Selenium Regulates Gene Expression of Selenoprotein W in Chicken Gastrointestinal Tract

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Abstract Selenoprotein W (SelW) is an existing form of selenium (Se). Se influences the levels of SelW in mammals. However, little is known about the pattern of SelW expression in the gastrointestinal tract tissue of bird. The present paper describes the effects of different dietary levels of Se on the SelW mRNA expression in the gastrointestinal tract tissue of chicken. The expression levels of SelW mRNA and the Se contents in the gastrointestinal tract tissues (glandular stomach, gizzard, duodenum, small intestine, and rectum) were determined on days 15, 25, 35, 45, and 55, respectively. The results showed that the Se contents and the SelW mRNA expression were significantly higher ($p<0.05$) in the high-Se group, and the Se contents and SelW mRNA expression in the low-Se group were significantly lower ($p<0.05$) than in the controls. The Se contents were the highest in the duodenum and the lowest in the rectum, while the SelW mRNA expression was the highest in the gizzard and the lowest in the rectum. In addition, the SelW mRNA levels in the gastrointestinal tract tissue were found to increase in a time-dependent manner with increasing feeding time. Furthermore, the expression of the SelW mRNA in the gastrointestinal tract tissues of chickens was found to correlate with the dietary Se concentrations, but not with the tissue Se contents.

Introduction

Selenium (Se) is a nutritionally essential trace element for a wide range of species, including birds. Previous data have suggested that Se plays an important role in chemo-prevention [1, 2], neurobiology [3], aging [4], immune functions [5, 6], muscle metabolism [7], reproduction [8], redox reactions [5], and many other aspects of health [9, 10]. The biological functions of Se are primarily implemented through its presence in a family of Se-containing proteins [11]. Se in the form of selenocysteine (Sec) is incorporated into a selected group of proteins, such as selenoproteins at UGA codons [12, 13]. Therefore, Se availability affects selenoprotein levels and the turnover of selenoprotein mRNA levels.

Selenoprotein W (SelW), which is the smallest identified selenoprotein, consists of approximately 85–88 amino acids and contains Sec at residue 13 and cysteine at residue 10 and 37 in various species [14–16]. The sequences of SelW from rats, mice, monkeys, humans, pigs, sheep [17], zebra fish [18] and chickens [19] have been obtained. SelW is highly conserved among mammalian species, all of which contain the Trx-like motif CXXU. Our laboratory has cloned the avian SelW gene (GenBank accession no. GQ919055) and observed that the structure of chicken SelW is similar to that of mammals. SelW has since been shown to prevent White Muscle Disease in domestic animals [20], which suggests that it functions as a catalyst in biological redox reactions. Whereas the biological characteristics of SelW are already well known in rodents, little is known about them in avians. In our previous study,

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the cDNA for SelW in chickens has been sequenced, which can be used for future research on the characteristics of the SelW in avians.

The gastrointestinal tract of any animal is important in converting the food they eat into the nutrients their body needs for maintenance, growth, and reproduction. Because chickens have no teeth and their feed tends to be coarse, their gastrointestinal tract is much stronger than that of humans and other mammalian species. Low intake of Se has been implicated with increased incidence of disease, and Se supplementation lowers cancer incidence and mortality from colorectal cancer [21]. It has been reported that adequate supplementation of Se elicits a significant increase in SelW mRNA levels in human intestinal Caco-2 cells [22]. In a previous study from our laboratory, SelW mRNA was determined in the gastrointestinal tract tissues of chickens fed diets containing sodium selenite at 1, 2, 3, and 5 mg/kg, respectively [23]. However, the effects of Se deficiency on SelW mRNA in avian gastrointestinal tract tissues were unknown until now. Therefore, the purpose of the present study was to re-examine SelW mRNA expression to determine whether it was more widespread throughout the gastrointestinal tract tissues than we had initially anticipated. Levels of 0.033, 0.15, and 1.5 mg dietary Se per kilogram diet were used. The purpose of the present study was to determine the influence of deficient and adequate levels of dietary Se on SelW mRNA expression in the gastrointestinal tract tissues of chickens.

Materials and Methods

Birds and Diets

All procedures used in the present study were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. One hundred and fifty chickens (1 day old; Weiwei Co. Ltd., Harbin, China) were randomly divided into three groups (50 chickens per group). The chickens were maintained either on Se-deficient granulated diet (L group, from Se deficiency region of Heilongjiang Province in China, containing 0.033 mg/kg) or on the same feed supplemented with Se though the addition of 0.15 (M group) or 1.50 (H group) mg Se/kg as sodium selenite for 55 days. Feed and tap water were supplied ad libitum. Following euthanasia with sodium pentobarbital, the gastrointestinal tract tissues (including gizzard, glandular stomach, duodenum, small intestine, and rectum) were quickly collected at 15, 25, 35, 45, and 55 days old. The tissues were blotted, rinsed with ice-cold sterile deionized water, frozen immediately in liquid nitrogen, and stored at −80°C until required. The Se contents and mRNA levels were measured in these tissues.

Determination of Se Contents in Tissues

The Se contents in the gastrointestinal tract tissues were estimated by the method described by Hasunuma et al. [24]. 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 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 and 520 nm, respectively. The Se contents were calculated by reference to a standard curve.

Determination of the SelW mRNA Level by Quantitative RT-PCR

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.

Primer Premier Software (PREMIER Biosoft International, USA) was used to design specific primers for SelW and GADPH based on known chicken sequences (Table 1).

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. Quantitative real-time PCR was performed on an ABI PRISM 7500 Detection System (Applied Biosystems, USA). Reactions were performed in a 20-μl reaction mixture containing 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, and 6.8 μl of PCR-grade water. The PCR procedure for SelW and GADPH consisted of 95°C for 30 s followed by 40 cycles of 95°C for 15 s, 60°C for 30 s and 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

Table 1 Primers used for quantitative real-time PCR

Target gene	GenBank accession number	Primer	Sequence(5′–3′)	PCR fragment length (bp)
Chicken SelW	GQ919055	Forward	5′-CTCCGCGTCACCGTGCTC-3′	150
		Reverse	5′-CACCGTCACCTCGAACCATCCC-3′	
GADPH	K01458	Forward	5′-AGAACATCATCCCAGCGT-3′	182
		Reverse	5′-AGCCTTCACTACCCTCTTG-3′	

program [25]. The mRNA relative abundance was calculated according to the method of Pfaffl [26], accounting for gene-specific efficiencies and was normalized to the mean expression of GADPH and expressed as the ratio of Se dietary contents.

Statistical Analysis

Statistical analysis of Se concentrations and mRNA level were performed using SPSS statistical software for Windows (version 13; SPSS Inc., Chicago, IL, USA). When a significant value ($p<0.05$) was obtained by one-way analysis of variance, further analysis was done. All data showed a normal distribution and passed equal variance testing. Differences between means were assessed by Tukey's honestly significant difference test for post hoc multiple comparisons. The relationship between Se concentrations in tissues and the abundance of SelW mRNA were assessed using Pearson's correlation coefficient. Data are expressed as mean±standard deviation. Differences were considered to be significant at $p<0.05$.

Results

Se Contents in Gastrointestinal Tract Tissues

The effects of the different concentrations of dietary Se on the contents of Se in the gastrointestinal tract tissues (glandular stomach, gizzard, duodenum, small intestine, and rectum) are shown for each sampling time point in Table 2. Se contents in the gastrointestinal tract tissues increased with increasing dietary Se contents. Se contents were significantly lower in the Se-deficient groups than that in the Se-supplemented groups at each sampling time point. The H group was significantly higher ($p<0.05$) than the M group. As the experiment progressed, a significant decrease in the Se contents of the gastrointestinal tract tissues was observed for chickens fed a Se-deficient basal diet compared to chickens fed a Se-supplemented diet. In contrast, the Se contents increased in the gastrointestinal tract tissue of chickens fed Se-supplemented diets compared to chickens fed a Se-deficient diet. The Se contents in the H and M groups had significantly increased ($p<0.05$) compared to the L group on day 55. In all the groups, the Se contents in the gastrointestinal tract tissues were the highest in the duodenum and the lowest in the rectum at each sampling time point.

Effect of Dietary Se on the mRNA Levels of SelW in Glandular Stomach of Chicken

The distribution of the SelW mRNA in the glandular stomach was determined by quantitative RT-PCR (Fig 1). The SelW mRNA levels in the L group were significantly lower ($p<0.05$) at each sampling time point, and the SelW mRNA levels in the H group were significantly higher ($p<0.05$) on days 35, 45, and 55 than in the M groups. Notably, the SelW mRNA expression in the L group was the lowest on day 55. The most significant increase in SelW mRNA expression in the H group was observed on day 55 (Fig 1).

Effect of Dietary Se on the mRNA Levels of SelW in Gizzard of Chicken

The mRNA level of SelW in the gizzard of chicken was determined (Fig 2). Compared with the M groups, a significant increase ($p<0.05$) of SelW mRNA levels was detected in the H group on days 35, 45, and 55, whereas a significant decrease ($p<0.05$) in SelW mRNA levels was observed in the L group at each sampling time point (Fig 2). In chickens that were fed a Se-deficient basal diet, significant decreases ($p<0.05$) of SelW mRNA levels were detected on days 35, 45, and 55 compared to that at the first sampling time point (day 15). Notably, the levels of SelW mRNA at the last sampling time point (day 55) for the L group were the lowest among all groups in the present study. The most significant increase in SelW mRNA expression among the groups was observed on day 55 in the H group (Fig 2).

Effect of Dietary Se on the mRNA Levels of SelW in Duodenum of Chicken

The distribution of the SelW mRNA in the duodenum was determined by quantitative RT-PCR (Fig 3). Compared with

Table 2 Se contents in gastrointestinal tract tissues

Tissue		15 d	25 d	35 d	45 d	55 d
Glandular stomach	L group	0.140±0.001*	0.12±0.0032*	0.109±0.008*	0.083±0.001*	0.056±0.001*
	M group	0.341±0.007	0.368±0.049	0.381±0.031	0.415±0.002	0.465±0.021
	H group	0.513±0.036*	0.631±0.024*	0.715±0.017*	0.877±0.085*	0.906±0.011*
Gizzard	L group	0.097±0.009	0.063±0.002*	0.059±0.019*	0.048±0.006*	0.037±0.005*
	M group	0.137±0.013	0.142±0.013	0.150±0.033	0.155±0.036	0.163±0.011
	H group	0.191±0.024*	0.289±0.073*	0.346±0.031*	0.415±0.021*	0.557±0.038*
Duodenum	L group	0.241±0.019*	0.237±0.007*	0.221±0.007*	0.217±0.003*	0.207±0.028*
	M group	0.367±0.024	0.386±0.049	0.415±0.002	0.503±0.004	0.537±0.013
	H group	0.875±0.071*	0.926±0.057*	1.921±0.013*	1.107±0.024*	1.205±0.083*
Small intestine	L group	0.204±0.021	0.194±0.033*	0.181±0.022*	0.171±0.006*	0.161±0.011*
	M group	0.251±0.019	0.315±0.024	0.391±0.019	0.453±0.038	0.513±0.022
	H group	0.434±0.026*	0.509±0.019*	0.679±0.031*	0.818±0.146*	1.051±0.029*
Rectum	L group	0.036±0.005	0.052±0.004	0.047±0.009	0.041±0.007	0.036±0.006*
	M group	0.073±0.031	0.078±0.002	0.079±0.001	0.082±0.003	0.091±0.011
	H group	0.113±0.003*	0.109±0.012*	0.127±0.002*	0.129±0.019*	0.135±0.025*

The Se contents in gastrointestinal tract tissues of chickens fed diets containing various concentrations of Se at five sampling points (*d* days). Values are means±SD (*n*=5)

*$p<0.05$, indicating a significant difference between the control group (M group) and treatment groups (L and H groups) at the same time of sampling

the M group, the SelW mRNA levels in the L group were significantly decreased at all sampling time points ($p<0.05$) with the exception of day 15. There was a significant increase ($p<0.05$) of SelW mRNA in the H group on days 35, 45, and 55 compared to that in the M group. Notably, the lowest levels of SelW mRNA were detected in the L group on day 55 in the present study (Fig 3).

Effect of Dietary Se on the mRNA Levels of SelW in Small Intestine of Chicken

The SelW mRNA in the small intestine of chickens was measured by quantitative RT-PCR (Fig 4). Compared with the M group, the SelW mRNA levels in the L group significantly decreased ($p<0.05$) at each sampling time
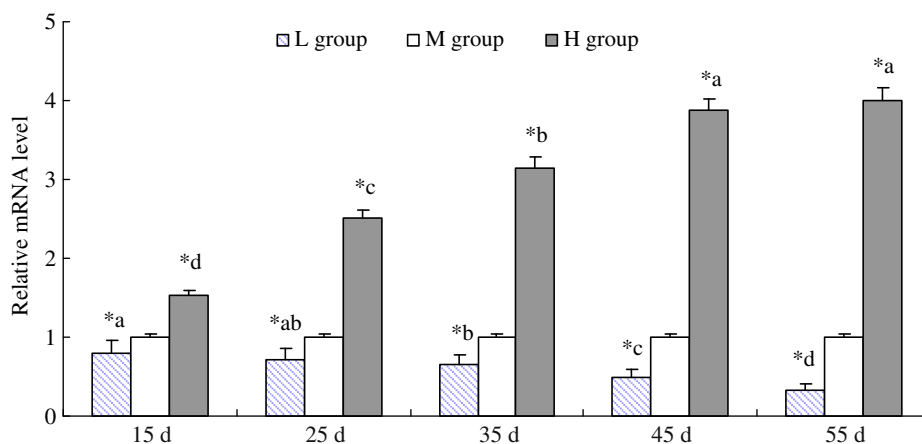

Fig. 1 Effects of different concentrations of Se on the abundance of SelW mRNA in the glandular stomach tissues of chickens at five sampling time points (*d*=days). Each value represented the means± SD of five individuals. At the same sampling time, *significant differences ($p<0.05$) between the control group (M group) and treatment groups (L and H groups). At the same concentrations of dietary Se, *bars with different small letter* are statistically significantly different from 15 days by one-way analysis of varianc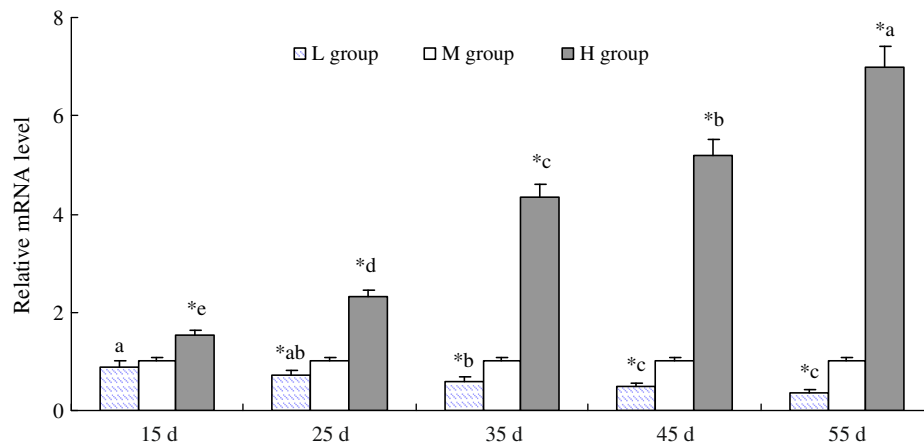e followed by Tukey–Kramer multiple comparison test ($p<0.05$), *bars sharing a common letter* are not significantly different ($p>0.05$)

Fig. 2 Effects of different concentrations of Se on the abundance of SelW mRNA in the gizzard tissues of chicken at five sampling time points (*d*=days). Each value represented the means±SD of five individuals. At the same sampling time, *significant differences (*p*< 0.05) between the control group (M group) and treatment groups (L and H groups). At the same concentrations of dietary Se, *bars with different small letter* are statistically significantly different from 15 days by one-way analysis of variance followed by Tukey–Kramer multiple comparison test (*p*<0.05), *bars sharing a common letter* are not significantly different (*p*>0.05)

point. A significant increase (*p*<0.05) of SelW mRNA in the H group was detected on days 35, 45, and 55 compared to that in the M group. Notably, the lowest levels of SelW mRNA were detected in the L group on day 55. The most significant increase in SelW mRNA expression among the groups was observed on day 55 in the H group (Fig 4).

Effect of Dietary Se on the mRNA Levels of SelW in Rectum of Chicken

The distribution of the SelW mRNA in the rectum was determined by quantitative RT-PCR (Fig 5). Compared with the M group, the SelW mRNA levels in the L group significantly decreased (*p*<0.05) at each sampling time point. A significant increase (*p*<0.05) of SelW mRNA was detected in the H group on day 55 compared to that in the M group. Notably, the lowest levels of SelW mRNA were detected in the L group on day 55. The most significant increase in SelW mRNA expression was observed in the H group on day 55 (Fig 5).

Discussion

Se plays an important role in normal physiology in a wide range of species, including birds. Poultry diets lacking Se result in slow growth and development, reduced egg production, decreased hatchability, pancreatic degeneration,
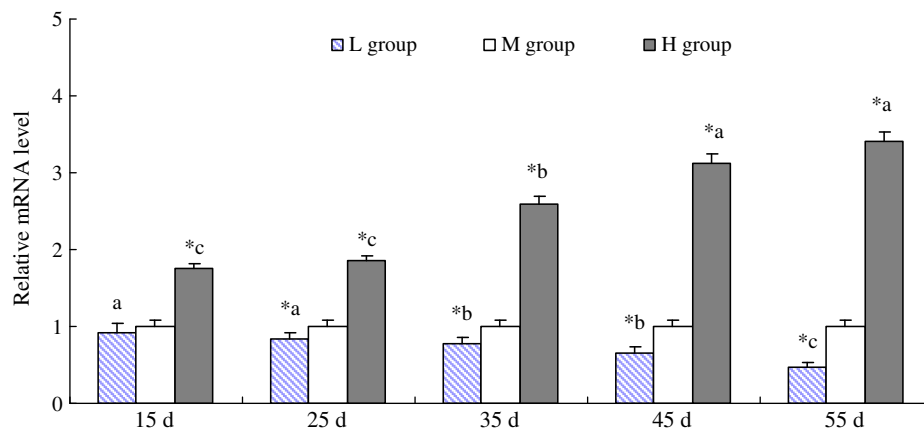

Fig. 3 Effects of different concentrations of Se on the abundance of SelW mRNA in the duodenum tissues of chicken at five sampling time points (*d*=days). Each value represented the means±SD of five individuals. At the same sampling time, *significant differences (*p*< 0.05) between the control group (M group) and treatment groups (L and H groups). At the same concentrations of dietary Se, *bars with different small letters* are statistically significantly different from 15 days by one-way analysis of variance followed by Tukey–Kramer multiple comparison test (*p*<0.05), *bars sharing a common letter* are not significantly different (*p*>0.05)
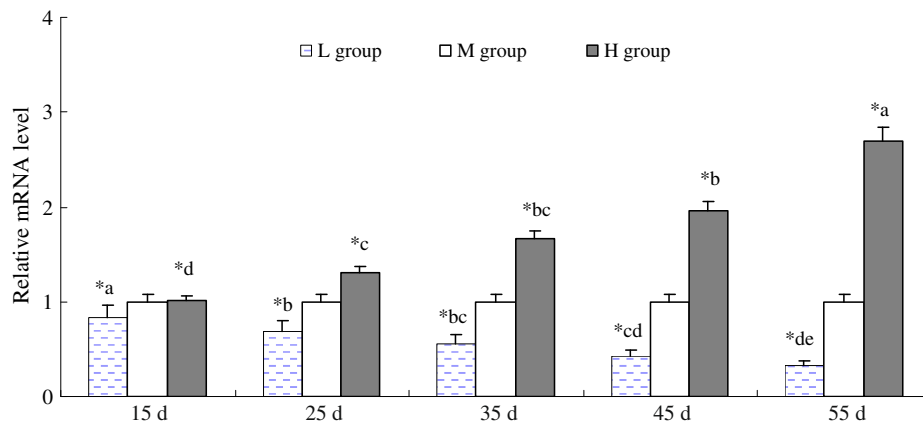
Fig. 4 Effects of different concentrations of Se on the abundance of SelW mRNA in the small intestine tissues of chicken at five sampling time points (d=days). Each value represented the means±SD of five individuals. At the same sampling time, *significant differences ($p<$ 0.05) between the control group (M group) and treatment groups (L and H groups). At the same concentrations of dietary Se, *bars with different small letters* are statistically significantly different from 15 days by one-way analysis of variance followed by Tukey–Kramer multiple comparison test ($p<0.05$), *bars sharing a common letter* are not significantly different ($p>0.05$)

nutritional muscular dystrophy, and necrotic lesions in the liver, muscle, and heart [27–30]. Excess dietary Se induces decreased relative weight, lesions, and apoptosis of chicken bursa of Fabricius [31]. Both excess and deficiency of Se supply lead to impaired growth. In our study, Se contents in the gastrointestinal tract tissues increased in a dose-dependent manner with increasing dietary Se concentrations. Compared with the chickens fed Se-supplemented diets, the chickens fed the Se-deficient basal diet had lower Se contents in its gastrointestinal tract tissues at each sampling time point (Table 2). The data indicated that Se contents in gastrointestinal tract tissues correlated with ingested Se levels. These results confirm previous studies showing that Se affects the expression of SelW [23, 32, 33].

SelW is a small protein that was initially purified from rat muscle [20]. Studies have indicated that the SelW is expressed in various animals, such as rodents [34–36], primates [15, 37], pigs [38], and sheep. It mainly exists in the cytosol, but small amounts are associated with membranes [34]. SelW mRNA and protein expression in muscles, hearts, testis, and spleen in rats and sheep are sensitive to Se [34, 35, 39, 40]. However, there was a significant difference in terms of the changes in gastrointestinal SelW expression between rats and sheep in response to dietary supplementation with Se. When animals were fed in Se-supplemented diets, higher levels of SelW were found in the tongue (rats and sheep) and colon (rats), whereas an indistinct increase in the SelW levels was observed in the
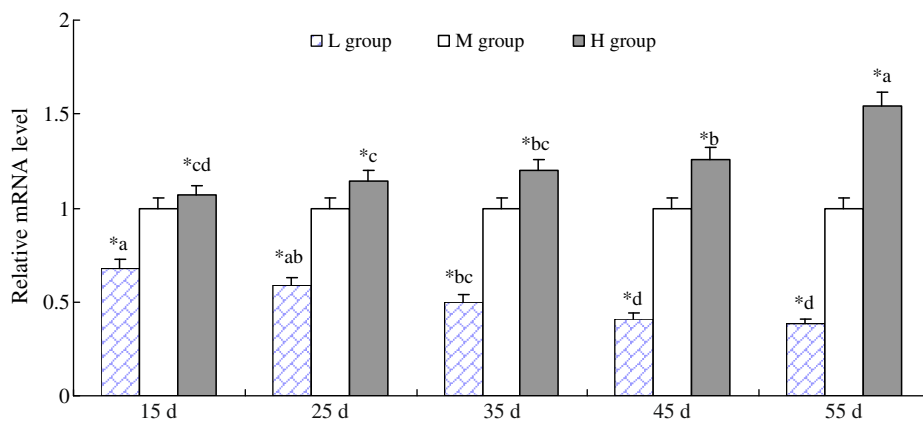

Fig. 5 Effects of different concentrations of Se on the abundance of SelW mRNA in the rectum tissues of chicken at five sampling time points (d=days). Each value represented the means±SD of five individuals. At the same sampling time, *significant differences ($p<$ 0.05) between the control group (M group) and treatment groups (L and H groups). At the same concentrations of dietary Se, *bars with different small letters* are statistically significantly different from 15 days by one-way analysis of variance followed by Tukey–Kramer multiple comparison test ($p<0.05$), *bars sharing a common letter* are not significantly different ($p>0.05$)

esophagus, stomach, and small intestine of rats [22, 36, 39]. Previous studies in our lab have shown that the cDNA for chicken SelW can be cloned from Se-supplemented chicken cerebral tissue; we also found that SelW is ubiquitously expressed in diverse tissues of chicken [19, 41]. The SelW mRNA distribution in the liver, pancreas, gastrointestinal tissues, and immune organs with different dietary selenium levels have yet to be determined [23, 32, 33, 42]. In the present study, the results of feeding two levels of dietary Se (0.15 and 1.5 mg) for 55 days indicated that SelW gene expression in the chicken gastrointestinal tract was sensitive to dietary Se concentrations and was consistent with these earlier studies. It has been reported that the expression of SelW in the liver and immune organs of chickens is dependent on the concentration of Se [32, 33]. A significant increase in SelW mRNA levels was observed in the gastrointestinal tract tissues of chickens fed diets containing sodium selenite at 1, 2, and 3 mg/kg, while the SelW mRNA levels decreased in the chickens fed diet containing sodium selenite at 5 mg/kg [23]. Previous studies mainly describe the effects on SelW mRNA in avians that were fed Se-supplemented diets. However, the effects of the Se-deficient basal diet on the regulation of SelW mRNA in avian gastrointestinal tract tissues were previously unknown. Se deficiency decreases the expression of SelW in mammals [43]. Some in vitro experiments have shown that Se depletion mediates decreases in SelW mRNA levels in human intestinal Caco-2 cells and neuronal cell line (SH-SY5Y) [22, 38]. Most studies have shown that SelW mRNA levels are significantly lower in the small intestine of Se-deficient rats [40]. In the present study, we observed that the effect of Se-deficient basal diet level (0.033 mg dietary Se per kilogram diet) on SelW mRNA levels at different time points. Moreover, our results indicated that SelW mRNA levels in the gastrointestinal tract tissue decreased in a time-dependent manner with increasing feeding time. To our knowledge, this is the first report to study the effects of Se deficiency on SelW mRNA levels in chickens. The results of present study may be helpful in understanding the mechanisms of Se-mediated regulation of SelW gene expression in birds.

Previous studies have reported that there were no correlations between the relative tissue distributions of Se and SelW [37, 39]. The results of present study support this conclusion. The most significant increase in SelW mRNA levels was observed in the gizzard (Fig 2), whereas the most significant increase in Se contents was detected in the duodenum (Table 1). Nevertheless, we identified a significant direct relationship between tissue Se contents and the SelW mRNA levels in the glandular stomach, gizzard, duodenum, small intestine, and rectum of chickens fed Se-supplemented diets and Se-deficient diets. The results of the present study confirm that SelW gene expression in the gastrointestinal tract of chickens was not correlated with tissue Se contents.

In conclusion, the present study indicated that the SelW gene was highly expressed in gastrointestinal tract tissues of chickens. The expression level of SelW mRNA in the gastrointestinal tract tissues of chickens correlated with dietary Se concentrations but not with the tissue Se contents. To our knowledge, this is the first report to study SelW mRNA levels caused by Se deficiency in chicken. These findings may be helpful in understanding the mechanisms of Se-mediated regulation of SelW gene expression.

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