from control by one-way analysis of variance followed by Tukey's multiple comparison test ($P<0.05$)

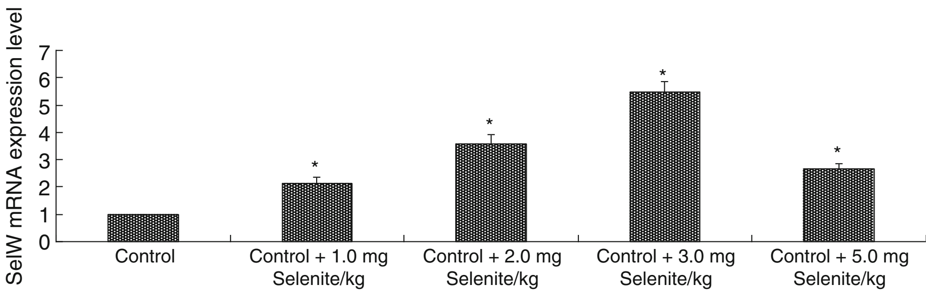

Fig. 2 Effects of different concentrations of Se on the abundance of SelW mRNA in the livers of chickens. *Bars* represent mean±standard deviation (n=3/group). *Bars with asterisk* are statistically significantly different from control by one-way analysis of variance followed by Tukey's multiple comparison test (P<0.05)

However, after reaching the maximal level, further increases of sodium selenite dose actually led to a reduction of SelW mRNA expression (Fig 2).

Effect of Se-supplemented Diet on the mRNA Expression of Components of the Selenoprotein Biosynthesis Machinery

The enzymes in selenocysteine biosynthesis (SecS, SPS-1) mRNA abundance measured by quantitative RT-PCR are shown in Figs. 3 and 4. There was a significant increase (P<0.05) of SecS and SPS-1 in the livers of Se-supplemented group chicken compared with the control group. The greatest increases in SecS mRNA expression were observed in chickens fed the diet containing 3 mg/kg sodium selenite (P<0.05; Fig 3). SPS-1 showed changes in fluctuations in Se-supplemented diet group.

Discussion

In previous work, the cDNA for SelW from chicken has been sequenced and found that SelW is expressed ubiquitously in various tissues [22]. Several experimental data denote that the expression of SelW depends on the concentrations of Se. The SelW mRNA levels in all tissues of sheep except the brain are sensitive to selenium status [23, 24]. Rodents

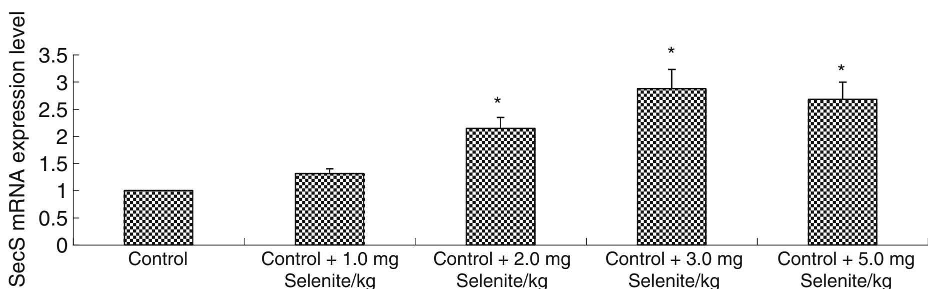

Fig. 3 Effects of different concentrations of Se on the abundance of SecS mRNA in the livers of chickens. *Bars* represent mean±standard deviation (n=3/group). *Bars with asterisk* are statistically significantly different from control by one-way analysis of variance followed by Tukey's multiple comparison test (P<0.05)

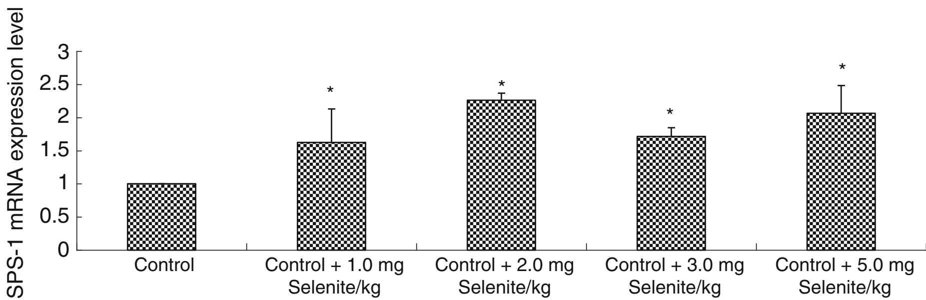

Fig. 4 Effects of different concentrations of Se on the abundance of SPS-1 mRNA in the livers of chickens. *Bars* represent mean±standard deviation (n=3/group). *Bars with asterisk* are statistically significantly different from control by one-way analysis of variance followed by Tukey's multiple comparison test (P<0.05)

[25–27], primates [28, 29], pigs [30] are also generally sensitive to Se status. In this study, we observed the mRNA expression of SelW in the liver tissues of 90-day-old male chickens. The chickens fed diets containing 1–3 mg/kg sodium selenite showed increased Se concentration and expression of SelW mRNA in the livers. However, the content of sodium selenite>3 mg/kg in diet, SelW mRNA levels decreased (Fig 2). In response to Se supplementation, SelW was observed to reach maximal activity at 3 mg/kg, but the Se concentration consistently continued to increase still further (Fig 1). The results of the present work confirm that SelW gene expression in the birds liver is also sensitive to dietary Se content, which is consistent with results of other investigators [24].

Trace elements are keys to many metabolic pathways, Se compounds have hepato-protective effects against different types of oxidative stress [31, 32] and decrease DNA damage [33]. It is shown that dietary Se supplementation has been reported to have protective effect in the development of primary liver cancer and Se-enriched malt showed a better chemopreventive efficiency in decreasing the number of hepatoma nodules. It was also showed that Se deficiency leads to necrotic degeneration of liver [34]. Both excess and deficiency of Se supply lead to impaired growth. Se has a window between deficiency and excess. Controlled mechanisms must be in place to sustain optimal concentrations of Se [35]. Therefore, we hypothesized that in birds Se and SelW may play an important role in the function of the liver and the liver diseases. SelW is vital in body development and further studies are needed to determine the mechanism of SelW gene expression in birds.

In this work, the impact of Se-supplemented on expression of two enzymes in selenocysteine biosynthesis was examined: SPS-1 and SecS. The mRNA levels of SecS were slightly enhanced with adding Se in the diet. Se upregulated the mRNA expression of SecS. Thus, the action of Se is not limited to upregulation of SelW expression but extends to components of the selenoprotein biosynthesis machinery. Prior study was to assess the consequences of removal of SPS-1 in a cell culture model. The reduction in SPS-1 expression had no effect on selenoprotein expression [36]. In addition, mammalian SPS-1 was not active in synthesis of selenophosphate [15]. In the course of this study, the presence of SPS-1 in liver indicates that this protein is involved in a pathway unrelated to selenoprotein biosynthesis. SPS-1 forms a complex with several proteins involved in the biosynthesis of selenocysteine [15], which suggests that it has a role in some aspect of selenium metabolism. Future studies will be required to establish its function in selenoprotein synthesis.

Evidence is presented that the Se-supplemented diet can make the SelW expression level higher and it is suggested that the transcription of the SelW gene in the livers of birds are so sensitive to Se. Se also has effect on the mRNA levels of SecS, one key enzyme essential for selenoprotein biosynthesis.

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