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Original Research Article

Milk selenium content and speciation in response to supranutritional selenium yeast supplementation in cows

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ABSTRACT

The effects of selenium (Se) yeast supplementation on performance, blood biochemical and antioxidant parameters, and milk Se content and speciation were evaluated. Thirty-six mid-lactation Holstein dairy cows were randomly assigned to 1 of 3 treatments: 1) control (basal diet containing Se at 0.11 mg/kg DM), 2) basal diet + 0.5 mg supplemental Se/kg DM (SY-0.5), and 3) basal diet + 5 mg supplemental Se/kg DM (SY-5). Selenium was supplemented as Se yeast. The trial consisted of a 1-week pretrial period and an 8-week experimental period. Milk somatic cell score decreased with SY-5 supplementation ($P < 0.05$), but other performance parameters were not affected ($P > 0.05$). The serum Se concentration increased with the increasing levels of Se yeast supplementation ($P < 0.05$), however, blood biochemical parameters showed few treatment effects. The antioxidant capacity of dairy cows was improved with Se yeast supplementation reflected in increased serum glutathione peroxidase activity ($P < 0.05$) and total antioxidant capacity ($P = 0.08$), and decreased malondialdehyde concentration ($P < 0.05$). Milk total Se concentration increased with Se dose ($P < 0.05$). Also, the selenomethionine concentration increased with Se dose from 13.0 ± 0.7 μg/kg in control to 33.1 ± 2.1 μg/kg in SY-0.5 and 530.4 ± 17.5 μg/kg in SY-5 cows ($P < 0.05$). Similarly, selenocystine concentration increased from 15.6 ± 0.9 μg/kg in control and 18.9 ± 1.1 μg/kg in SY-0.5 to 22.2 ± 1.5 μg/kg in SY-5 cows ($P < 0.05$). In conclusion, Se yeast is a good organic Se source to produce Se-enriched cow milk with increased Se species including selenomethionine and selenocystine. The results can provide useful information on milk Se species when a high dose Se yeast was supplemented in the cow diet.

1. Introduction

Selenium (Se) is a trace element important for overall human and animal health and it exerts its physiological functions (i.e. maintaining redox homeostasis) mainly in the form of selenoproteins such as glutathione peroxidase (GSH-Px), thioredoxin reductases or iodothyronine deiodinases (Brown and Arthur, 2001). Insufficient or excessive Se intakes are associated with many chronic and acute diseases (Fairweather-Tait et al., 2011; Manzanares and Hardy, 2016). In humans, health problems linked to Se deficiency such as decreased immunological potential, compromised fertility and increased incidence of cancer (Weiss

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et al., 1990), are more common than its toxicity caused by excessive Se intake. Increasing Se content in foods by nutritional biofortification are useful to overcome Se deficiency and improve human health (Hu et al., 2020). Cereals, meats, aquatic products, and dairy products are the major dietary sources of Se (Dos Santos et al., 2017; Dinh et al., 2018), and some Se-enriched functional products have been manufactured in many countries, such as Se-enriched cereal crops and Se-enriched eggs (Fisinin et al., 2008; Mao et al., 2014). The beneficial or detrimental effects of Se are not only related to total Se concentration but also associated with Se speciation (Rayman, 2019).

Information on chemical speciation is critical in understanding metabolic pathway, bioavailability and physiological functions (Trinta et al., 2020). Accordingly, the chemical speciation of Se intake is very important besides the quantity. Selenium exists in various forms of inorganic (e.g. selenite and selenate) and organic (e.g. selenoamino acids). In general, selenoamino acids such as selenomethionine (SeMet) and selenocystine ($SeCys_2$) are easier to absorb and have lower toxicity than inorganic Se species (Rayman, 2019). For example, inorgnaic Se must undergo a metabolic transformation before it is assimilated into selenocysteine (SeCys) and then incorporated into selenoprotein (Weiss, 2005). However, SeMet can be absorbed directively via the methionine pathway and incorporated into general proteins, which act as a biological pool of Se (Weiss, 2005). Therefore, using only the total Se concentration to evaluate the quality of Se-enriched livestock products is not appropriate, and Se speciation should also be considered. However, few previous studies have undertaken to analyze the Se speciation in livestock products although the advancement and development of modern instrumental analysis technology has achieved reliable chemical speciation analysis.

In terms of animal-based foods, milk is a good source of Se for humans, as Se in milk protein is highly bioavailable (Uglietta et al., 2008) and is effective in reducing diseases such as colon cancer (Hu et al., 2008). The bioavailability and bioactivity of organic Se has been shown to be greater than inorganic sources of Se (Knowles et al., 1999), and more effectively retained through nonspecific incorporation into milk proteins (Burk et al., 2001). Selenium yeast is a popular organic Se source. Many studies have been undertaken to produce Se-enriched milk using high dose Se yeast (Givens et al., 2004; Heard et al., 2004; Doyle et al., 2011), however, little information is available on the contents of Se speciation when dairy cows were exposed to high dose Se yeast to produce Se-enriched milk.

Therefore, the main objective of the current study was to evaluate the changes of Se speciation and percentages of different species of Se-enriched milk produced by high dose dietary Se yeast supplementation, and also to indicate the changes of performance, blood biochemical and antioxidant enzyme parameters. We hypothesis that not only the total milk Se concentration but the Se speciation will increase with the increasing dosages of Se yeast supplementation. The results might provide useful data to better evaluate the quality of Se-enriched milk produced by high dose Se yeast.

2. Materials and methods

2.1. Ethics statement

The experimental protocol was approved by the State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agriculture Sciences, Beijing, China (No. IAS20180115). The experiment was carried out at Sino Farm (Beijing, China). All animals in this study were handled and raised following the standards established by the Institute of Animal Science, Chinese Academy of Agricultural Sciences Care and Use Committee.

2.2. Animals, experiment design, and management

All cows were housed in free stall barns with rice husks for bedding and fed ad libitum with free access to water. All cows were inspected by a veterinary surgeon at Sino Farm, prior to the initiation of the study. A total of 36 multiparous mid-lactation Holstein dairy cows were selected according to body weight (BW, 574 ± 36 kg), days in milk (DIM, 131 ± 19 d), parity (2 ± 1), and milk yield (30.2 ± 1.3 kg). Cows were randomly allocated to 1 of 3 treatments with 12 cows assigned to each treatment according to a complete randomized design, which were balanced for BW, DIM, parity and milk yield. The experiment included a 1-week pretrial period, and an 8-week experimental period. During the pretrial period, the cows were adapted to the new environment, and all cows ate the regular diet of the farm. During the experimental period, all cows consumed the basal diet (Se content of 0.105 mg/kg DM) with the corresponding Se yeast treatments. The treatments were negative control (basal diet containing 0.105 mg of Se/kg DM, control, no Se supplementation), basal diet + 0.5 mg supplemental Se/kg DM (SY-0.5), basal diet + 5 mg supplemental Se/kg DM (SY-5). Selenium was supplemented as Se yeast (Angel Yeast Co., Ltd; Beijing, China), which contained 2,000 mg/kg of Se. The total mixed ration (TMR) was formulated using a premix without Se (Sanyuan

Table 1
Ingredients and chemical composition of the basal diet fed during the experiment (% of DM).

Item	Content
Ingredients	
Alfalfa hay	13.6
Oat hay	4.38
Corn silage	17.8
Soybean meal	10.1
Extruded soybean	3.71
Molasses meal[1]	1.01
Steam-flaked corn[2]	18.2
Soybean hull	6.23
Cottonseed	7.34
Ground corn	8.00
Milk power	1.48
Distillers dried grains with solubles	1.75
Fat-bergafat[3]	1.62
Calcium carbonate	1.02
Potassium bicarbonate	1.03
Dicalcium phosphate	0.06
Sodium bicarbonate	1.32
Magnesium oxide	0.36
Salt	0.43
Zeolite	0.45
Premix[4]	0.19
Nutrient composition	
Neutral detergent fiber	28.0
Acid detergent fiber	18.7
Crude protein	17.5
Ether extract	5.98
Calcium	0.91
Phosphorous	0.44
Selenium, mg/kg DM	0.105
NE_L[5], Mcal/kg	1.68

[1] Vedan, Yantai, Shandong Province, China.
[2] Steam-flaked corn was processed to flake density of 0.36 kg/L.
[3] A saturated free fatty acid supplement (Berg + Schmidt Co., Germany).
[4] Contained (per kilogram of diet DM) a maximum 240,000 IU of vitamin A; 85,000 IU of vitamin D; 3,000 IU of vitamin E; 400 mg Fe; 600 mg Cu; 2,100 mg Zn; 500 mg Mn; 35 mg I; and 60 mg Co.
[5] NE_L = Net energy for lactation, calculated value according to Feeding Standard of Dairy Cows in China (NY/T 34, 2004).

seed technology Co., Ltd; Beijing, China) to meet or slightly exceed nutritional requirements except for Se (NRC, 2001, Table 1). Cows were individually fed twice daily at 07:00 and 14:00. The appropriate quantity of Se yeast was weighed then mixed with 100 g of ground corn and top-dressed on the TMR of each cow at the morning feeding. All cows were fed using a computerized monitoring system (RIC system, Insentec B.V., Marknesse, Netherlands) as reported previously (Zhou et al., 2015). Cows were milked 3 times daily at 06:30, 13:30, and 20:30 and milk yields were recorded for all cows at each milking. Orts were collected at the morning milking time, and fresh feed offered was adjusted allowing a 5% refusal. Cows were evaluated daily for clinical Se poisoning signs, but no signs of poisoning were observed during the experimental period. Two cows had mastitis: one in the control and one in the SY-0.5 groups; and their data and samples were excluded from the analysis.

2.3. Sampling procedures

Representative samples of the offered TMR and refusals were obtained 3 times per week for nutritional and total Se concentration analysis. Milk samples were taken weekly (50 mL per cow in duplicate). On sampling days, samples from the 3 milkings were pooled in a 4:3:3 ratio (40, 30, 30 mL; Sun et al., 2011) and split in 2 aliquots. One of the 50 mL samples was analyzed for milk composition, and the other was stored at −20 °C for total Se concentration and Se speciation analysis. Two 10-mL blood samples were taken before the morning feeding (07:00) at 0 (the beginning), 4 and 8 weeks of the treatment period for all cows. Blood samples were collected via coccygeal vein into Vacutainer tubes (BD Biosciences, San Jose, CA) without additives, allowed to clot for a minimum of 30 min at room temperature and stored in a 4-°C refrigerator overnight, and then centrifuged at 3,000 × *g* at 4 °C for 15 min to obtain serum. Samples were stored at −20 °C for analysis of antioxidant and biochemical parameters, and total Se concentration.

2.4. Feed analysis

The weekly TMR samples were pooled and dried in an oven at 65 °C until static weight was achieved, then ground by a fodder grinder (Arthur H. Thomas Co., Philadelphia, PA), and passed through a 1-mm mesh screen for subsequent analysis. Dry matter was analyzed by drying at 105 °C for 4 h. Neutral detergent fiber (NDF) was measured following the method described by Van Soest et al. (1991) using α-amylase and sodium sulfide. Acid detergent fiber (ADF, method 973.18C; AOAC, 1990), crude protein (CP, method 984.13; AOAC, 1990) and ether extract (EE, method 920.39; AOAC, 1990) were analyzed according to the Association of Official Analytical Chemists (AOAC) methods. Ash content was determined by incineration in a muffle furnace at 550 °C. The values of nutritional components are shown in Table 1.

2.5. Milk composition

Weekly milk samples were analyzed for milk protein, fat, lactose, solids matter, and milk solid not fat using infrared reflectance spectroscopy (Foss 120 Milko-Scan, Foss Electric, Hillerød, Denmark). Somatic cell counts (SCC) were analyzed by a somatic cell counter (Fossomatic 5000, Foss Electric A/S) and transformed to a somatic cell score (SCS) using the following formula (Shook, 2006):

$$SCS = \log_2 [SCC \text{ (cells per mL)}/100{,}000] + 3$$

The SCC below 100,000 cells/mL (SCS = 3) is considered as healthy udder quarters (Schwarz et al., 2011), whereas SCC ≥400,000 cells/mL (SCS = 5) is used for the diagnosis of subclinical mastitis (Schepers et al., 1997; De Haas et al., 2004).

2.6. Serum antioxidant and biochemical parameters

Serum samples were analyzed for GSH-Px activity, total superoxide dismutase (T-SOD) activity, total antioxidant capacity (T-AOC) and malondialdehyde (MDA) content using commercial detection kits (A005, A001-3, A015, A003-1; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and following manufacturer's instructions. The intra- and interassay coefficients of variation for GSH-Px, T-SOD, T-AOC and MDA were 3.1% and 4.3%, 1.7% and 3.5%, 3.2% and 6.8%, and 3.5% and 4.1%; respectively. These serum samples were also analyzed for biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholinesterase (CHE), creatine kinase (CK), alkaline phosphatase (ALP), creatinine (CR), urea, and glutamate dehydrogenase (GLDH) using an automatic biochemical analyzer (Hitachi 7080, Ltd., Tokyo, JPN) and commercial kits. The intra-and interassay coefficients of variation for ALT, AST, CHE, CK, ALP, CR, urea (Hitachi 7080), and GLDH (A125-1-1, Nanjing Jiancheng Bioengineering Institute) were 1.9% and 2.9%, 2.5% and 3.7%, 1.3% and 1.9%, 2.6% and 3.4%, 3.5% and 4.0%, 1.9% and 2.1%,1.6% and 1.8%, 5.6% and 4.4%; respectively.

2.7. Selenium analysis

The pooled weekly TMR samples, serum samples at 0, 4 and 8 weeks, and milk samples at 0 and 8 weeks were used for Se analysis. Total Se concentrations were determined according to the methods of the National Standard of the People's Republic of China: National Food Safety Standard-Determination of Multi-elements in Foods (Stands Press of China, Beijing; GB5009.268-2016). Briefly, 0.2 g of TMR samples (accurate to 0.001 g) or 1 mL of milk or serum, were added to 5 mL of hydrochloric acid, incubated for 1 h or overnight, and then digested (3 steps:120 °C for 15 min, 150 °C for 15 min, 190 °C for 25 min). After cooling, samples were placed in a heated block for 30 min at 100 °C. The volume was then adjusted to 25 or 50 mL with water and the mixed solution was used to analyze total Se concentration by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 8800, Agilent Co., CA, USA).

The milk samples as described above also were used to analyze Se speciation according to the methods described by Bierla et al. (2008) with modifications. Briefly, 1 mL of milk sample and 10 mL of extracting solution (composition: Tris−HCl buffer and protease) were added to a 15 mL tube, vortexed, sonicated for 20 min, centrifugated at 4,000 × *g* at 4 °C for 10 min, and the supernatant was collected. This step was repeated 3 times. The supernatant was evaporated to 5 mL under N_2 flow, then volume was adjusted to 10 mL with water and filtered through 0.45-μm pore size (Millipore, Bedford, MA, USA). Then Se speciation was determined by high performance liquid chromatography (HPLC, Waters 2659)-ICP-MS. The ICP-MS operating parameters were as follows: radio frequency power = 1,400 W, cooling gas flow = 13.6 L/min, plasma gas flow = 15 L/min, nebulizer = 0.87, sampling depth = 180. H_2/He mixed gas flow = 5.6 mL/min, auxiliary gas flow = 0.72 L/min. Flowing phase: 15 mmol/L $NH_4H_2PO_4$ and tetrabutylammonium bromide mixed solution, flow rate = 0.8 mL/min.

2.8. Statistical analysis

Data were analyzed by repeated measures using the PROC MIXED procedures of SAS (version 9.4, SAS Institute Inc., Cary,

NC). An autoregressive covariance structure (AR1) was utilized for the performance and milk composition and an unstructured covariance was used for blood parameters. The statistical model includes fixed effects of week, treatment, and the interaction of treatment and week and the random effect of cow. Week effect was not included in the model when total Se concentration and speciation in milk were analyzed. The mean value of DMI and milk yield during the adaptation period, and other parameters measured at 0 week were used as covariates. Data were shown as covariate-adjusted least square means and standard error of the means. Tukey's multiple range test was used for the evaluation of differences between the treatments. The statistical significance was declared at $P < 0.05$ and the trends at $0.05 \leq P < 0.1$.

3. Results

3.1. Production performance

The effects of Se yeast treatment on production performance are presented in Table 2 and Fig. 1A, B, and C. All the parameters changed with time (week; $P < 0.01$). The SCS was lower in SY-5 supplemented cows compared with control and SY-0.5 supplemented cows (Table 2; $P < 0.05$). There were tendencies in the treatment by week interaction in DMI ($P = 0.10$, Fig. 1A), milk yield ($P = 0.10$, Fig. 1B), and SCS ($P = 0.08$, Fig. 1C). The DMI was increased in SY-5 cows from week 7 and 8 ($P < 0.05$), but it did not differ between treatments from week 1 to 6. The milk yield did not differ between treatments from week 1 to 4, but it was increased in SY-5 cows during week 5 to 8 ($P < 0.05$). The SCS did not differ between treatments from week 1 to 6, but it was decreased in SY-0.5 and SY-5 cows by week 7 and 8 ($P < 0.05$).

3.2. Serum biochemical parameters

The effects of supplemental Se yeast on serum biochemical parameters, Se concentration and antioxidant indicators are presented in Table 3 and Fig. 1D. The AST, urea, and CK changed with time (week; $P < 0.05$). There was a treatment by week interaction ($P = 0.02$) for CHE activity, as it did not differ between treatments in week 4 but it was decreased in SY-5 cows by week 8 (Fig. 1D). There were no other effects of Se yeast supplementation on biochemical parameters in serum ($P > 0.05$).

3.3. Selenium concentration and antioxidant indicators in serum

The effects of supplemental Se yeast on serum Se concentration and antioxidant indicators are presented in Table 4. Total Se concentrations in serum increased with Se dose and changed with time ($P < 0.05$). The activities of GSH-Px and T-SOD and the capacity of T-AOC changed with time (week; $P < 0.05$). The GSH-Px activity was higher in SY-5 supplemented cows compared with control and SY-0.5 ones ($P < 0.05$), which did not differ. Supplementing Se yeast had no effect on T-SOD activity ($P > 0.05$). The T-AOC tended to increase ($P = 0.08$) with the Se yeast supplementation, but it did not differ between SY-0.5 and SY-5 treatments. The MDA concentration decreased ($P < 0.05$) with Se yeast supplementation, but there was no difference between SY-0.5 and SY-5 treatments ($P > 0.05$).

3.4. Total Se concentration and Se speciation in milk

The effects of supplemental Se yeast on total Se concentration and Se speciation in milk are presented in Fig. 2. Overall, milk Se concentration increased with Se dose from 39 ± 3 μg/kg in control to 86 ± 6 μg/kg in SY-0.5 and 583 ± 13 μg/kg in SY-5 cows (Fig. 2A). The SeMet concentration increased with Se dose from 13.0 ± 0.7 μg/kg in control to 33.1 ± 2.1 μg/kg in SY-0.5 and 530.4 ± 17.5 μg/kg in SY-5 cows ($P < 0.05$, Fig. 2B). Similarly, $SeCys_2$ concentration increased with Se dose, and the concentrations were 15.6 ± 0.9 μg/kg in control, 18.9 ± 1.1 μg/kg in SY-0.5 and 22.2 ± 1.5 μg/kg in SY-5 cows ($P < 0.05$, Fig. 2C). Fig. 2D shows the proportions of each species in milk after different doses of Se yeast supplementation for 8 weeks. The proportion of SeMet was higher under SY-5 treatment which accounted for 91.0% compared with 33.3% in control and 38.5% in SY-0.5 treatments. The percentage of $SeCys_2$ decreased from 40.0% in control to 22.0% in SY-0.5 and 3.8% in SY-5 treatments. Unidentified Se species accounted for 26.7% of total Se content in control, 39.5% in SY-0.5 treatment, and 5.2% in SY-5 treatment.

Table 2
The effects of selenium yeast supplementation on dry matter intake, milk production and composition.

Item	Treatment[1]			SEM	*P*-value		
	Control	SY-0.5	SY-5		Treatment	Week	Treatment × Week
Dry matter intake, kg/d	22.4	22.2	22.7	0.13	0.32	<0.01	0.10
Production, kg/d							
Milk yield	31.2	30.1	33.2	1.32	0.46	<0.01	0.10
4% FCM[2]	31.8	29.9	34.9	1.53	0.17	<0.01	0.31
ECM[3]	35.4	33.4	38.8	1.62	0.17	<0.01	0.24
Protein yield	1.12	1.06	1.21	0.04	0.20	<0.01	0.23
Fat yield	1.29	1.20	1.44	0.07	0.12	<0.01	0.38
Lactose yield	1.49	1.41	1.61	0.06	0.35	<0.01	0.15
Total solids field	4.08	3.45	4.46	0.29	0.19	<0.01	0.21
Total solids-not fat field	2.81	2.67	3.04	0.11	0.27	<0.01	0.19
Composition, %							
Protein	3.63	3.54	3.65	0.03	0.60	<0.01	0.53
Fat	4.15	4.02	4.36	0.10	0.45	<0.01	0.84
Lactose	4.74	4.70	4.84	0.04	0.24	<0.01	1.00
Total solids	13.1	12.9	13.4	0.21	0.31	<0.01	0.66
Solids-not fat	9.01	8.93	9.13	0.12	0.19	<0.01	0.94
SCS[4]	4.48[a]	4.07[a]	3.42[b]	0.11	0.01	0.01	0.08

[a,b] Values in the same row with a different superscript differ significantly.
[1] Control, SY-0.5 and SY-5 were supplemented with selenium yeast at 0.5 and 5 mg Se/kg DM, respectively.
[2] 4% FCM = 4% fat-corrected milk = 0.4 × Milk yield (kg) + 15.0 × Milk fat yield (kg).
[3] ECM = energy-corrected milk = 0.327 × Milk yield (kg) +12.95 × Milk fat yield (kg) +7.20 × Milk protein yield (kg).
[4] SCS = somatic cell score = $\log_2$ [SCC (cells per mL)/100,000] + 3.

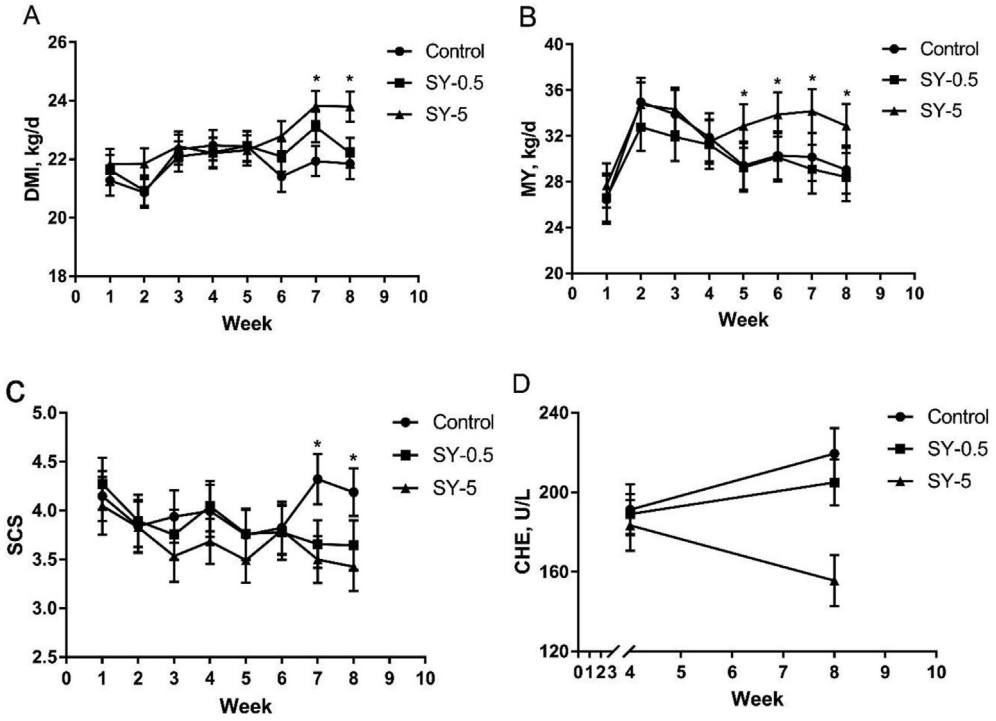

Fig. 1. Effects of selenium (Se) yeast on dry matter intake (DMI; A), milk yield (MY; B), somatic cell score (SCS; C) and cholinesterase (CHE; D) in lactating Holstein cows. The lines with circles represent control group (no Se supplementation). The lines with squares represent 0.5 mg of Se/kg of DM Se yeast supplementation (SY-0.5). The lines with triangles represent 5 mg of Se/kg of DM Se yeast supplementation (SY-5). The asterisks represent a significant difference ($P < 0.05$) between weeks. Error bar represent standard error of mean.

Table 3
The effects of selenium yeast supplementation on biochemical parameters in serum.

Item	Treatment[1]			SEM	*P*-value		
	Control	SY-0.5	SY-5		Treatment	Week	Treatment × Week
Aspartate aminotransferase, U/L	111	113	97	5.1	0.17	0.02	0.65
Urea, mmol/L	5.32	5.31	5.12	0.12	0.61	<0.01	0.31
Creatinine, μmol/L	53.1	58.2	55.1	1.21	0.49	0.09	0.38
Uric acid, μmol/L	50.2	47.3	49.1	2.22	0.78	0.15	0.21
Creatine kinase, U/L	146	132	125	3.4	0.15	0.04	0.12
Alkaline phosphatase, U/L	50.0	54.2	56.2	2.36	0.80	0.18	0.26
Cholinesterase, U/L	205[a]	197[a]	170[b]	5.1	0.03	0.46	0.02
Glutamate dehydrogenase, U/L	34.1	32.2	37.1	2.28	0.27	0.52	0.52

[a,b] Values in the same row with a different superscript differ significantly.
[1] Control, SY-0.5 and SY-5 were supplemented with selenium yeast at 0.5 and 5 mg Se/kg DM, respectively.

Table 4
The effects of selenium yeast supplementation on selenium content and antioxidant indicators in serum.

Item	Treatment[1]			SEM	*P*-value		
	Control	SY-0.5	SY-5		Treatment	Week	Treatment × Week
Total selenium in serum, μg/kg	93[c]	106[b]	683[a]	44.2	<0.01	0.02	0.78
Glutathione peroxidase, U/mL	143[b]	155[b]	189[a]	4.3	<0.01	<0.01	0.46
Superoxide dismutase, U/mL	95	98	100	2.4	0.60	0.01	0.76
Total antioxidant capacity, U/mL	1.52	1.91	2.01	0.12	0.08	<0.01	0.73
Malondialdehyde, nmol/mL	5.62[a]	4.31[b]	4.82[b]	0.24	0.04	0.59	0.39

[a–c] Values in the same row with a different superscript differ significantly.
[1] Control, SY-0.5 and SY-5 were supplemented with selenium yeast at 0.5 and 5 mg Se/kg DM, respectively.

4. Discussion

Supplementing Se at very high doses might improve dairy cow health and maximize milk Se excretion, which is an attractive strategy to increase Se intake in humans. However, Se toxicity is a concern. For ruminants, the NRC (2005) establishes maximum tolerable doses of Se at 5 mg of Se/kg DM. We wanted to make further efforts to examine the effects of Se yeast at a tolerant dose on lactating dairy cows under Chinese feeding conditions, so we chose SY-5 as a high-dose. In the current study, supplementing at this level had no negative effects on performance as demonstrated by the lack of differences relative to 0.5 mg/kg DM supplementation. The results support the reports by Palacios et al. (2005) and Juniper et al. (2006) in which even greater doses of Se were fed with

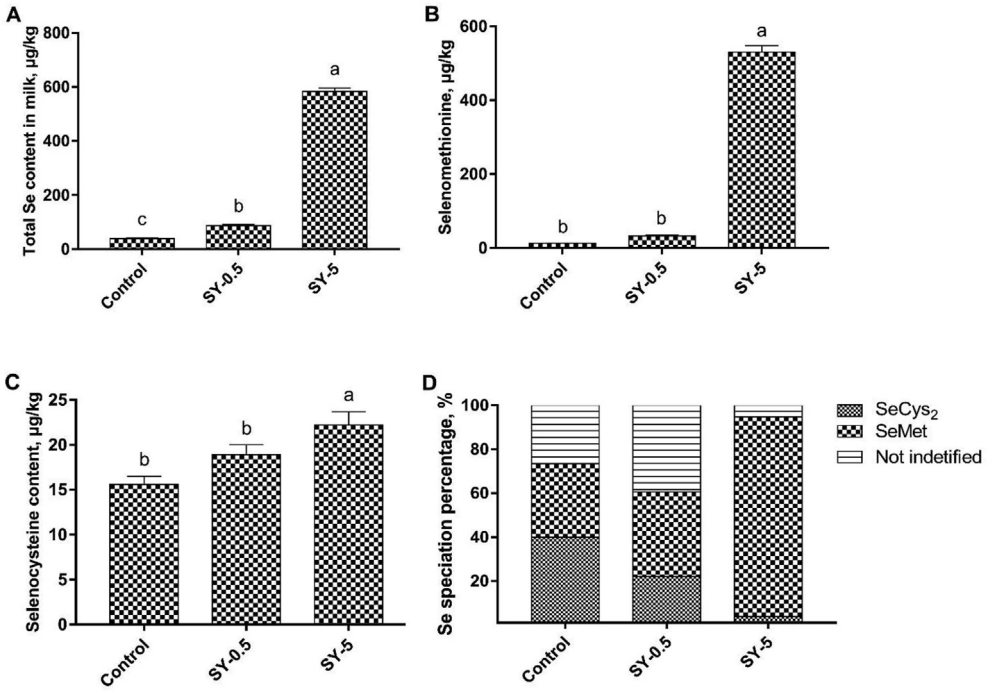

Fig. 2. Mean total selenium (Se; A), selenomethionine (SeMet; B) and selenocysteine ($SeCys_2$; C) contents in milk of dairy cows offered diets supplemented with different Se yeast levels and distributions of Se species in milk with different Se treatments (D). Control = 0 mg of Se/kg of DM (no Se supplementation); SY-0.5 = 0.5 mg of Se/kg of DM Se yeast supplementation; SY-5 = 5 mg of Se/kg of DM Se yeast supplementation. Different letters denote significant differences among treatments.

no overt negative effects. In addition, SCC, which are mainly constituted by lymphocytes and when increased can suggest an infectious process in the mammary gland, decreased with SY-5 supplementation. The current result agrees with Weiss et al. (1990) who established a negative relationship between increasing circulating Se concentration and bulk tank SCC in dairy herds.

Blood Se is an adequate indicator of Se status in dairy cows (Pavlata et al., 2000), especially after short term dietary Se changes (Thompson et al., 1991). At the highest supplementation level, serum Se concentration was 683 μg/kg (0.7 mg/L), which is well below the 2.5 to 3.5 mg/L range, previously established as the limit for chronic selenosis (Underwood, 1993). Selenium poisoning can lead to necrosis of tissues such as liver and kidney, thereby causing changes in blood biochemical parameters (Cristaldi et al., 2005). To further exclude Se toxicity, markers of liver, renal and other organs function such as ALT, AST, ALP, UR, CHE and CK were measured in blood and no differences were detected except for CHE, which decreased in SY-5 cows by week 8, but remained within the normal range (Aiello et al., 2016). Besides that, as a liver-specific biomarker (Engelking, 2015), GLDH in SY-5 treatment was numerically higher than the other two groups. This implies that potential adverse effects may occur for long-term supplementation of high dose Se yeast, therefore, for a short period 5 mg/kg DM Se yeast can be acceptable, but for the purpose increasing milk Se, a longer time at lower intake needs to be evaluated.

Glutathione peroxidase was the first selenoprotein described (Rotruck et al., 1973). Its antioxidant capacity is based on the catalyzation of the reduction of lipid hydroperoxides and hydrogen peroxide, disposing of excess free radicals (Lyons et al., 2007). Previous research in dairy cows reported dietary supplementation of Se improved the activity of GSH-Px in blood (Salman et al., 2013). Further, Gong et al. (2014) demonstrated a higher antioxidant capacity when supplementing Se as Se yeast compared to inorganic Se selenite. In the current study, serum GSH-Px activity increased with Se supplementation, and the effects were significant even at 0.5 mg Se yeast/kg DM. Similar to the GSH-Px activity, T-AOC, an indicator of the overall capacity to counteract oxidative stress (Cao et al., 2014), tended to increase with Se yeast supplementation. Accordingly, MDA, an important marker of lipid peroxidation (Gawel et al., 2004), decreased with the Se yeast supplementation. In summary, the markers of antioxidant capacity and lipid peroxidation were improved with Se yeast supplementation, with a supraphysiological dose (5 mg/kg DM) being further beneficial in some parameters (i.e. GSH-Px).

The total Se concentration in milk increased from 39 μg/kg (control) to 583 μg/kg (110 mg/d Se derived from Se yeast) with an intermediate value of 86 μg/kg (11 mg/d Se derived from Se yeast), which showed a significant dose effect of the Se yeast dietary supplementation and supported the results obtained by Givens et al. (2004), Heard et al. (2007) and Doyle et al. (2011) where 2 to 40 mg/d Se yeast was fed to dairy cows. The recommended consumption of milk and dairy products by the Chinese Dietary Guidelines and Food Pagoda is 300 g, and the recommended Se intake is 55 μg of Se/d for humans (FAO, 2001). The consumption of 300 g milk from dairy cows supplemented with SY-0.5 and SY-5 can provide 25.8 and 174.9 μg Se intake for humans, which are lower than the upper tolerable intake (400 μg/d of Se). However, the recommended intakes of milk and dairy products in many countries are higher than 300 g/d per capita (i.e. 500 mL milk for Norway; FAO, 2013), so individuals should consider the upper tolerable intake when they consume Se-enriched milk produced by 5 g/kg DM Se yeast supplementation in the diet of dairy cows, which can result in 583 μg/kg Se concentration in milk.

The Se speciation in cow milk is important to its biological function, metabolic pathway and bioavailability of Se, and it can be affected by Se source and supplementation level in the diet (Phipps et al., 2008). We detected Se speciation including selenite, selenate, SeMet and $SeCys_2$ using HPLC-ICP-MS. Unfortunately, selenite and selenate were not identified in cow milk samples, so the

data were not shown. Selenite and selenate were absorbed in the small intestine mainly via a passive mechanism and were used almost exclusively to produce selenospecfic enzymes (Weiss, 2005). Therefore, the uptake of inorganic Se mainly existed in the form of selenoenzymes. Selenomethionine is the predominant form of Se yeast, which can be incorporated into selenoenzymes or general proteins that contain Met and is quite efficient (Weiss, 2005). The concentration of SeMet in SY-5 treatment was significantly higher than the SY-0.5 and control groups. In addition, the proportions of SeMet in milk increased with the increasing of Se yeast doses, accounting for 91.0% of total Se in SY-5 treatment, which is consistent with the results reported by Juniper et al. (2008). The increase of concentration and proportion of SeMet with the increasing of Se yeast supplementation levels suggested a high efficiency of SeMet incorporation. The SeMet can be directly assimilated into SeCys or via an intermediate step for the transformation (Kajander et al., 1991). Selenocystine, the metabolite of unstable SeCys, is a proteinaceous Se species in milk of mammals (He et al., 2019). As expected, the absolute concentration of $SeCys_2$ increased with the increasing levels of Se yeast supplementation. However, the proportions of $SeCys_2$ from the total Se decreased with the increasing of Se yeast doses, which may be the result of the changes from SeMet and other unidentified Se species. A portion of Se species had not been identified in the current study, which may have important physiological function, and linear and quadratic responses of Se speciation with the Se yeast supplementation doses were not determined due to the large differences in Se supplementation in the current study, therefore, further study is required.

5. Conclusion

As expected, the SCS and antioxidant parameters of dairy cows were improved by Se yeast supplementation. More importantly, Se speciation can be significantly affected by Se yeast supplementation level. The concentration of SeMet and $SeCys_2$ increased with the increasing of Se yeast dosages in the diet. Therefore, Se yeast is a good organic Se source to produce Se-enriched dairy cow milk with increased Se species including SeMet and $SeCys_2$. The results presented here contain some unique and important data about Se yeast (i.e. Se speciation) and can provide useful information on Se species for evaluation of the quality of Se-enriched milk produced by high dose Se yeast.

Author contributions

Lingling Sun: investigation, data curation, writing - original draft. Gentao Liu: investigation. **Dongmei Xu**: investigation. **Zhaohai Wu**: conceptualization, methodology. **Lu Ma**: conceptualization, methodology. **Sanz-Fernandez M. Victoria**: conceptualization, writing - review&editing. **Lance H. Baumgard**: conceptualization, writing - review&editing. **Dengpan Bu**: conceptualization, methodology, writing - review&editing, supervision.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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