https://doi.org/10.17221/24/2018-CJAS

Effects of Selenium on Selenoprotein Synthesis and Antioxidant Parameters of Bovine Mammary Epithelial Cells

*Yongmei Guo[1], Jian Gong[1,2], Binlin Shi[1], Xiaoyu Guo[1], Sumei Yan[1]**

[1]*College of Animal Science, Inner Mongolia Agricultural University, Hohhot, P.R. China*

[2]*College of Life Science and Technology, Inner Mongolia Normal University, Hohhot, P.R. China*

**Corresponding author: yansmimau@163.com*

ABSTRACT

Guo Y., Gong J., Shi B., Guo X., Yan S. (2018): **Effects of selenium on selenoprotein synthesis and antioxidant parameters of bovine mammary epithelial cells**. Czech J. Anim. Sci., 63, 313–322.

This study aimed to investigate the effects of selenium (Se) on the selenoproteins synthesis and antioxidant parameters of bovine mammary epithelial cells (BMECs). The experiment was conducted as a single factor completely randomized design to explore the effect of different levels of Se supplementation (0, 10, 20, 50, and 100 nmol/l) on selenoproteins synthesis and antioxidant parameters of BMECs, and to screen the appropriate dose of Se supplementation ensuring a better antioxidant function. Se supplementation increased cell proliferation, the activities of glutathione peroxidase (GPx) and superoxide dismutase, total antioxidant capacity and seleoprotein P (SelP) content, and decreased reactive oxygen species and malondialdehyde levels in a dose-dependent manner. Se supplementation of 50–100 nmol/l had a better effect. Se supplementation also increased thioredoxin reductase (TrxR) activity in a dose-dependent manner, and Se supplementation of 20–50 nmol/l had a better promoting effect. The dose-dependent response between Se supplementation and mRNA and protein expression of GPx1 and TrxR1, as well as SelP mRNA expression was also observed in this experiment. The mRNA and protein expression of GPx1 was up-regulated with the addition of 50–100 nmol/l Se, and the mRNA expression of TrxR1 and SelP was up-regulated with the addition of 20–100 nmol/l Se. Results indicated that Se supplementation of 50 nmol/l had a better promoting effect on the selenoproteins synthesis and antioxidant parameters of BMECs.

Keywords: trace element; dairy cows; glutathione peroxidase; thioredoxin reductase; antioxidant function

List of abbreviations: SE = selenium, ROS = reactive oxygen species, BMECs = bovine mammary epithelial cells, GPx = glutathione peroxidase, TrxR = thioredoxin reductase, BW = body weight, DMSO = dimethyl sulfoxide, DMEM/F12 = Dulbecco's Modified Eagle's Medium/F12, T-AOC = total antioxidant capacity, SOD = superoxide dismutase, CAT = catalase, MDA = malondialdehyde, SELP = selenoprotein P, MTT = 3-(4,5-dimethylthiazol1622-yl)-2,5-diphenyltetrazolium bromide salt, RT-PCR = reverse transcription-polymerase chain reaction, BAECs = bovine aorta endothelial cells

Oxidative stress in animals is not only a major cause of economic loss in the dairy industry, but it is also a major problem in ensuring milk quality and the health of dairy cows. Normally, there is a balance between the reactive oxygen species (ROS) production and the antioxidant function of mammary gland. Since the late pregnancy till the peak of lactation, bovine mammary epithelial cells

Supported by the National Natural Science Foundation of China (Project No. 31560650).

https://doi.org/10.17221/24/2018-CJAS

(BMECs) exhibit a high metabolic rate and then produce large amounts of ROS and lipid peroxides *in vivo*. This is related to the rapid development of mammary gland and a large-scale synthesis and secretion of milk. This effect is exacerbated in high-yielding dairy cows. If these excessive ROS could not be cleared in time, fatty acid chain reaction would be triggered, oxidizing cellular lipids, proteins, and DNA, eventually resulting in apoptosis and tissue damage (Sordillo 2013). Therefore, improving the antioxidant function of dairy cows is one of the important measures of ensuring their health, as well as improving milk performance and milk quality.

Certain trace minerals, such as selenium (Se), could be effective in reducing oxidative stress and the severity of several pro-inflammatory-based dairy cow diseases such as mastitis and metritis (Sordillo and Aitken 2009). Se in the form of selenoproteins plays a range of biological functions. Certain selenoproteins associated with antioxidant functions in dairy cows such as glutathione peroxidase (GPx), thioredoxin reductase (TrxR), and selenoprotein P (SelP) can clean oxygen free radicals and maintain redox balance in tissues and cells. Most studies about rat aortas (Stupin et al. 2017), mice liver (Murano et al. 2018), chicken dendritic cells (Sun et al. 2017), and bovine vascular endothelial cells (Sunde et al. 2009) indicated that the lack of Se or Se supplementation significantly decreased or increased the mRNA level and the activity of GPx1. Lack of Se significantly decreased the mRNA level of *TrxR2* in Caco-2 cells of human colonic mucosa (Pagmantidis et al. 2005) and Se supplementation increased the mRNA level of *TrxR1* in human Caco-2 cells (Barrera et al. 2012). Se supplementation significantly increased the mRNA level of *SelP* in human liver and endothelial cells (Steinbrenner et al. 2006; Zhang et al. 2017). These results demonstrate that Se may affect the antioxidant function of animal tissues or cells by regulating the synthesis of selenoprotein. However, little information is available about the effects of Se on the selenoproteins synthesis and antioxidant functions of BMECs. Based on these observations, a hypothesis is proposed that the antioxidant function and selenoprotein synthesis in BMECs are regulated by Se.

In view of this, the present study explores the effects of Se on the antioxidant function of BMECs *in vitro* and on the regulation of selenoprotein synthesis at the transcription and translation level, providing a basis for the further study of the mechanism of Se effects on the antioxidant function of mammary gland.

MATERIAL AND METHODS

BMECs culture. Mammary tissue was collected from healthy lactating Holstein cows (parity: 3, age: 5 years, body weight (BW): 650 ± 50 kg, milk yield: 20.75 ± 0.50 kg/day), kept on ice, and transported to the laboratory without delay. Tissue samples were pared and minced, sequentially washed with phosphate balanced solution and 75% ethanol. Mammary epithelial cells were cultured by the method of collagenase digestion. Sample pieces enriched with acini were transferred into a tube with 0.05% collagenase type II (Gibco BRL, USA) and minced thoroughly to separate BMECs from erythrocytes. The BMECs were cultured at 37°C in 95% humidified air containing 5% CO_2 using Dulbecco's Modified Eagle's Medium (DMEM/F12) containing 10% fetal bovine serum (FBS), 10 ng/ml epidermal growth factor, 1 μg/ml hydrocortisone, 5 μg/ml insulin transferrin Se, 5 μg/ml prolactin, 100 IU/ml penicillin and 100 ug/ml streptomycin, 2.5 μg/ml amphotericin B (all Sigma Chemical Co., USA), the rest was basal medium (15.6 g DMEM/F12 powder, 1000 ml ultra pure water, and 2.4 g sodium bicarbonate). For experimentation, BMECs were plated at 25 cm^2 cell culture flasks at an approximate density after quantified by cytometry (Cytorecon; GE Healthcare Co., USA). Third-generation BMECs were plated at different density (1×10^4, 1×10^5, 3×10^5, and 5×10^5 cells/ml) at 96-well plates, 6-well plates, 60-mm culture dishes, and 25 cm^2 culture flasks, and incubated in the atmosphere of 5% CO_2 at 37°C for 24 h. Medium was decanted when the anchorage-dependent rates of BMECs were up to 80–90%, and then the cells were serum-starved for 24 h.

Experimental design. The experiment was conducted as a single-factor arrangement. After serum-starved for 24 h, BMECs were randomly divided into five treatments, consisting of 6 replicates. Group 1 was the control group, the remaining 4 groups were selenium treatment groups, cultured with medium of selenomethionine at concentrations of 10, 20, 50, and 100 nmol/l for 24 h.

https://doi.org/10.17221/24/2018-CJAS

Measurement of cell viability. Cell viability was assessed by 3-(4,5-dimethylthiazol1622-yl)-2,5-diphenyltetrazolium bromide salt (MTT) assay. After culturing for 24 h according to the above-given experimental design, 20 µl of 5 mg/ml MTT was added for 4 h, then the medium was decanted. Cells were dissolved in dimethyl sulfoxide (DMSO; Sigma Chemical Co.) and shaken for 10 min. The optical density value was analyzed with automatic microplate reader (Synergy H4; BioTek Co., Japan) at 490 nm. Cell viability of control group was expressed as 100%.

Measurement of antioxidant parameters. The cell culture solution and cell suspension were separately collected in a 1.5 ml centrifuge tube. Firstly, the protein concentrations of the cell homogenate samples were measured according to the method of bicinchoninic acid. Briefly, The SelP content in culture solution was measured by a radioimmunoassay (Hill et al. 1996). The TrxR activity in cells was measured using a 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) reduction assay, as described previously (Hill et al. 1997). The malondialdehyde (MDA) concentration in cells was measured by a thiobarbituric acid positive reactants (TBAR) assay (Ohkawa et al. 1979). The ROS level in cells was analyzed using commercial ELISA kits (Beijing Neobioscience Co., China). The superoxide dismutase (SOD), GPx, catalase (CAT) activities, and the total antioxidant capacity (T-AOC) were measured using commercial colorimetric assay kits (Nanjing Jiancheng Bioengineering Institute, China) following the manufacturer's protocol. The absorbance was measured using a spectrophotometer at various wavelengths.

Relative expression of selenoprotein mRNA in BMECs. Total RNA extraction was isolated following the manufacturer's protocol of a RNAiso™ Plus kit (TaKaRa Inc., China). The cDNA probes were obtained by PrimeScript™ reverse transcription kit (TaKaRa Inc.) according to the manufacturer's instruction. The primers for reverse transcription-polymerase chain reaction (RT-PCR) were designed using Oligo and Premier software based on data from GenBank (Table 1). General PCRs were first performed to confirm the specificity of the primers. Relative mRNA expressions of GPx1, GPx4, TrxR1, and SelP in BMECs were determined according to the manufacturer's instruction of SYBR® Premix Ex *Taq*™ kit (TaKaRa Inc.) by fluorescence-based real-time PCR (SYBR® Green I). *β-actin, UXT,* and *18SrRNA* were used as internal controls, and the qPCR data were normalized using the geometric mean of the three internal control genes.

Table 1. Sequences of reverse transcription-polymerase chain reaction primers for genes

Genes	Accession No.[1]	Primer sequences (5'-3')		Length (bp)	Annealing temperature (°C)
GPx1	NM_174076.3	F:	AGTGCGAGGTGARATGGCGAGARA	328	60
		R:	TGGGCARAARATCCCTGGAGAGCA		
GPx4	NM_174770.3	F:	ATCARAAGAGTTCGCCGCTGGCT	295	60
		R:	TCGGARACACAGGCARACAGGCTT		
TrxR1	NM_174625.3	F:	AGGAGARAAGCTGTGGAGARAA	94	60
		R:	TTATCCCTTGATGGARATCGT		
SelP	NM_174459.3	F:	CTTCATCACCACCACCACAG	331	60
		R:	GAGGCARAACGTCACTGTCARA		
β-actin	NM_173979.3	F:	AACTCCATCATGAAGTGTGACG	234	60
		R:	GATCCACATCTGCTGGAAGG		
UTX	NM_001037471	F:	CATTGAGCGACTCCAGGAAG	112	60
		R:	GGCCACATAGATCCGTGAAG		
18S rRNA	Robinson et al. 2007	F:	GTAACCCGTTGAACCCCATT	152	60
		R:	CCATCCAATCGGTAGTAGCG		

GPx = glutathione peroxidase, *TrxR1* = thioredoxin reductase 1, *SelP* = selenoprotein P, F = forward primer, R = reverse primer

[1]mRNA sequences of the related genes were obtained from NCBI Entrez Nucleotide (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide&itool=toolbar)

https://doi.org/10.17221/24/2018-CJAS

The $2^{-\Delta\Delta Ct}$ method was used to analyse the data. Integrity and size of the PCR products for each sample were assessed by formaldehyde-agarose-gel electrophoresis. Sequence analysis was carried out by Shanghai Sangon Biotech, China.

Western blotting analysis. After culturing for 24 h in 25 cm^2 culture flasks, cells were scraped and then lysed for 5 min in RIPA lysis at 4°C. Protein expression of GPx1 and TrxR1 in cells was determined by Western blotting on the basis of the internal reference gene *β-actin*. Cell lysates containing 30 μg of protein were boiled in 1/4 volume of 4 × sample loading buffer at 95°C for 5 min and separated on 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) with a 5% stacking gel in electrophoretic buffer. Proteins were electrotransferred to a polyvinylidene fluorine membrane. The membranes were blocked with blocking buffer for 1 h at room temperature. Then the membrane was incubated with diluted polyclonal rabbit anti-β-actin (1 : 2000) (Proteintech Co., USA), polyclonal rabbit anti-GPx1 (1 : 1000), and polyclonal rabbit anti-TrxR1 (1 : 500) (both Abcam Co., USA) overnight at 4°C. After incubation with goat anti-rabbit IgG (1 : 1000) (KPL Co., USA) for 1 h at room temperature, colour development was carried out according to manufacturer's instruction of an ECL ultra sensitive luminescence kit (Thermo Fisher Scientific, USA). Signals were visualized and photographed with ImageQuant (GE Healthcare Co., USA). The image intensity was analysed with Quantity One software (Bio-Rad Laboratories Inc., USA), and the relative expression of target protein was calculated.

Statistical analysis. The data were analysed using the one-way ANOVA procedure of SAS software (Statistical Analysis System, Version 9.0, 2003) and Duncan multiple comparison tests. The linear and quadratic effects of Se level on all the indexes were analysed with regression analysis. Statistical significance was considered at $P < 0.05$.

RESULTS

Effect of Se on cell viability. The BMECs proliferation rate presented in Table 2 showed a non-significant change in 10 nmol/l and 20 nmol/l Se treated groups, a significant increase in 50 nmol/l and 100 nmol/l Se treated groups, when compared with the control group. The 100 nmol/l Se treated group showed a slight decrease when compared with the 50 nmol/l Se treated group (Table 2). The results of regression analysis indicated that with the Se level increasing the cell viability showed a significant quadratic increase ($P = 0.0002$), and the maximum value was observed at 50 nmol/l Se.

Effect of Se on antioxidant parameters in BMECs. The GPx activity was significantly increased in all the Se treated groups, when compared with the control group ($P = 0.0001$) (Table 3), and in 50 nmol/l and 100 nmol/l Se treated groups it was significantly higher than in the other groups. A linear and quadratic dose-dependent relationship between the level of Se and the GPx activity was observed ($P = 0.0001$). Compared with the control group, the TrxR activity presented in Table 3 showed a non-significant change in 10 nmol/l Se treated group, and a significant increase in 20 nmol/l and 50 nmol/l Se treated groups ($P = 0.0001$). In the 100 nmol/l Se treated group it was significantly decreased when compared with 20 nmol/l and 50 nmol/l Se treated groups, but it showed a non-significant change compared with 10 nmol/l Se treated group and the control group. The results of regression analysis indicated that with the Se level increasing, the TrxR activity showed a significant quadratic increase ($P = 0.0001$). The TrxR activity in the groups treated

Table 2. Effect of Se on cell viability

Items	Level of Se (nmol/l)					*P*-value[1]	SEM	*P*-value[2]	
	0	10	20	50	100			linear	quadratic
Cell viability (%)	100.0^{c}	104.2^{bc}	104.5^{bc}	111.5^{a}	108.0^{ab}	0.0018	1.51	0.0041	0.0002

SEM = standard error of the mean

[1]values in the same row without the same superscript letters mean significant difference ($P < 0.05$), $0.05 < P < 0.10$ means significant statistical trend

[2]$P < 0.05$ means significant regression relation, and $0.05 < P < 0.10$ means regression relation tends to be significant

https://doi.org/10.17221/24/2018-CJAS

with 20 nmol/l and 50 nmol/l Se was higher than in the other groups, especially in the 50 nmol/l Se treated group. The results of Se treated groups showed a significant increase (P = 0.001) in SelP content when compared with the control group (Table 3), while there was no significant difference between the Se treated groups. With increasing the level of Se, SelP content showed a significant quadratic increase (P = 0.0012). SelP content was higher when the level of Se was 20–50 nmol/l.

The SOD and T-AOC activity presented in Table 3 showed a significant increase in 50 nmol/l and 100 nmol/l Se treated groups, non-significant change in 10 nmol/l Se treated group, but there was a trend toward increase when compared with the control group. With increasing the level of Se, SOD and T-AOC activity showed a significant quadratic increase (P = 0.0049, P = 0.0001). However, CAT activity presented in Table 3 showed a non-significant change in all the Se treated groups (P = 0.1164) though it showed an increasing trend in 50 nmol/l and 100 nmol/l Se treated groups compared with the control group.

The MDA and ROS content presented in Table 3 showed a significant decrease in all the Se treated groups (P = 0.0003, P = 0.0001). With increasing the level of Se, MDA and ROS contents showed a significant quadratic decrease (P = 0.0001). The MDA and ROS contents were lower when the level of Se was 50–100 nmol/l.

Effect of Se on selenoprotein mRNA expression in BMECs. Results revealed that the mRNA expression of *GPx1* was significantly higher in groups treated with Se in comparison with the control group (P = 0.0001), and in 50 nmol/l and 100 nmol/l Se treated groups it was significantly higher than in 10 nmol/l and 20 nmol/l Se treated groups. The results of regression analysis indicated that a linear or quadratic dose-dependent relationship was observed between the level of Se and the mRNA expression of *GPx1* (P = 0.0001). The mRNA expression of *GPx1* was higher when the level of Se was 50–100 nmol/l, and in 50 nmol/l Se treated group it was the highest. However, results revealed (Table 4) that the mRNA expression of *GPx4* was non-significantly changed in groups treated with Se in comparison with the control group (P = 0.2596).

Compared with the control group, the mRNA expression of *TrxR1* and *SelP* was significantly increased in 20 nmol/l, 50 nmol/l, and 100 nmol/l Se treated groups, and higher than that in 10 nmol/l Se treated group (P = 0.0001), however, it did not significantly change in 10 nmol/l Se treated group. The results of regression analysis indicated that with increasing the level of Se, the mRNA expression of *TrxR1* showed a significant quadratic increase (P = 0.0001), and the mRNA expression of *SelP* showed a significant linear and quadratic increase (P = 0.0005). The higher mRNA expression

Table 3. Effect of Se on antioxidant measurements in bovine mammalian epithelial cells

Items	Level of Se (nmol/l)					P-value[1]	SEM	P-value[2]	
	0	10	20	50	100			linear	quadratic
GPx (U/mg protein)	127.70^{d}	167.48^{c}	208.65^{b}	259.09^{a}	264.00^{a}	0.0001	9.80	0.0001	0.0001
TrxR (U/g protein)	2.95^{b}	3.46^{b}	8.06^{a}	8.49^{a}	3.56^{b}	0.0001	0.54	0.7583	0.0001
SelP (mg/ml)	0.24^{b}	0.41^{a}	0.54^{a}	0.52^{a}	0.47^{a}	0.0010	0.04	0.0495	0.0012
SOD (U/ml)	8.88^{b}	10.46^{ab}	10.95^{ab}	2.23^{a}	12.06^{a}	0.0325	0.69	0.0093	0.0049
CAT (U/ml)	2.46	2.63	2.85	3.61	3.47	0.1164	0.69	0.0165	0.0243
T-AOC (U/ml)	1.48^{c}	2.10^{c}	6.46^{b}	9.42^{a}	9.56^{a}	0.0001	0.45	0.0001	0.0001
MDA (nmol/mg protein)	3.63^{a}	2.45^{b}	2.05^{bc}	1.56^{bc}	1.17^{c}	0.0003	0.33	0.0002	0.0001
ROS (U/ml)	127.5^{a}	116.3^{b}	103.6^{c}	102.3^{c}	100.3^{c}	0.0001	2.82	0.0003	0.0001

GPX = glutathione peroxidase, TrxR = thioredoxin reductase, SelP = selenoprotein P, SOD = superoxide dismutase, CAT = catalase, T-AOC = total antioxidant capacity, MDA = malondialdehyde, ROS = reactive oxygen species, SEM = standard error of the mean

[1]values in the same row without the same superscript letters mean significant difference (P < 0.05), 0.05 < P < 0.10 means significant statistical trend

[2]P < 0.05 means significant regression relation, and 0.05 < P < 0.10 means regression relation tends to be significant

https://doi.org/10.17221/24/2018-CJAS

Table 4. Effect of Se on selenoprotein mRNA expression in bovine mammalian epithelial cells

Items	Level of Se (nmol/l)					*P*-value[1]	SEM	*P*-value[2]	
	0	10	20	50	100			linear	quadratic
GPx1	0.0051[c]	0.0115[b]	0.0153[b]	0.0201[a]	0.0195[a]	0.0001	0.0012	0.0001	0.0001
GPx4	0.2142[a]	0.2519[a]	0.2256[a]	0.2354[a]	0.2004[a]	0.2596	0.0155	0.1492	0.1923
TrxR1	0.0038[b]	0.0050[b]	0.0064[a]	0.0076[a]	0.0067[a]	0.0001	0.0004	0.0036	0.0001
SelP	0.0072[b]	0.0076[b]	0.0115[a]	0.0128[a]	0.0139[a]	0.0026	0.0012	0.0005	0.0005

GPx = glutathione peroxidase, *TrxR1* = thioredoxin reductase 1, *SelP* = selenoprotein P, SEM = standard error of the mean

[1]values in the same row without the same superscript letters mean significant difference ($P < 0.05$), $0.05 < P < 0.10$ means significant statistical trend

[2]$P < 0.05$ means significant regression relation, and $0.05 < P < 0.10$ means regression relation tends to be significant

Table 5. Effect of Se on selenoprotein expression in bovine mammalian epithelial cells

Items	Level of Se (nmol/l)					*P*-value[1]	SEM	*P*-value[2]	
	0	10	20	50	100			linear	quadratic
GPx1	0.987[c]	3.203[b]	3.256[b]	4.649[a]	4.597[a]	0.0001	0.0522	0.0001	0.0001
TrxR1	0.980[b]	1.003[b]	2.567[a]	2.678[a]	2.544[a]	0.0001	0.0521	0.0001	0.0001

GPx1 = glutathione peroxidase 1, TrxR1 = thioredoxin reductase 1, SEM = standard error of the mean

[1]values in the same row without the same superscript letters mean significant difference ($P < 0.05$), $0.05 < P < 0.10$ means significant statistical trend

[2]$P < 0.05$ means significant regression relation, and $0.05 < P < 0.10$ means regression relation tends to be significant

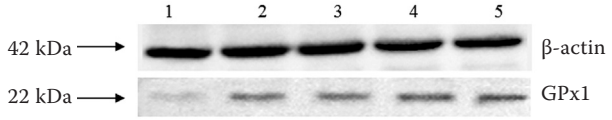

Figure 1. Effect of Se on GPx1 protein expression in cells
1 = Control, 2 = 10 nmol/l Se treated group, 3 = 20 nmol/l Se treated group, 4 = 50 nmol/l Se treated group, 5 = 100 nmol/l Se treated group

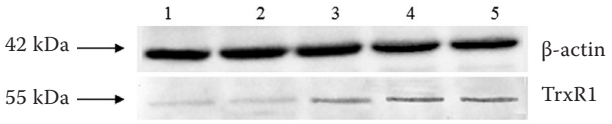

Figure 2. Effect of Se on TrxR1 protein expression in cells
1 = Control, 2 = 10 nmol/l Se treated group, 3 = 20 nmol/l Se treated group, 4 = 50 nmol/l Se treated group, 5 = 100 nmol/l Se treated group

of *TrxR1* and *SelP* was found in BMECs cultured in 20–100 nmol/l Se.

Effect of Se on selenoprotein expression in BMECs. As show the results in Table 5 and Figures 1 and 2, the protein expression of GPx1 and TrxR1 was significantly higher in groups treated with Se in comparison with the control group ($P = 0.0001$), and in 50 nmol/l and 100 nmol/l Se treated groups it was significantly higher than in 10 nmol/l and 20 nmol/l Se treated groups ($P = 0.0001$). According to the results of regression analysis, there was a linear and quadratic increase of GPx1 and TrxR1 protein expression in BMECs cultured with the increasing level of Se ($P = 0.0001$). The highest protein expression of GPx1 and TrxR1 was observed in 50 nmol/l Se treated group.

DISCUSSION

Effects of Se on proliferation of BMECs. Regulated by a variety of factors, cell proliferation and death are normal processes in the maintenance of internal homeostasis. Zeng (2002) reported that selenite or selenomethionine (250 nmol/l) enhanced the growth of HL-60 cells and affected the cell cycle progression. Yeo and Kang (2007) reported that sodium Se effectively protected neurons from apoptotic cell death after a traumatic brain injury. Mosca et al. (2002) found that a supplement with

https://doi.org/10.17221/24/2018-CJAS

Se reduced susceptibility of lymphocytes to apoptosis. Selenomethionine supplementation protects BMECs from apoptotic cell death and increases proliferation and cell viability under conditions of oxidative stress (Miranda et al. 2011) despite the limited documentation related to this. The results in the current study indicated that cell viability was significantly increased by Se addition. There was a quadratic dose-dependent relation between the level of Se and the cell viability, and Se at 50 nmol/l had the best promotion effect. This result was consistent with the report for BMECs (Miranda et al. 2011).

The effect of Se on cell proliferation and apoptosis is directly related to its dose. Se advances cell cycle progression and prevents cell death following the nutritional dose. In contrast, at supranutritional doses that are greater than the nutritional requirement but not toxic, Se induces cell cycle arrest and apoptosis (Zeng 2009). Several reports related to the anti-cancerogenic effect of Se drew the same conclusion (Goncalves et al. 2013). Zeng (2009) summarized a lot about the regulatory effect of Se on cell apoptosis and proliferation of Se. A nutritional dose at the range of 50–250 nmol/l could stimulate cell proliferation and prevent cell apoptosis. This experiment also confirmed that 10–20 nmol/l of Se non-significantly affected the BMECs viability, however, 50–100 nmol/l of Se significantly increased cell viability. However, the Se addition above 100 nmol/l requires further investigation.

At present, it is not clear what mechanisms mediate these effects of Se on cell proliferation and apoptosis. Several studies suggested that Se may regulate cell proliferation and apoptosis by affecting the cell cycle. Cyclin/cdk1 is thought to be the primary kinase that initiates the onset of mitosis, and cdk2 and cyclin B are essential for the transition of S/G2 phases, initiation of DNA synthesis, activation of cdk1, and entry into mitosis in higher eukaryotes (Hu et al. 2001). It was found out that the G1 phase cell distribution in Se-deficient cells decreased and the G2 phase cell distribution in Se-deficient cells significantly increased. Selenite or selenomethionine at nutritional dose significantly up-regulate the mRNA expression of many cell cycle-related genes (including *cdk1, cdk2, cyclinB,* and *cyclinC* etc.), furthermore they increase cell proliferation (Zeng 2009).

Effect of Se on antioxidant parameters of BMECs. A strong antioxidant system prevents body from oxidative damage and maintains the intracellular redox balance. The antioxidant system is very complex, composed of a number of key antioxidant enzymes and non-enzymatic antioxidants. The activity of SOD and CAT and the generation of T-AOC, MDA, and ROS are parameters commonly used to evaluate the antioxidant capacity of tissues and cells. In the former investigation we revealed that increasing Se level contributes to the decrease of ROS in the serum and the increase of CAT and T-AOC activities, moreover to improving the antioxidant capacity in dairy cows (Gong et al. 2014). But the research on the effect of Se on the antioxidant capacity of BMECs is relatively poor. Our study provided evidence that the addition of Se significantly increased the activity of SOD and T-AOC in culture solution and decreased the content of ROS and MDA in cells. At the same time, there was a significant linear and quadratic dose-dependent relation between the antioxidant index and the dose of Se. These results indicated that Se could enhance the antioxidant capacity of BMECs.

Selenoprotein is the main indicator reflecting the body antioxidant capacity. As the composition of selenoprotein, Se plays a moderating role on its activity, and then affects the antioxidant function. Some selenoproteins with the activities of antioxidant enzymes have been widely studied, including GPx1, GPx4, TrxR1, and SelP. Hara et al. (2001) examined the effects of Se deficiency on the activity of selenoproteins in bovine aorta endothelial cells (BAECs); the activity of TrxR1 and GPx1 was reduced in Se-deficient cells but the Se deficiency did not affect the GPx4 activity. The activity of GPx1 and TrxR1 in BAECs grown in Se-deficient medium for 3 days was reduced to about 60% and 70% of that in Se-sufficient cells, respectively. Bruzelius et al. (2010) found that there was no change in the mRNA expression of *GPx4* after supplementing the cells with sodium selenite, while the expression of *SelP* and *GPx1* was found to increase with increasing doses of Se. On the basis of the experiments *in vivo*, this experiment *in vitro* further explored the effects of Se on the synthesis and antioxidant function of selenoprotein in BMECs. The GPx and TrxR activities as well as the content of SelP were significantly increased by the addition of Se in BMECs. The results were consistent with previous studies, and there was a linear and quadratic dose-dependent relation

https://doi.org/10.17221/24/2018-CJAS

between the level of Se and the indexes mentioned above. These results indicated that the addition of Se significantly promoted the antioxidant function of BMECs in a dose-dependent manner.

Most studies indicated that Se affected the synthesis and activity of selenoprotein by regulating the mRNA expression of selenoprotein. Se deficiency or sufficiency significantly decreased or increased the level of *GPx1* mRNA (Stupin et al. 2017; Sun et al. 2017; Murano et al. 2018). The mRNA expression of *GPx1* was down-regulated and the activity of GPx1 was nearly undetectable in rabbit liver when Se was deficient (Muller and Pallauf 2002). Se-depletion contributed to statistically significant falls in *GPx1* mRNA in human intestinal Caco-2 cells and colonic mucosa cells of rats (Pagmantidis et al. 2005). Compared with *GPx1*, Se deficiency caused a modest decrease of *TrxR1* mRNA in liver of rats (Hadley and Sunde 2001). The mRNA level of *SelP* was also reduced by Se deficiency; however, the extent of reduction was small compared with the *GPx1* mRNA level in BAEC (Hara et al. 2001). The *GPx4* mRNA expression was not generally affected by Se deficiency or addition (Kipp et al. 2009). The data about the effects of Se on the bovine mammary gland or gene expression of selenoprotein in BMECs is limited. Bruzelius et al. (2007) came to the conclusion that the mRNA expression of *SelP* and *GPx1* was influenced by the Se status, but the expression of *GPx4* and *TrxR1* was not. Our experiment shows the addition did not affect the mRNA expression of *GPx4*, but significantly increased the *GPx1*, *TrxR1,* and *SelP* mRNA expression; compared with the control group, the indexes of 50 nmol/l Se treated group mentioned above were increased by 12, 1, and 0.78, respectively.

The regulatory effect of Se on selenoproteins synthesis varies in selenoprotein types. The *GPx1* mRNA expression is highly sensitive to supplement or deficiency of Se, whereas the *GPx4* mRNA expression is usually not (Barnes et al. 2009). Compared with the control group, 10 nmol/l of Se significantly increased the *GPx1* mRNA expression, but the mRNA expression of *TrxR1* and *SelP* did not change; when the dose of Se increased to 20 nmol/l, the *TrxR1* and *SelP* mRNA expression was significantly increased, while the supplement of Se did not affect that of *GPx4*. Our results are similar to those of the above-mentioned studies.

At present, the mechanism of Se regulating the selenoproteins synthesis is not very clear, it is a complicated issue and concerned with multiple levels of regulation. The available data indicates that Se deprivation regulates the abundance of cytoplasmic *GPx1* mRNA by eliciting the nonsense mediated decay of cytoplasmic mRNA rather than by affecting the production and transcription rate of *GPx1* mRNA (Moriarty et al. 1998). Nonsense mediated decay appears to require the UGA codon to be positioned > 55 nt upstream of an intron splice junction (Nagy and Maquat 1998). However, the first UGA codon of SelP is 26 nt upstream of the splice junction and mRNA levels of *SelP* decrease dramatically in Se deficiency (Sunde et al. 2009). Therefore, there may exist other mechanisms about the effect of Se on the mRNA level of selenoprotein. Se was found to regulate the synthesis of selenoprotein at the translational level. It was notable that the addition of SBP2 promoted GPx4 synthesis in reticulocyte of rats by 25-fold. Some argued that the binding activity of SBP2 might be involved in preventing termination at the UGA/Sec codon, improving the stability of mRNA, and then the selenoprotein expression level (Copeland et al. 2000), others considered that Se affected the protein translation level of selenoprotein by regulating the level of tRNAsec. This issue is poorly understood, it may be considered for further study.

The results of this research indicated that the addition of Se had a significant effect on the activity of GPx, TrxR, SOD, T-AOC and the content of SelP, MDA, and ROS. Se (50 nmol/l) played a significant role in promoting the antioxidant function of BMECs; when the dose of Se increased to 100 nmol/l, some antioxidant parameters changed to the disadvantageous direction of antioxidant function, suggesting that Se at high doses might not be beneficial to the antioxidant function. Yet the results implied that further study about the effect of over 100 nmol/l of Se on antioxidant function is warranted.

CONCLUSION

Taking the antioxidant and immune function into consideration, Se has a significant effect on the selenoproteins synthesis and antioxidant parameters in BMECs, and the enhancement was greater with 50 nmol/l of Se.

https://doi.org/10.17221/24/2018-CJAS

REFERENCES

Barnes K.M., Evenson J.K., Raines A.M., Sunde R.A. (2009): Transcript analysis of the selenoproteome indicates that dietary selenium requirements of rats based on selenium-regulated selenoprotein mRNA levels are uniformly less than those based on glutathione peroxidase activity. The Journal of Nutrition, 139, 199–206.

Barrera L.N., Cassidy A., Wang W., Wei T., Belshaw N.J., Johnson I.T., Brigelius-Flohe R., Bao Y. (2012): TrxR1 and GPx2 are potently induced by isothiocyanates and selenium, and mutually cooperate to protect caco-2 cells against free radical-mediated cell death. Biochimica et Biophysica Acta, 1823, 1914–1924.

Bruzelius K., Hoac T., Sundler R., Onning G., Akesson B. (2007): Occurrence of selenoprotein enzyme activities and mRNA in bovine mammary tissue. Journal of Dairy Science, 90, 918–927.

Bruzelius K., Sundler R., Pagmantidis V., Akesson B. (2010): Regulation of selenoprotein mRNA expression by hormones and retinoic acid in bovine mammary cells. Journal of Trace Elements in Medicine and Biology, 24, 251–256.

Copeland P.R., Fletcher J.E., Carlson B.A., Hatfield D.L., Driscoll D.M. (2000): A novel RNA binding protein, SBP2, is required for the translation of mammalian selenoprotein mRNAs. Embo Journal, 19, 306–314.

Goncalves A.C., Barbosaribeiro A., Alves V., Silva T., Sarmentoribeiro A.B. (2013): Selenium compounds induced ROS-dependent apoptosis in myelodysplasia cells. Biological Trace Element Research, 154, 440–447.

Gong J., Ni L., Wang D., Shi B., Yan S. (2014): Effect of dietary organic selenium on milk selenium concentration and antioxidant and immune status in midlactation dairy cows. Livestock Science, 170, 84–90.

Hadley K.B., Sunde R.A. (2001): Selenium regulation of thioredoxin reductase activity and mRNA levels in rat liver. Journal of Nutritional Biochemistry, 12, 693–702.

Hara S., Shoji Y., Sakurai A., Yuasa K., Himeno S., Imura N. (2001): Effects of selenium deficiency on expression of selenoproteins in bovine arterial endothelial cells. Biological and Pharmaceutical Bulletin, 24, 754–759.

Hill K.E., Xia Y., Akesson B., Boeglin M.E., Burk R.F. (1996): Selenoprotein P concentration in plasma is an index of selenium status in selenium-deficient and selenium-supplemented Chinese subjects. Journal of Nutrition, 126, 138–145.

Hill K.E., McCollum G.W., Burk R.F. (1997): Determination of thioredoxin reductase activity in rat liver supernatant. Analytical Biochemistry, 253, 123–125.

Hu B., Mitra J., van den Heuvel S., Enders G.H. (2001): S and G2 phase roles for Cdk2 revealed by inducible expression of a dominant-negative mutant in human cells. Molecular and Cellular Biology, 21, 2755–2766.

Kipp A., Banning A., van Schothorst E.M., Meplan C., Schomburg L., Evelo C., Coort S., Gaj S., Keijer K., Hesketh J., Brigelius-Flohe R. (2009): Four selenoproteins, protein biosynthesis, and Wnt signalling are particularly sensitive to limited selenium intake in mouse colon. Molecular Nutrition and Food Research, 53, 1561–1572.

Miranda S.G., Purdie N.G., Osborne V.R., Coomber B.L., Cant J.P. (2011): Selenomethionine increases proliferation and reduces apoptosis in bovine mammary epithelial cells under oxidative stress. Journal of Dairy Science, 94, 165–173.

Moriarty P.M., Reddy C.C., Maquat L.E. (1998): Selenium deficiency reduces the abundance of mRNA for Se-dependent glutathione peroxidase 1 by a UGA-dependent mechanism likely to be nonsense codon-mediated decay of cytoplasmic mRNA. Molecular and Cellular Biology, 18, 2932–2939.

Mosca L., Marcellini S., Perluigi M., Mastroiacovo P., Moretti S., Famularo G., Peluso I., Santini G., De Simone C. (2002): Modulation of apoptosis and improved redox metabolism with the use of a new antioxidant formula. Biochemical Pharmacology, 63, 1305–1314.

Muller A.S., Pallauf J. (2002): Down-regulation of GPx1 mRNA and the loss of GPx1 activity causes cellular damage in the liver of selenium-deficient rabbits. Journal of Animal Physiology and Animal Nutrition, 86, 273–287.

Murano K., Ogino H., Okuno T., Arakawa T., Ueno H. (2018): Role of supplementary selenium on the induction of insulin resistance and oxidative stress in NSY mice fed a high fat diet. Biological and Pharmaceutical Bulletin, 41, 92–98.

Nagy E., Maquat L.E. (1998): A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. Trends in Biochemical Sciences, 23, 198–199.

Ohkawa H., Ohishi N., Yagi K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95, 351–358.

Pagmantidis V., Bermano G., Villette S., Broom I., Arthur J., Hesketh J. (2005): Effects of Se-depletion on glutathione peroxidase and selenoprotein W gene expression in the colon. FEBS Letters, 579, 792–796.

Robinson T.L., Sutherland I.A., Sutherland J. (2007): Validation of candidate bovine reference genes for use with real-time PCR. Veterinary Immunology and Immunopathology, 115, 160–165.

Sordillo L.M. (2013): Selenium-dependent regulation of oxidative stress and immunity in periparturient dairy cattle. Veterinary Medicine International, 4, 1–8.

https://doi.org/10.17221/24/2018-CJAS

Sordillo L.M., Aitken S.L. (2009): Impact of oxidative stress on the health and immune function of dairy cattle. Veterinary Immunology and Immunopathology, 128, 104–109.

Steinbrenner H., Bilgic E., Alili L., Sies H., Brenneisen P. (2006): Selenoprotein P protects endothelial cells from oxidative damage by stimulation of glutathione peroxidase expression and activity. Free Radical Research, 40, 936–943.

Stupin A., Cosic A., Novak S., Vesel M., Jukic I., Popovic B., Karalic K., Loncaric Z., Drenjancevic I. (2017): Reduced dietary selenium impairs vascular function by increasing oxidative stress in Sprague-Dawley rat aortas. International Journal of Environmental Research and Public Health, 14: 591.

Sun Z., Liu C., Pan T., Yao H., Li S. (2017): Selenium accelerates chicken dendritic cells differentiation and affects selenoproteins expression. Developmental and Comparative Immunology, 77: 30.

Sunde R.A., Raines A.M., Barnes K.M., Evenson J.K. (2009): Selenium status highly regulates selenoprotein mRNA levels for only a subset of the selenoproteins in the selenoproteome. Bioscience Reports, 29, 329–338.

Yeo J.E., Kang S.K. (2007): Selenium effectively inhibits ROS-mediated apoptotic neural precursor cell death in vitro and in vivo in traumatic brain injury. Biochimica et Biophysica Acta, 1772, 1199–1210.

Zeng H. (2002): Selenite and selenomethionine promote HL-60 cell cycle progression. Journal of Nutrition, 132, 674–679.

Zeng H. (2009): Selenium as an essential micronutrient: roles in cell cycle and apoptosis. Molecules, 14, 1263–1278.

Zhang Z., Guo Y., Qiu C., Deng G., Guo M. (2017): Protective action of Se-supplement against acute alcoholism is regulated by selenoprotein P (SelP) in the liver. Biological Trace Element Research, 175, 1–13.

Received: 2018–02–27
Accepted after corrections: 2018–06–04