Effects of Different Selenium Sources on Selenium Concentrations in Plasma and Milk and Serum Antioxidant Capacity in Lactating Dairy Cows under Short-Term Experimental Conditions: Postprint

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Abstract

This study aimed to compare the effects of hydroxy-selenomethionine analog (HMSeBA) and sodium selenite (SS) on selenium content in plasma and milk and serum antioxidant capacity in lactating dairy cows under short-term experimental conditions. A completely randomized design was adopted, and 8 Chinese Holstein dairy cows with similar parity, days in milk, and milk yield were randomly divided into 2 groups (4 cows per group), supplemented with 0.3 mg/kg DM of HMSeBA or SS in the basal diet, respectively. The preliminary period lasted for 2 weeks, and the experimental period lasted for 4 weeks. The results showed: 1) The milk fat percentage (P=0.060 3) and milk fat yield (P=0.055 2) of cows in the HMSeBA group tended to decrease compared with the SS group; dry matter intake, milk yield, and the yield and percentage of other milk components were not significantly affected by selenium source (P>0.05); 2) The selenium content in plasma and milk of cows in the HMSeBA group was significantly higher than that in the SS group (P<0.05); 3) Serum glutathione peroxidase activity was not significantly affected by selenium source (P>0.05); however, serum total antioxidant capacity and superoxide dismutase activity in the HMSeBA group were significantly higher than those in the SS group (P<0.05), while serum malondialdehyde content was significantly lower than that in the SS group (P<0.05). In conclusion, under short-term experimental conditions, dietary supplementation of 0.3 mg/kg DM HMSeBA compared with the same dose of SS could significantly increase selenium content and antioxidant capacity in plasma and milk of dairy cows.

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Full Text

Effects of Different Selenium Sources on Selenium Content in Plasma and Milk, and Serum Antioxidant Capacity of Lactating Dairy Cows under Short-Term Trial Conditions

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Abstract

This study aimed to compare the effects of 2-hydroxy-4-methylselenobutanoic acid (HMSeBA) and sodium selenite (SS) on selenium content in plasma and milk, and serum antioxidant capacity of lactating dairy cows under short-term trial conditions. Using a completely randomized design, eight Chinese Holstein cows similar in parity, days in milk, and milk yield were randomly divided into two groups (n=4) and supplemented with 0.3 mg/kg DM of either HMSeBA or SS in the basal diet. The pre-trial period lasted for 2 weeks, followed by a 4-week formal trial period. The results showed that: (1) Milk fat percentage ($P=0.0603$) and milk fat yield ($P=0.0552$) in the HMSeBA group tended to be lower compared with the SS group, while dry matter intake, milk yield, and other milk component yields and percentages were not significantly affected by selenium source ($P>0.05$); (2) Selenium content in both plasma and milk of cows in the HMSeBA group was significantly higher than that in the SS group ($P<0.05$); (3) Serum glutathione peroxidase activity was not significantly affected by selenium source ($P>0.05$), but serum total antioxidant capacity and superoxide dismutase activity in the HMSeBA group were significantly higher than those in the SS group ($P<0.05$), while serum malondialdehyde content was significantly lower ($P<0.05$). In conclusion, under short-term trial conditions, dietary supplementation with 0.3 mg/kg DM HMSeBA significantly increased selenium content in plasma and milk, and enhanced serum antioxidant capacity compared with supplementation of the same level of SS.

Keywords: 2-hydroxy-4-methylselenobutanoic acid; sodium selenite; dairy cow; selenium content in milk; serum antioxidant capacity

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Selenium is an essential trace element for maintaining animal and human health, performing numerous important biological functions in the body, such as enhancing antioxidant capacity, promoting absorption of vitamins A and K, strengthening immunity, and regulating basal metabolism [1]. Selenium deficiency affects normal physiological metabolism and harms growth and reproduction in livestock, with many studies indicating that over 20 human diseases are associated with selenium nutritional status [2]. To meet normal physiological metabolic needs, adults should maintain adequate daily selenium intake. Diet represents the direct pathway for human selenium acquisition, and supplementing selenium in animal diets not only meets nutritional requirements but also produces selenium-enriched animal products that are important for human dietary selenium supplementation. Selenium-enriched milk is a particularly important animal product for human selenium supplementation.

Studies have shown that adding selenium to dairy cow diets can increase milk selenium content [3-6]. Selenium exists in two common forms: inexpensive but highly toxic inorganic selenium with lower biological utilization, such as selenate and selenite; and expensive but low-toxicity, high-efficiency organic selenium, such as selenium yeast [7]. Selenium absorption occurs primarily in the small intestine, where selenomethionine from organic selenium is absorbed through the methionine transport system at approximately 80% efficiency, whereas inorganic selenium is mainly absorbed through passive diffusion at about 50% efficiency [8]. Meta-analysis results indicate that dietary supplementation with selenium yeast significantly increases milk selenium concentration compared with inorganic selenium forms [9]. 2-Hydroxy-4-methylselenobutanoic acid (HMSeBA) is a novel organic selenium source that differs from selenomethionine in that the amino group on the 2-carbon atom is replaced by a hydroxyl group. The European Union approved HMSeBA as a feed additive for animal production in 2013, and studies in monogastric animals such as pigs [10] and chickens [11] have demonstrated its effectiveness as a new organic selenium additive. Our previous research indicated that under long-term trial conditions, HMSeBA improved antioxidant capacity and nutrient apparent digestibility in dairy cows compared with inorganic selenium [12]. However, the high cost of organic selenium severely limits its long-term application in production practice. Furthermore, as a novel organic selenium additive, the potential of HMSeBA to increase milk selenium content has not been reported. Therefore, this trial compared the effects of HMSeBA and sodium selenite (SS) on selenium content in plasma and milk, and antioxidant capacity of lactating dairy cows under short-term trial conditions to provide scientific reference for its application in selenium-enriched milk production.

1.1 Experimental Materials

HMSeBA, containing 2% selenium, was provided by Adisseo (Shanghai) Co., Ltd. SS was an analytically pure chemical reagent with purity $ $99.5%.

1.2 Experimental Design and Animal Management

The trial employed a completely randomized design. Eight healthy Chinese Holstein cows with similar parity (2.01±1.23), $daysinmilk[(158\pm27)d], andmilkyield[(25.3\pm2.8)$ kg/d] were randomly divided into two groups (n=4) and supplemented with 0.3 mg/kg DM of either HMSeBA or SS in the basal diet. The pre-trial period lasted 2 weeks, followed by a 4-week formal trial period.

The trial was conducted at the Changping Base Environmental Control Chamber of the State Key Laboratory of Animal Nutrition. Cows were fed twice daily (07:00 and 19:00). HMSeBA and SS were each mixed with ground corn and sprinkled on the diet surface during morning feeding to ensure complete consumption. Cows had free access to feed and water, and were milked twice daily (07:00 and 19:00). The basal diet was prepared daily using a selenium-free premix (Sanyuan Seed Industry Technology Co., Ltd.), and the measured selenium content of the basal diet was 0.05 mg/kg DM. The composition and nutrient levels of the basal diet are shown in Table 1 .

1.3 Sample Collection and Analysis

1.3.1 Feed Intake During the 7 consecutive days of week 4 of the formal trial period, feed offered to all cows was recorded, and refusals were collected and weighed before morning feeding to calculate daily feed intake for each cow. Representative feed and refusal samples were collected daily during this period to determine dry matter content for calculating daily dry matter intake (DMI) for each cow.

1.3.2 Milk Yield and Composition During the 7 consecutive days of week 4 of the formal trial period, daily milk yield was recorded for each cow. The following formulas were used to calculate 4% fat-corrected milk yield and feed conversion efficiency:

4% fat-corrected milk yield (kg/d) = 0.4 × milk yield (kg/d) + 15 × milk fat yield (kg/d)
Feed conversion efficiency = 4% fat-corrected milk yield (kg/d) / DMI (kg/d)

Milk samples were collected each morning and evening and mixed at a 1:1 ratio. A 50 mL milk sample was placed in an analysis tube containing potassium dichromate preservative, stored overnight at 4°C, and sent the following day to the Ministry of Agriculture Milk and Dairy Product Quality Testing and Supervision Center (Beijing) for determination of milk composition using a MilkoScan™ FT 6000 analyzer (FOSS, Denmark) and somatic cell count (SCC) using a Fossomatic 5000 analyzer (FOSS, Denmark). Milk somatic cell score was calculated using the following formula:

Milk somatic cell score = $\log_2$[SCC (cells/mL) / 100,000] + 3

Another 50 mL milk sample was aliquoted into 10 mL centrifuge tubes and stored at -20°C. Milk selenium content was determined according to the method described by Heard et al. [13], where 1.0 g of milk sample was digested with 10 mL of nitric acid and perchloric acid mixture, followed by addition of 2 mL hydrochloric acid, and analyzed using inductively coupled plasma mass spectrometry (ICP-MS/MS) (Agilent 8800, Agilent Technologies, USA).

1.3.3 Serum Antioxidant Indices and Plasma Selenium Content On the final 2 days of week 4 of the formal trial period, blood was collected from the tail vein 3 h after morning feeding into 10 mL vacuum tubes in two portions. One portion was used for plasma separation: blood was immediately centrifuged at 3,000×g for 15 min at 4°C to obtain plasma, which was stored at -20°C for plasma selenium determination. Plasma selenium content was determined using the same method as for milk selenium, but with 0.5 g of plasma. Selenium transfer efficiency was calculated using the following formula:

Selenium transfer efficiency (%) = [milk yield (kg/d) × milk selenium content (µg/kg)] / [DMI (kg/d) × dietary selenium content (mg/kg)] × 1,000

The other portion was used for serum separation: blood was left at room temperature for 30 min, refrigerated overnight at 4°C, then centrifuged at 3,000×g for 15 min at 4°C to obtain serum, which was stored at -20°C for antioxidant capacity determination. Serum total antioxidant capacity (T-AOC), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, and malondialdehyde (MDA) content were measured using assay kits provided by Nanjing Jiancheng Bioengineering Institute.

1.4 Statistical Analysis

Trial data were subjected to statistical analysis using the MIXED procedure of SAS 9.4 software. In the statistical model, cows were considered random factors and treatment as a fixed factor. $P<0.05$ was considered statistically significant, while $0.05 \leq P<0.10$ indicated a trend toward significance.

2.1 DMI, Milk Yield, and Milk Composition

As shown in Table 2 , dietary supplementation with different selenium sources had no significant effects on DMI, milk yield, 4% fat-corrected milk yield, feed conversion efficiency, milk protein percentage, milk protein yield, lactose percentage, lactose yield, milk solids content, milk non-fat solids content, or milk somatic cell score of lactating dairy cows ($P>0.05$). Compared with the SS group, the HMSeBA group showed a trend toward decreased milk fat percentage ($P=0.0603$) and milk fat yield ($P=0.0552$).

2.2 Selenium Content in Plasma and Milk

As shown in Table 3 , plasma and milk selenium contents in the HMSeBA group were significantly higher than those in the SS group ($P<0.05$), increasing by

39.63% and 77.96%, respectively. Selenium transfer efficiency in the HMSeBA group was 15.7%, higher than the 9.0% observed in the SS group.

2.3 Serum Antioxidant Capacity

As shown in Table 4 , dietary supplementation with different selenium sources had no significant effect on serum GSH-Px activity in dairy cows ($P>0.05$). However, compared with the SS group, serum T-AOC and SOD activity in the HMSeBA group were significantly higher ($P<0.05$), while MDA content was significantly lower ($P<0.05$).

3 Discussion

From a practical production perspective, omitting selenium supplementation results in sub-health status in dairy cows, with inorganic selenium typically used in practice. However, compared with inorganic selenium, organic selenium offers greater absorption efficiency [14], higher biological activity [15], greater tissue retention [11], and lower toxicity. As this trial aimed to compare the effects of a novel organic selenium source, HMSeBA, with SS on milk selenium retention rate under short-term conditions, only organic and inorganic selenium groups were established. The U.S. Food and Drug Administration (FDA) recommends a selenium supplementation rate of 0.3 mg/kg DM (including both organic and inorganic forms), as this dietary selenium level meets the nutritional requirements of dairy cows. Therefore, this trial set the selenium supplementation level at 0.3 mg/kg DM.

3.1 Effects of Dietary Selenium Sources on DMI, Milk Yield, and Milk Composition of Lactating Dairy Cows

Selenium is an indispensable trace element for maintaining normal animal growth. When dietary selenium concentrations are extremely deficient or at toxic doses, animal performance may be affected [16]. When animals have insufficient selenium stores, supplementation with appropriate dietary selenium levels can promote growth, with studies indicating that organic selenium is more effective than inorganic selenium [17-19]. This trial used the FDA-recommended selenium supplementation level to add HMSeBA and SS to the diet. At this supplementation rate, cows in the HMSeBA and SS groups consumed an average of 5.7 and 5.6 mg selenium daily, respectively, with plasma selenium contents of 84.4 and 60.4 µg/kg, both within the appropriate plasma selenium range (51-85 µg/kg) recommended by Villar et al. [20]. Consequently, no significant differences in production performance were observed, consistent with long-term trial results reported by Calamari et al. [21]. However, unlike these studies, the current trial showed a trend toward decreased milk fat percentage and milk fat yield in the HMSeBA group compared with the SS group. Fraser et al. [22] previously reported a negative correlation between milk fat yield and blood selenium content during the first season of selenium supplementation, while Pehrson et al. [23] attributed this negative relationship to selenium' s involvement in the oxidation process of the tricarboxylic acid cycle and fatty acid metabolism.

3.2 Effects of Dietary Selenium Sources on Selenium Content in Plasma and Milk of Lactating Dairy Cows

Absorbed selenium in blood combines with α-globulin, β-globulin, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and albumin, with plasma selenium primarily bound to albumin [24]. Villar et al. [20] reported that appropriate plasma selenium content ranges from 51 to 85 μg/kg, requiring certain dietary selenium supplementation levels to achieve this range. Studies have shown that equivalent doses of organic selenium are more effective than inorganic selenium in increasing blood selenium content in dairy cows [3-5]. Weiss et al. [25] suggested that blood selenium content can differ by approximately 20% between cows fed organic versus inorganic selenium. In this trial, plasma selenium content was within the normal range, with the HMSeBA group showing a 39.6% higher plasma selenium content than the SS group, exceeding the reference value proposed by Weiss et al. [25] and demonstrating that HMSeBA as a novel organic selenium source is more effective in increasing plasma selenium content.

Rowntree et al. [26] reported that plasma selenium content is 3-5 times higher than milk selenium content, and elevated plasma selenium leads to increased milk selenium. The mammary gland preferentially absorbs and incorporates selenomethionine from organic selenium to synthesize milk proteins [23], which explains why many studies have shown organic selenium to be superior to inorganic selenium in increasing milk selenium content [6]. Juniper et al. [3] reported that dietary supplementation with selenium yeast increased milk selenium content by 34% compared with equivalent SS supplementation. Zhu et al. [27] found that cows consuming 15 g of nano-selenium daily reached a milk selenium content of 34 μg/kg on day 30. In this trial, milk selenium content in the HMSeBA group was 39.88 μg/kg, 78.0% higher than that in the SS group and exceeding results obtained with selenium yeast and nano-selenium in previous studies. This suggests that HMSeBA may be a more effective organic selenium additive for increasing milk selenium content. Givens et al. [28] reported that average milk selenium content in the UK is 10 μg/kg, and that replacing SS with selenium yeast in dairy cow diets can alleviate human dietary selenium deficiency by increasing milk selenium content. The current trial results suggest that HMSeBA may be superior to selenium yeast in increasing milk selenium content, making it suitable for selenium-enriched milk production with noticeable effects even under short-term conditions. The selenium transfer efficiency (dietary selenium converted to milk selenium) in the HMSeBA group was 15.7%, higher than the 9.0% in the SS group, consistent with findings by Calamari et al. [21] and further demonstrating that dairy cows have greater potential to utilize HMSeBA for selenium-enriched milk production compared with SS.

3.3 Effects of Dietary Selenium Sources on Serum Antioxidant Capacity of Lactating Dairy Cows

Selenoprotein GSH-Px is an important indicator of antioxidant capacity. As selenium is the active component of GSH-Px, supplementing dairy cow diets with appropriate selenium levels can increase blood GSH-Px activity and thereby enhance antioxidant capacity [29]. However, reports on the effects of selenium source on blood GSH-Px activity are controversial. Studies by Cao et al. [30], Tian et al. [31], and Xu et al. [32] in weaned piglets, broilers, and dairy cows, respectively, showed that appropriate selenium supplementation increased blood GSH-Px activity, with selenium yeast being more effective than equivalent inorganic selenium doses. In contrast, Juniper et al. [3] found that blood GSH-Px activity was not affected by selenium source at week 5 in lactating dairy cows, consistent with Weiss [6] who summarized nine reports on selenium source effects on blood GSH-Px activity. Ortman et al. [15] observed similar results at week 6, but found significantly higher blood GSH-Px activity in the selenium yeast group compared with the inorganic selenium group when the trial progressed from week 6 to 12. The current trial showed no significant difference in serum GSH-Px activity between the two groups at week 4, inconsistent with the 12-week trial results of Wang et al. [12], possibly because the 4-week trial duration was insufficient to significantly affect serum GSH-Px activity.

Blood T-AOC and SOD activity are also important indicators of antioxidant capacity, where T-AOC reflects the body' s compensatory capacity to external stimuli, and blood SOD activity reflects the ability to scavenge free radicals [33]. The current trial results showed that serum T-AOC and SOD activity in the HMSeBA group were higher than in the SS group, increasing by 43.46% and 23.32%, respectively. These findings are consistent with results from Xu et al. [32] and Huang et al. [34] comparing selenium yeast and SS effects on dairy cow antioxidant capacity. Notably, Xu et al. [32] reported that selenium yeast increased blood T-AOC and SOD activity by 37.7% and 15.4%, respectively, compared with SS—both lower than the increases observed in this trial, indicating that HMSeBA has better short-term effects on improving dairy cow antioxidant capacity. MDA is an important product of membrane lipid peroxidation, generated when free radicals act on lipids to cause peroxidation reactions. It can cause cross-linking and polymerization of vital macromolecules such as proteins and nucleic acids, exacerbate membrane damage, and exhibits cytotoxicity [35]. In this trial, the HMSeBA group significantly inhibited MDA generation compared with the SS group, consistent with findings by Gong et al. [36] and Huang et al. [34], demonstrating that HMSeBA is superior to SS in reducing lipid peroxidation.

In conclusion, under short-term trial conditions, dairy cows fed the novel organic selenium additive HMSeBA showed higher efficiency in converting dietary selenium to milk selenium than those fed SS, significantly increasing milk selenium content. The potential for selenium-enriched milk production was evident even under short-term conditions, without adversely affecting milk yield or composition, while significantly enhancing serum antioxidant capacity.

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Note: Figure translations are in progress. See original paper for figures.