Hydroxy-selenomethionine: A novel organic selenium source that improves antioxidant status and selenium concentrations in milk and plasma of mid-lactation dairy cows

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ABSTRACT

This study aimed to evaluate the effect of hydroxy-selenomethionine (HMSeBA), a novel organic selenium (Se) source, on milk performance, antioxidative status, and Se concentrations in the milk and plasma of mid-lactation dairy cows compared with that of sodium selenite (SS). Fifty mid-lactation dairy cows with similar days in milk, milk yield, and parity received the same basal diet containing 0.06 mg of Se/kg of DM. They were assigned to 1 of 5 treatments according to a randomized complete block design: negative control (without Se supplementation), SS supplementation (0.3 mg of Se/kg of DM; SS-0.3) or HMSeBA supplementation (0.1, 0.3, or 0.5 mg of Se/kg of DM: SO-0.1, SO-0.3, and SO-0.5, respectively). The experiment lasted for 10 wk, including a pretrial period of 2 wk. The results indicated that neither Se supplementation nor Se source affected dry matter intake, milk yield, milk composition, or blood biochemical parameters, except for milk fat percentage. Simultaneously, milk fat percentage and milk fat yield increased linearly as the quantity of HMSeBA supplementation was increased. Production of 4% FCM and ECM was elevated linearly as dietary HMSeBA increased. The SO-0.3 group showed higher serum activity of glutathione peroxidase, total antioxidant capacity, and superoxide dismutase than the SS-0.3 group, but malondialdehyde content was not affected by Se source. Furthermore, HMSeBA supplementation linearly increased the activities of serum glutathione peroxidase and superoxide dismutase,

but decreased malondialdehyde content. Compared with the SS-0.3 group, the SO-0.3 group showed augmented concentrations of total Se in milk and plasma, and total Se milk-to-plasma concentration ratio. In addition, increasing doses of HMSeBA linearly increased the concentrations of total Se in the milk and plasma. This study demonstrates that HMSeBA improves antioxidant status and increases milk and plasma Se concentrations more effectively than SS, indicating that HMSeBA could replace SS as an effective organic Se source for lactating dairy cows.

Key words: hydroxy-selenomethionine, dairy cow, antioxidant status, milk and plasma Se

INTRODUCTION

Reactive oxygen species (**ROS**) are produced during normal cellular respiration, in which energy is generated to meet cellular needs (Prasad, 2014). The ROS generated are neutralized by antioxidant defense systems to maintain normal physiological status (redox homeostasis). When the production of ROS overwhelms the capacity of these systems, oxidative stress develops. Overproduction and accumulation of ROS predisposes animals to metabolic diseases (Gong and Xiao, 2016); therefore, antioxidant status must be improved to remedy the problems associated with oxidative stress. During dairy cow lactation, when a large amount of energy is required for rapid development of the mammary gland and for synthesis of large quantities of milk constituents, dairy cows are usually exposed to oxidative stress (Gong and Xiao, 2016). The antioxidant capacity of dairy cows declines during early lactation, and therefore the risk of oxidative stress increases (Castillo et al., 2005).

Selenium is a trace element that is necessary to maintain antioxidant status in humans and animals and

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which often requires supplementation in the diet of production animals (Schwarz and Moltz, 1957). Previous studies show that providing sufficient Se in the diet of dairy cows improves their antioxidant status, increases the activity of glutathione peroxidase (**GSH-Px**), decreases the malondialdehyde (**MDA**) concentration in plasma, and reduces SCC in milk (Stewart et al., 2012). Due to the contrasting soil Se levels in different parts of China, feed produced in areas deficient in Se does not fulfill the dietary requirement for Se (Zheng et al., 1982). Therefore, supplementation of the diet of dairy cows with Se is important to avoid deficiency.

Both inorganic and organic forms of Se are common in dietary supplements on dairy farms. Because of their low prices, inorganic forms of Se [sodium selenite (**SS**) and sodium selenate] are widely used. However, compared with inorganic Se, organic Se has a higher absorption rate (Boldizarova et al., 2005), greater biological activity (Ortman and Pehrson, 1999), a higher rate of tissue accumulation (Briens et al., 2014), and lower toxicity. Organic and inorganic forms of Se are metabolized differently in the body (Calamari et al., 2010). Selenium yeast (**SY**) improves antioxidant status (Gong et al., 2014), increases whole blood and milk Se concentrations (Slavik et al., 2008), and improves the Se status of ruminants more effectively than **SS** (Doucha et al., 2009). Calamari et al. (2010) concluded that a greater proportion of Se reached the milk from the circulation when **SY** was provided to cows, rather than **SS**.

Hydroxy-selenomethionine (**HMSeBA**), namely Selenio (**SO**, Adisseo France S. A. S., Antony, France), is an organic bioavailable source of Se for all animals, which can be added to feed premixes (Briens et al., 2013). In contrast to selenomethionine, which is the predominant form of Se in **SY** (Korhola et al., 1986), **HMSeBA** is synthetic R,S-2-hydroxy-4-methylselenobutanoic acid, possessing a hydroxyl group instead of an amino group on its second carbon atom. The European Commission has approved the use of **HMSeBA** for animals as a feed additive (EFSA, 2013a). To date, the benefits of **HMSeBA** have been described in monogastric animals, such as broilers (Briens et al., 2013, 2014), layers (Jlali et al., 2013), and pigs (Jlali et al., 2014), but not in ruminants. We hypothesized that the use of **HMSeBA** as a feed additive may be effective as a novel source of Se for dairy cows. Therefore, the objectives of the present study were to evaluate the effects of different sources of Se and different doses of **HMSeBA** on milk production, biochemical parameters, and serum antioxidant status, as well as Se concentrations, in the milk and plasma of dairy cows.

MATERIALS AND METHODS

This study was conducted at Beijing Sino Farm (Beijing, China). All procedures were conducted using protocols approved by the Institute of Animal Science, Chinese Academy of Agricultural Sciences (Beijing, China). All animals used in the current study were raised according to standards established by the Institute of Animal Science, Chinese Academy of Agricultural Sciences (Beijing, China). All cows were housed in a freestall barn with free access to fresh water. Total mixed ration was fed ad libitum to all cows. Before the commencement of the feeding trial and throughout the experimental period, the health condition of the cows was monitored and recorded.

Sources of Se

Two types of Se were used in this study. The **HMSeBA** was provided by Adisseo Life Science Co. Ltd. (Shanghai, China). The minimum contents of Se and **HMSeBA** were 2.0 and 5.0%, respectively. The **SS** was provided by Henan XiuCang Chemical Products Co. Ltd. (Henan Province, China).

Cows, Diets and Experimental Design

The current study was performed as a randomized complete block design. Fifty multiparous Holstein cows ($\text{DIM} = 153 \pm 18$; milk yield = 28.9 ± 1.5 kg/d; and parity = 2.4 ± 1.1) were assigned to 1 of 5 treatments ($n = 10$), which were balanced for DIM, milk yield, and parity. All cows received the same TMR without Se supplementation. The background Se concentration in the TMR was 0.06 mg/kg on a DM basis. The dietary supplements differed only in the source and amount of Se they contained, specifically negative control (without Se supplementation), **SS** supplementation (0.3 mg of Se/kg of DM; **SS-0.3**), or **HMSeBA** supplementation (0.1, 0.3, and 0.5 mg of Se/kg of DM: **SO-0.1**, **SO-0.3**, and **SO-0.5**, respectively).

The TMR (Table 1) without Se supplementation (Beijing Sanyuan Seed Technology Co. Ltd., Beijing, China) was formulated to meet nutrient requirements according to NRC (2001). A computerized monitoring system (RIC system, Insentec B.V., Marknesse, the Netherlands) was used as described previously (Zhou et al., 2015). Each cow had an electronic ear tag that gave access to a single feed tank, and the RIC system recorded its daily feed intake. Feed was provided 3 times a day, after each milking, at 0730, 1400, and 2030 h, with a 5 to 10% refusal rate. The refusals were removed every morning. The **HMSeBA** and **SS** were

weighted and separately packed for each cow before the start of the experiment. The appropriate quantity of HMSeBA or SS was mixed with 100 g of ground corn during milking and fed with the TMR during the morning feed. The pretrial period was 2 wk and the experimental period lasted 8 wk.

Sampling and Analysis

Samples of feed offered and refused by each cow were collected consecutively on 3 d per week during the treatment period to measure DM content. Individual daily feed intake was calculated by subtracting the refusals

from the total fresh TMR offered, on a DM basis. The samples of feed were then combined, oven-dried at 65°C for 48 h, and ground in a fodder grinder using a 1-mm screen. Dry matter content was measured by drying at 105°C for 4 h to a constant weight, and CP and ether extract were measured using standard procedures. The NDF and ADF contents were determined using the procedures of Van Soest et al. (1991). The composition and nutrient levels of TMR are presented in Table 1.

Cows were milked daily at 0700, 1330, and 2000 h, and individual milk production was recorded at each milking. Milk samples from each cow were collected every week during the treatment period at 3 consecutive milkings, and combined in the proportion 4:3:3 (Sun et al., 2011). Preservative (Bronopol tablet; D&F Control System Inc., Dublin, ON, Canada) was added to 1 subsample, and this was stored at 4°C for the subsequent analysis of fat, protein, lactose, and TS using infrared analysis (Foss MilkoScan 2000, Foss Food Technology Corp., Eden Prairie, MN). Another milk subsample, which was obtained at the end of the experiment, was frozen and stored at -20°C for the determination of Se concentration.

Blood samples were collected from the coccygeal vein of all cows using vacutainer tubes (BD Biosciences, San Jose, CA) at the end of the experiment, before the morning feeding. Plasma and serum were separated from vacutainer tubes containing heparin anticoagulant and no coagulant by centrifugation at $3,000 \times g$ for 15 min at 4°C. Serum was stored at -20°C for the subsequent determination of biochemical parameters, including aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), alkaline phosphatase (**ALP**), cholinesterase (**CHE**), creatine kinase (**CK**), creatinine (**CR**), glucose (**GLU**), and urea, using an automated biochemical analyzer (HITACHI 7080, Hitachi Valve Ltd., Tokyo, Japan). The intra- and interassay coefficients of variation for AST, ALT, ALP, CHE, CK, CR, GLU, and urea were 2.52 and 3.66, 1.91 and 2.95, 3.52 and 4.01, 1.27 and 1.99, 2.64 and 3.43, 1.88 and 2.11, 1.44 and 1.85, and 1.56 and 2.01%, respectively.

Indexes reflecting antioxidant capacity were also measured in the serum, including total antioxidant capacity (**T-AOC**), GSH-Px, superoxide dismutase (**SOD**), and MDA, using the commercial assay kits (Nanjing Jian Cheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. For the estimation of T-AOC, the ferric-reducing antioxidant power assay (**FRAP**) was used, as previously described (Emin et al., 2010; Momeni and Eskandari, 2017) with some modifications. The principle of FRAP is based on the reduction of Fe^{3+} to Fe^{2+} , which can combine with 2,4,6-tri(2-pyridyl)-1,3,5-triazine (**TPTZ**) to form a colored Fe^{2+} -TPTZ complex. The maximum absorption

Table 1. Ingredients and chemical composition of the basal diet (% as-fed DM, unless noted)

Item	%
Ingredient	
Alfalfa hay	12.54
Oat hay	4.21
Corn silage	18.85
Ground corn	7.35
Soybean meal	9.71
Rapeseed meal	1.76
Extruded soybeans	2.40
Distillers dried grains with solubles	1.81
Flaked corn	18.09
Soybean hull	6.81
Whole cottonseed	8.02
CMS 1404163 ¹	1.33
Salt	0.39
Calcium carbonate 38%	1.06
Sodium bicarbonate	1.41
Magnesium oxide	0.24
Potassium bicarbonate	0.92
Dicalcium phosphate	0.05
Zeolite ²	0.05
Fat ³	1.79
Yeast culture ⁴	0.63
Premix ⁵	0.58
Nutrient composition ⁶	
Ether extract	6.08
CP	17.05
NDF	28.67
ADF	19.51
Ca	0.87
P	0.49
Ash	8.42
NE _L ⁷ (mcals/kg)	1.71

¹A syrup supplement (Vedan, Yantai, Shandong Province, China).

²Zeolite, Xinyang Huaiye Zeolite Powder Co., Ltd., Xinyang, Henan Province, China.

³Bergafat, a saturated free fatty acid supplement (Berg + Schmidt, Hamburg, Germany).

⁴Diamond V XP yeast culture supplement (Diamond V, Cedar Rapids, IA).

⁵Contained (DM basis) 250,000 IU/kg of vitamin A; 65,000 IU/kg of vitamin D; 2,100 IU/kg of vitamin E; 400 mg/kg of Fe; 540 mg/kg of Cu; 2,100 mg/kg of Zn; 560 mg/kg of Mn; 35 mg/kg of I; and 68 mg/kg of Co.

⁶Analyzed value.

⁷Calculated value (based on China NY/t 34, 2004).

of the complex was 593 nm. Briefly, 100 μ L of serum was diluted with distilled water (vol/vol, 1:1) and then mixed with 900 μ L of freshly prepared and pretempered FRAP working agent, which contained acetate buffer, TPTZ, and FeCl_3 (Emin et al., 2010). The mixture was incubated for 10 min at 37°C. Absorbance was measured using a spectrophotometer (MD, Spectramax M3, Beckman Co., Fullerton, CA) at 593 nm and compared with a blank mixture, in which 100 μ L of distilled water had been added to the FRAP working reagent instead of serum. The absorbance of other antioxidant indexes was also measured spectrophotometrically at various wavelengths, as described by Gong et al. (2014). The intra- and interassay coefficients of variation for T-AOC, GSH-Px, SOD, and MDA were 3.14 and 4.33, 2.42 and 3.59, 2.04 and 3.27, and 2.11 and 3.89%, respectively. Plasma was also stored at -20°C for the analysis of Se concentration.

Analysis of Se Content of Plasma and Milk

The concentrations of total Se in milk and plasma were assayed as described by Heard et al. (2007), with some modifications. Briefly, samples of milk (1.00 g) or plasma (0.50 g) were digested in a mixture of nitric acid and perchloric acids (vol/vol, 10:1, 10 mL) in an electrically heated digestion block, followed by the addition of hydrochloric acid (2 mL). Thereafter, inductively coupled plasma mass spectrometry/vapor generation (Agilent 8800, Agilent Technologies, Santa Clara, CA) was used to assay total Se. The analyses were run in duplicate and the result is stated as the average of the 2 replicates and the confidence interval to twice the standard deviation between the replicates.

Statistical Analysis

All data were analyzed using the MIXED procedure in SAS software (version 9.3, SAS Institute Inc., Cary, NC). The repeated measures model contained fixed effects of week ($df = 7$), treatment ($df = 4$), and the interaction of treatment and week ($df = 28$) and the random effect of cow ($df = 45$). Contrasts were constructed to examine treatment effects, HMSeBA supplementation level, with orthogonal polynomials accounting for unequal spacing of SO dosage levels, and Se source (SO-0.3 vs. SS-0.3). Tukey's multiple range test was used for the evaluation of differences between the groups. Degrees of freedom were determined according to the Kenward-Roger method (Littell et al., 1998). Data are presented as the least squares means and standard error of the mean. Statistical significance was set at $P < 0.05$, and trends are reported when $0.05 \leq P < 0.10$.

RESULTS

Performance of the Cows

The results of Se supplementation on DMI, milk production, and milk composition are presented in Table 2. Because samples of feed and milk were collected every week during the treatment period, the variables in Table 2 were tested for treatment, week, and treatment $\times$ week interactions. Although the week of sampling had a significant effect on milk yield, 4% FCM, ECM, percentage of milk protein, fat, lactose, and TS, treatment and treatment $\times$ week interaction were not significant for all variables (except for milk fat percentage). As shown in Table 2, neither the level of Se supplementation nor the Se source affected DMI, lactation performance, and milk composition, except for the percentage of milk fat ($P < 0.05$) and a trend toward an effect on milk fat yield ($P = 0.08$). Simultaneously, milk fat percentage and milk fat yield both increased linearly as the level of supplementation with HMSeBA increased ($P = 0.01$). Furthermore, the production of 4% FCM and ECM increased linearly with increasing amounts of HMSeBA in the diet ($P < 0.05$).

Biochemical Parameters and Antioxidant Indicators in the Serum

The effects of dietary supplementation with different sources of Se on serum biochemical parameters and antioxidant indicators are shown in Table 3 and Table 4. Because blood samples were obtained only at the end of the trial, we found no significant effects of week or the treatment $\times$ week interaction for any variable. The concentrations of serum biochemical parameters were not affected by Se supplementation or Se source. As shown in Table 4, cows supplemented with HMSeBA had higher GSH-Px and SOD activities than did cows in the control and SS-0.3 groups ($P < 0.01$). Furthermore, T-AOC was highest in the SO-0.3 group and the content of MDA was lowest in the SO-0.5 group ($P < 0.01$). Compared with the SS-0.3 group, the SO-0.3 group showed higher serum GSH-Px, T-AOC, and SOD activity ($P < 0.05$); however, MDA content was not affected by Se source. In addition, a positive effect of supplementation level was observed on serum GSH-Px and SOD activity ($P < 0.01$), whereas a negative effect was detected on serum MDA content ($P < 0.01$) as the level of HMSeBA supplementation was increased.

Total Se Concentrations in the Milk and Plasma

The effects of dietary supplementation with different sources of Se on Se concentrations in milk and plasma

Table 2. Dry matter intake, milk production, and milk composition of dairy cows fed different sources of Se

Item	Treatment mean ¹					P-value ³				
	Control (n = 10)	SS-0.3 (n = 10)	SO-0.1 (n = 10)	SO-0.3 (n = 10)	SO-0.5 (n = 10)	SEM ²	Treatment ⁴	Source ⁵	SO level ⁶	
									Linear	Quadratic
DMI, kg/d	19.1	19.2	19.5	20.2	20.3	0.250	0.71	0.70	0.25	0.59
Milk yield, kg/d	28.36	29.97	29.40	30.69	30.79	1.736	0.85	0.78	0.28	0.67
4% FCM, kg/d	25.77	28.11	28.34	29.85	31.72	1.678	0.17	0.48	0.02	0.66
ECM, kg/d	28.68	31.14	31.25	32.84	34.37	1.835	0.27	0.52	0.03	0.62
Fat yield, kg/d	0.99	1.06	1.16	1.17	1.29	0.076	0.08	0.32	0.01	0.66
Protein yield, kg/d	0.92	0.95	0.99	1.00	0.99	0.046	0.68	0.44	0.35	0.36
Lactose yield, kg/d	1.40	1.44	1.50	1.53	1.52	0.069	0.65	0.36	0.27	0.39
TS yield, kg/d	3.44	3.58	3.79	3.75	3.95	0.176	0.31	0.52	0.09	0.69
Fat, %	3.41 ^b	3.46 ^{ab}	3.87 ^{ab}	3.88 ^{ab}	4.20 ^a	0.194	0.04	0.14	0.01	0.69
Protein, %	3.21	3.18	3.28	3.26	3.24	0.066	0.82	0.40	0.94	0.54
Lactose, %	4.93	4.89	4.91	4.98	4.92	0.048	0.75	0.19	0.89	0.55
TS, %	12.00	11.96	12.52	12.27	12.85	0.241	0.07	0.37	0.05	0.80

^{a,b}Mean values within treatment with different superscripts are significantly different ($P < 0.05$).

¹Treatment mean: control = 0 mg of Se/kg of DM (without Se supplementation); SS-0.3 = 0.3 mg of Se/kg of DM (sodium selenite supplementation); SO-0.1 = 0.1 mg of Se/kg of DM; SO-0.3 = 0.3 mg of Se/kg of DM; SO-0.5 = 0.5 mg of Se/kg of DM (hydroxy-analog of selenomethionine supplementation).

²For comparison between the 5 treatments.

³The statistical model contained fixed effects of week, treatment, and the interaction of week and treatment. Week was significant for milk yield, 4% FCM, ECM, percentage of milk protein, fat, lactose, and TS. Treatment $\times$ week was not significant for all variables.

⁴Treatment: includes control, SS-0.3, SO-0.1, SO-0.3, and SO-0.5, representing the difference between the overall treatments.

⁵Source: a contrast comparing SS-0.3 and SO-0.3.

⁶SO level: orthogonal polynomials for response due to SO (0, 0.1, 0.3 and 0.5).

are summarized in Table 5. The concentrations of total Se in milk and plasma in the SO-0.3 and SO-0.5 groups were greater than those in the other 3 groups ($P < 0.01$). The SO-0.3 group showed higher total Se levels in milk and plasma than did the SS-0.3 group ($P < 0.01$). Furthermore, the total Se milk-to-plasma concentration ratio was higher in the SO-0.3 group than in the SS-0.3 group ($P < 0.01$). In addition, as HMSeBA supplementation level increased, total Se concentrations in plasma and milk increased linearly ($P < 0.01$).

DISCUSSION

Selenium yeast is an organic Se feed supplementation that has been widely used for a long time and provides higher bioavailability in several animal species than SS (Boldizarova et al., 2005; Ran et al., 2010; Cao et al., 2014). However, although HMSeBA has been shown to improve the relative bioavailability of Se compared with SY in broiler chickens (Briens et al., 2013, 2014), little research has been conducted regarding the effects of HMSeBA on dairy cow production. Therefore, in the present study, we compared the bioavailability of HMSeBA and SS in mid-lactation dairy cows.

Many previous studies show that oral administration of various quantities of organic or inorganic Se does not affect milk yield and milk composition (Juniper et al., 2006; Calamari et al., 2010; Gong et al., 2014).

Calamari et al. (2010) suggested that neither the source of Se (SS or SY) nor its level of supplementation (0.31 or 0.50 mg of Se/kg of DM) affected milk yield and milk characteristics when dairy cows consumed the same basal diet containing 0.1 mg of Se/kg of DM. Similar to these previous findings, the DMI and milk yield of dairy cows were not affected by the inclusion of different Se sources (SS or HMSeBA) or the level of HMSeBA supplementation (0.1, 0.3, or 0.5 mg of Se/kg of DM) in the present study. No differences were observed in milk composition, except for milk fat content and a trend toward an effect on milk fat yield, which were dependent on the level of supplementation with HMSeBA. The reason for the increase in milk fat percentage might be related to the antioxidant function of Se, because previous studies showed that vitamin E could reduce the severity of diet-induced milk fat suppression and increase milk fat percentage (Kay et al., 2005). However, the detailed mechanism whereby milk fat percentage is affected by HMSeBA supplementation is still unclear and requires further exploration.

Publications to date have shown that Se supplementation has little effects on blood chemistry (Juniper et al., 2006; Phipps et al., 2008). In agreement with the published literature, dietary supplementation with Se did not affect serum biochemical indices in the present study. Only AST activity tended to rise linearly as the level of supplementation with HMSeBA increased, but

Table 3. Biochemical parameters in dairy cows fed different sources of Se

Item	Treatment mean ¹					P-value			
	Control (n = 10)	SS-0.3 (n = 10)	SO-0.1 (n = 10)	SO-0.3 (n = 10)	SO-0.5 (n = 10)	SEM ²	Treatment ³	Source ⁴	SO level ⁵
Aspartate aminotransferase, U/L	82.33	91.88	89.44	103.33	98.63	7.531	0.32	0.29	Linear 0.08 Quadratic 0.25
Alanine aminotransferase, U/L	25.33	27.75	27.33	24.44	24.63	1.301	0.26	0.08	Linear 0.32 Quadratic 0.83
Alkaline phosphatase, U/L	59.68	61.44	54.02	51.73	52.26	4.640	0.47	0.15	Linear 0.29 Quadratic 0.45
Cholinesterase, U/L	145.22	135.38	144.33	137.44	134.63	11.050	0.93	0.90	Linear 0.43 Quadratic 0.96
Creatine kinase, U/L	155.01	144.69	148.92	137.02	140.23	10.213	0.74	0.60	Linear 0.25 Quadratic 0.49
Creatinine, µmol/L	59.67	64.00	61.56	64.78	63.88	2.224	0.46	0.81	Linear 0.14 Quadratic 0.36
Glucose, mmol/L	3.08	3.13	3.13	3.05	3.08	0.076	0.94	0.49	Linear 0.81 Quadratic 0.97
Urea, mmol/L	4.70	4.78	4.66	4.78	4.68	0.163	0.97	0.99	Linear 0.94 Quadratic 0.73

¹Treatment mean: control = 0 mg of Se/kg of DM (without Se supplementation); SS-0.3 = 0.3 mg of Se/kg of DM (sodium selenite supplementation); SO-0.1 = 0.1 mg of Se/kg of DM; SO-0.3 = 0.3 mg of Se/kg of DM; SO-0.5 = 0.5 mg of Se/kg of DM (hydroxy-analog of selenomethionine supplementation).

²For comparison between the 5 treatments.

³Treatment: includes control, SS-0.3, SO-0.1, SO-0.3, and SO-0.5, representing the difference between the overall treatments.

⁴Source: a contrast comparing SS-0.3 and SO-0.3.

⁵SO level: orthogonal polynomials for response due to SO (0, 0.1, 0.3 and 0.5).

Table 4. Antioxidant indexes of dairy cows fed different sources of Se

Item	Treatment mean ¹					P-value			
	Control (n = 10)	SS-0.3 (n = 10)	SO-0.1 (n = 10)	SO-0.3 (n = 10)	SO-0.5 (n = 10)	SEM ²	Treatment ³	Source ⁴	SO level ⁵
Glutathione peroxidase, U/mL	107.87 ^c	113.60 ^c	145.17 ^b	165.92 ^a	178.41 ^a	4.290	<0.01	<0.01	<0.01
Total antioxidant capacity, U/mL	2.07 ^b	2.09 ^b	2.19 ^b	2.50 ^a	2.13 ^b	0.069	<0.01	<0.01	<0.01
Superoxide dismutase, U/mL	104.39 ^b	117.55 ^b	133.67 ^a	140.44 ^a	136.50 ^a	3.947	<0.01	<0.01	<0.01
Malondialdehyde, nmol/mL	6.22 ^a	6.30 ^a	6.00 ^a	5.71 ^a	4.57 ^b	0.237	<0.01	0.09	<0.01 0.13

^{a-c}Mean values within treatment with different superscripts are significantly different ($P < 0.05$).

¹Treatment mean: control = 0 mg of Se/kg of DM (without Se supplementation); SS-0.3 = 0.3 mg of Se/kg of DM (sodium selenite supplementation); SO-0.1 = 0.1 mg of Se/kg of DM; SO-0.3 = 0.3 mg of Se/kg of DM; SO-0.5 = 0.5 mg of Se/kg of DM (hydroxy-analog of selenomethionine supplementation).

²For comparison between the 5 treatments.

³Treatment: includes control, SS-0.3, SO-0.1, SO-0.3, and SO-0.5, representing the difference between the overall treatments.

⁴Source: a contrast comparing SS-0.3 and SO-0.3.

⁵SO level: orthogonal polynomials for response due to SO (0, 0.1, 0.3 and 0.5).

Table 5. Forms and concentrations of Se in milk and plasma of dairy cows fed different sources of Se

Item	Treatment mean ¹					SEM ²	P-value			
	Control (n = 10)	SS-0.3 (n = 10)	SO-0.1 (n = 10)	SO-0.3 (n = 10)	SO-0.5 (n = 10)		Treatment ³	Source ⁴	SO level ⁵	
									Linear	Quadratic
Milk										
Total Se, µg/kg	17.53 ^c	22.60 ^c	27.95 ^c	50.95 ^b	65.99 ^a	3.168	<0.01	<0.01	<0.01	0.33
Plasma										
Total Se, µg/kg	70.91 ^c	82.87 ^c	83.88 ^c	101.75 ^b	117.18 ^a	2.956	<0.01	<0.01	<0.01	0.30
Milk/plasma of total Se, %										
Ratio	24.79 ^b	27.41 ^b	33.56 ^b	49.39 ^a	56.29 ^a	2.552	<0.01	<0.01	<0.01	0.08

^{a-c}Mean values within treatment with different superscripts are significantly different ($P < 0.05$).

¹Treatment mean: control = 0 mg of Se/kg of DM (without Se supplementation); SS-0.3 = 0.3 mg of Se/kg of DM (sodium selenite supplementation); SO-0.1 = 0.1 mg of Se/kg of DM; SO-0.3 = 0.3 mg of Se/kg of DM; SO-0.5 = 0.5 mg of Se/kg of DM (hydroxy-analog of selenomethionine supplementation).

²For comparison between the 5 treatments.

³Treatment: includes control, SS-0.3, SO-0.1, SO-0.3, and SO-0.5, representing the difference between the overall treatments.

⁴Source: a contrast comparing SS-0.3 and SO-0.3.

⁵SO level: orthogonal polynomials for response due to SO (0, 0.1, 0.3, and 0.5).

the values remained within the normal range (Beers and Berkow, 1998).

Glutathione peroxidase is an enzyme that contains Se as selenocysteine in its catalytic site (Rotruck et al., 1973) and which catalyzes hydroperoxide detoxification, metabolizing hydrogen peroxide and lipid peroxides to water and harmless oxy compounds (Xu et al., 2007). Selenium status is usually evaluated directly by determining the concentration of Se, or indirectly by measuring the activity of GSH-Px in blood and tissues (Gong and Xiao, 2016). Whereas whole blood or erythrocyte GSH-Px reflects long-term Se status, serum or plasma GSH-Px reflects short-term Se status (Rowntree et al., 2004), and blood Se concentration is a good indicator of blood GSH-Px activity (Knowles et al., 1999). The present study showed that HMSeBA supplementation increases the activity of serum GSH-Px compared with SS. Similar results were also reported by Gong et al. (2014), who compared the effects of the same level of supplementation with SS and SY (0.3 mg/kg of DM) on the antioxidant status of dairy cows fed basal diets containing Se at 0.02 mg/kg of DM, and found that organic Se increased serum GSH-Px activity more efficiently than SS.

Glutathione peroxidase functions as a Se-dependent antioxidant enzyme, which can prevent the harmful effects of ROS accumulation (Gong and Xiao, 2016). The present study showed that HMSeBA is more effective at supporting antioxidant defense systems than SS. Furthermore, increasing levels of HMSeBA supplementation were associated with linear enhancements in serum GSH-Px activity, but with similar levels being achieved when 0.3 and 0.5 mg of Se/kg of DM were included. Thus, a dose effect existed when Se was supplemented

at a relatively low level and serum GSH-Px activity was not further affected when the level of supplementation rose above a threshold (Juniper et al., 2006).

Total antioxidant capacity is an indicator of the capacity for resistance to oxidative stress in the body (Cao et al., 2014), whereas MDA is one of the end-products of lipid peroxidation and a useful marker of oxidative stress (Gawel et al., 2004) and SOD is considered to be an important marker that reflects the antioxidant status of animals (Gong et al., 2014). In the present study, HMSeBA supplementation increased the activities of T-AOC and SOD compared with SS at 0.3 mg of Se/kg of DM. Superoxide dismutase activity rose but MDA content declined linearly as the supplementation level of HMSeBA increased. Therefore, supplementation with HMSeBA improved antioxidant activity in dairy cows more than SS. These results corroborate the findings of Gong et al. (2014), who found that SY was more effective at improving antioxidant status than SS.

Selenium status is an important indicator of animal health, and this is commonly assessed by measuring Se concentration in whole blood or serum and plasma (Pavlata et al., 2000). Some previous studies suggest that Se status is adequate if its serum concentration in dairy cows is in the range of 70 to 79 µg/L (Gong and Xiao, 2016). Cows fed the basal diet in the current study had a plasma Se concentration of 70.91 µg/kg, implying that they had achieved the required minimum level for Se. Compared with cows that received SS, total plasma Se concentrations in the cows supplemented with HMSeBA (SO-0.3) were 23% higher, which supports the general view that organic Se is more effective than inorganic Se at increasing blood Se concentrations (Givens et al., 2004; Juniper et al., 2006). The differ-

ences in total plasma Se concentration achieved following HMSeBA (SO-0.3) and SS supplementation may be because organic Se is more easily absorbed from the intestine. The European Food Safety Authority has validated that the use of HMSeBA would lead to similar to greater Se deposition in animal tissues and products (EFSA, 2013a). Furthermore, we observed a linear effect of supplementation level.

Compared with SS, HMSeBA (SO-0.3) supplementation increased the concentration of Se in milk, which agrees with most published findings (Ortman and Pehrson, 1999; Phipps et al., 2008; Gong et al., 2014). Moreover, milk Se concentration was approximately 125% higher in cows supplemented with HMSeBA (SO-0.3) than SS. Ran et al. (2010) reported that dietary supplementation with 5 mg of Se as SY resulted in 53% higher milk Se than supplementation with the same amount of SS on d 90 after calving when the cows consumed 0.2 mg of Se/kg of DM in their basal diet. These results indicate that HMSeBA, a novel organic source of Se, was more effective in increasing milk Se concentration in cows than SS. Variation in total milk Se concentrations was similar to that in the plasma, which supports the notion that milk Se originates from plasma Se. The milk-to-plasma Se concentration ratio in cows supplemented with HMSeBA was also higher than in those fed SS, implying greater transfer of Se from blood to milk after HMSeBA supplementation. This greater transfer might be due to different metabolic pathways being used for different forms of Se (Fairweather-Tait et al., 2010). Fairweather-Tait et al. (2010) demonstrated that Se as selenate or selenite is well absorbed, but less well retained in the body than organic Se, which might be reflected by the higher plasma and milk Se concentrations but similar fecal and urinary Se concentration (data not shown) despite the same intake of HMSeBA and SS in the present study. Juniper et al. (2006) assayed Se levels in the blood, milk, urine, and feces of cows that had been fed similar amounts of SY (0.27 mg of Se/kg of DM) and SS (0.25 mg of Se/kg of DM). They found similar Se concentrations in the urine and feces, but higher blood and milk Se concentrations in dairy cows fed with SY versus those fed with SS, implying that SY provided superior Se availability to SS.

According to the Referenced Dietary Nutrient Intake of Chinese people published by the Nutrition Council of China in 2000 (Ran et al., 2010), the recommended upper intake level of Se is 360 µg/d for people over 14 yr of age. Five hundred grams of milk from cows in the SO-0.3 and SO-0.5 groups in our study would provide approximately 25.48 and 33.00 µg of Se, which is below the required intake for people over 14 yr of age (50 µg/d; Ran et al., 2010). This implies that the concentration of Se achieved in the milk of cows fed

HMSeBA at 0.5 mg of Se/kg of DM would not pose a health risk to humans.

CONCLUSIONS

The present study showed that different Se sources did not influence DMI, milk yield, and milk composition, except for milk fat percentage. Higher levels of HMSeBA supplementation promoted the activities of GSH-Px and SOD and elevated milk and plasma Se concentrations. Compared with SS, dietary HMSeBA enhanced the activities of GSH-Px, T-AOC, and SOD and increased the total Se concentrations in milk and plasma and the milk-to-plasma Se ratio, but reduced MDA content. This implies that HMSeBA functions effectively as an organic Se source that improves antioxidant status and Se concentrations of plasma and milk and could replace SS in the diet of dairy cows.

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REFERENCES

- Beers, M. H., and R. Berkow. 1998. The Merck Veterinary Manual. Accessed May 15, 2005. <http://www.merck.com>.
- Boldizarova, K., L. Gresakova, S. Faix, M. Mellen, and L. Leng. 2005. Antioxidant status of lambs fed on diets supplemented with selenite or Se-yeast. *J. Anim. Feed Sci.* 14:245–253.
- Briens, M., Y. Mercier, F. Rouffineau, F. Mercierand, and P. A. Geraert. 2014. 2-Hydroxy-4-methylselenobutanoic acid induces additional tissue selenium enrichment in broiler chickens compared with other selenium sources. *Poult. Sci.* 93:85–93.
- Briens, M., Y. Mercier, F. Rouffineau, V. Vacchina, and P. A. Geraert. 2013. Comparative study of a new organic selenium source v. seleno-yeast and mineral selenium sources on muscle selenium enrichment and selenium digestibility in broiler chickens. *Br. J. Nutr.* 110:617–624.
- Calamari, L., F. Petrera, and G. Bertin. 2010. Effects of either sodium selenite or Se yeast (Sc CNCM I-3060) supplementation on selenium status and milk characteristics in dairy cows. *Livest. Sci.* 128:154–165.
- Cao, J., F. Guo, L. Zhang, B. Dong, and L. Gong. 2014. Effects of dietary selenomethionine supplementation on growth performance, antioxidant status, plasma selenium concentration, and immune function in weaning pigs. *J. Anim. Sci. Biotechnol.* 5:46.
- Castillo, C., J. Hernandez, A. Bravo, M. Lopez-Alonso, V. Pereira, and J. L. Bedito. 2005. Oxidative status during late pregnancy and early lactation in dairy cows. *Vet. J.* 169:286–292.
- China NY/t 34. 2004. Feeding Standard of Dairy Cattle. China Non-Ye HangYe BiaoZhun/Tuijian-34. China Agricultural Publisher, Beijing, China.

- Doucha, J., K. Livansky, V. Kotrbacek, and V. Zachleder. 2009. Production of *Chlorella* biomass enriched by selenium and its use in animal nutrition: A review. *Appl. Microbiol. Biotechnol.* 83:1001–1008.
- EFSA. 2013a. Scientific opinion on safety and efficacy of hydroxy-analogue of selenomethionine as feed additive for all species. *EFSA J.* 11:3046.
- Emin, S., K. Yordanova, D. Dimov, V. Ilieva, A. Koychev, G. Prakovska, and T. Vlaykova. 2010. Total antioxidant capacity of plasma determined as ferrous reducing ability of plasma in patients with COPD. *Trakia J. Sci.* 8:205–213.
- Fairweather-Tait, S. J., R. Collings, and R. Hurst. 2010. Selenium bio-availability: Current knowledge and future research requirements. *Am. J. Clin. Nutr.* 91:1484S–1491S.
- Gawel, S., M. Wardas, E. Niedworok, and P. Wardas. 2004. Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiad. Lek.* 57:453–455.
- Givens, D. I., R. Allison, B. Cottrill, and J. S. Blake. 2004. Enhancing the selenium content of bovine milk through alteration of the form and concentration of selenium in the diet of the dairy cow. *J. Sci. Food Agric.* 84:811–817.
- Gong, J., L. Ni, D. Wang, B. Shi, and S. Yan. 2014. Effect of dietary organic selenium on milk selenium concentration and antioxidant and immune status in midlactation dairy cows. *Livest. Sci.* 170:84–90.
- Gong, J., and M. Xiao. 2016. Selenium and antioxidant status in dairy cows at different stages of lactation. *Biol. Trace Elem. Res.* 171:89–93.
- Heard, J. W., C. R. Stockdale, G. P. Walker, C. M. Leddin, F. R. Dunshea, G. H. McIntosh, P. M. Shields, A. McKenna, G. P. Young, and P. T. Doyle. 2007. Increasing selenium concentration in milk: effects of amount of selenium from yeast and cereal grain supplements. *J. Dairy Sci.* 90:4117–4127.
- Jlali, M., M. Briens, F. Rouffineau, P. A. Geraert, and Y. Mercier. 2014. Evaluation of the efficacy of 2-hydroxy-4-methylselenobutanoic acid on growth performance and tissue selenium retention in growing pigs. *J. Anim. Sci.* 92:182–188.
- Jlali, M., M. Briens, F. Rouffineau, F. Mercierand, P. A. Geraert, and Y. Mercier. 2013. Effect of 2-hydroxy-4-methylselenobutanoic acid as a dietary selenium supplement to improve the selenium concentration of table eggs. *J. Anim. Sci.* 91:1745–1752.
- Juniper, D. T., R. H. Phipps, A. K. Jones, and G. Bertin. 2006. Selenium supplementation of lactating dairy cows: Effect on selenium concentration in blood, milk, urine, and feces. *J. Dairy Sci.* 89:3544–3551.
- Kay, J. K., J. R. Roche, E. S. Kolver, N. A. Thomson, and L. H. Baumgard. 2005. A comparison between feeding systems (pasture and TMR) and the effect of vitamin E supplementation on plasma and milk fatty acid profiles in dairy cows. *J. Dairy Res.* 72:322–332.
- Knowles, S. O., N. Grace, K. Wurms, and J. Lee. 1999. Significance of amount and form of dietary selenium on blood, milk, and casein selenium concentrations in grazing cows. *J. Dairy Sci.* 82:429–437.
- Korhola, M., A. Vainio, and K. Edelmann. 1986. Selenium yeast. *Ann. Clin. Res.* 18:65–68.
- Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76:1216–1231.
- Momeni, H. R., and N. Eskandari. 2017. Effect of curcumin on kidney histopathological changes, lipid peroxidation and total antioxidant capacity of serum in sodium arsenite-treated mice. *Exp. Toxicol. Pathol.* 69:93–97.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Ortman, K., and B. Pehrson. 1999. Effect of selenate as a feed supplement to dairy cows in comparison to selenite and selenium yeast. *J. Anim. Sci.* 77:3365–3370.
- Pavlati, L., A. Pechova, and J. Illek. 2000. Direct and indirect assessment of selenium status in cattle—A comparison. *Acta Vet. Brno* 69:281–287.
- Phipps, R. H., A. S. Grandison, A. K. Jones, D. T. Juniper, E. Ramos-Morales, and G. Bertin. 2008. Selenium supplementation of lactating dairy cows: Effects on milk production and total selenium content and speciation in blood, milk and cheese. *Animal* 2:1610–1618.
- Prasad, A. S. 2014. Zinc: An antioxidant and anti-inflammatory agent: role of zinc in degenerative disorders of aging. *J. Trace Elem. Med. Biol.* 28:364–371.
- Ran, L., X. Wu, X. Shen, K. Zhang, F. Ren, and K. Huang. 2010. Effects of selenium form on blood and milk selenium concentrations, milk component and milk fatty acid composition in dairy cows. *J. Sci. Food Agric.* 90:2214–2219.
- Rotruck, J. T., A. Pope, H. Ganther, A. Swanson, D. G. Hafeman, and W. Hoekstra. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. *Science* 179:588–590.
- Rowntree, J. E., G. Hill, D. Hawkins, J. Link, M. Rincker, G. Bednar, and R. Kreft. 2004. Effect of Se on selenoprotein activity and thyroid hormone metabolism in beef and dairy cows and calves. *J. Anim. Sci.* 82:2995–3005.
- Schwarz, K., and C. M. Moltz. 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc.* 79:3292–3293.
- Slavik, P., J. Illek, M. Brix, J. Hlavicova, R. Rajmon, and F. Jilek. 2008. Influence of organic versus inorganic dietary selenium supplementation on the concentration of selenium in colostrum, milk and blood of beef cows. *Acta Vet. Scand.* 50:43.
- Stewart, W. C., G. Bobe, G. Pirelli, W. Mosher, and J. Hall. 2012. Organic and inorganic selenium: III. Ewe and progeny performance. *J. Anim. Sci.* 90:4536–4543.
- Sun, P., J. Wang, J. Shen, and H. Wei. 2011. Residues of melamine and cyanuric acid in milk and tissues of dairy cows fed different doses of melamine. *J. Dairy Sci.* 94:3575–3582.
- Van Soest, P. J., J. Robertson, and B. Lewis. 1991. Symposium: Carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. *J. Dairy Sci.* 74:3583–3597.
- Xu, Z., L. Zhang, and J. Han. 2007. Effects of Se-yeast in dairy ration on somatic cell and anti-oxidation performance. *Chin. J. Anim. Nutr.* 19:753–757.
- Zheng, D., R. Li, and W. Wang. 1982. A preliminary exposition on low-selenium zone in the world. *Acta Sci. Circumstantiae* 3:241–250.
- Zhou, X. Q., Y. D. Zhang, M. Zhao, T. Zhang, D. Zhu, D. P. Bu, and J. Q. Wang. 2015. Effect of dietary energy source and level on nutrient digestibility, rumen microbial protein synthesis, and milk performance in lactating dairy cows. *J. Dairy Sci.* 98:7209–7217.