Article

Effect of subcutaneous selenium injection and supplementary selenium source on blood selenium and glutathione peroxidase in feedlot heifers

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Abstract — This study measured the effect on glutathione peroxidase (GSH-Px) and selenium (Se) in whole blood and plasma associated with subcutaneous Se injections in beef heifers fed organic or inorganic Se. Heifers (n = 120) were randomly divided into 2 groups, 1 of which received subcutaneous Se injections. Both groups were given the same total mixed ration with 3 mg of organic or inorganic Se daily. Until week 2, heifers that had received Se injections showed higher concentrations of plasma Se and GSH-Px and whole blood Se (P < 0.001) than those having had no injections. Concentrations of plasma Se and GSH-Px were higher in the group receiving organic Se than the group receiving inorganic Se. Whole blood GSH-Px concentrations increased significantly (P < 0.001) throughout a 12-week period but were not affected by Se source. Combination of Se injections and supplementation could help maintain normal Se and GSH-Px blood status in beef heifers during the first few weeks in the feedlot.

Introduction

In areas where soil is poor in selenium (Se), deficiencies of this trace element in pasture- and forage-fed cattle is widespread (1). Previous reports showed a deficiency of Se in both forages and grains grown in eastern Canada (2). In Quebec, beef calves weaned on pasture are generally Se-deficient (3). Selenium is an integral component of the enzyme glutathione peroxidase (GSH-Px), an important part of the cellular antioxidant system that can metabolize hydrogen peroxide and a range of organic peroxides (4). Chemical characterization of GSH-Px has shown that selenocysteine is at the active site of the enzyme and its replacement with cysteine causes a large decrease in enzyme activity (5).

The most prevalent forms of Se given to ruminants are selenomethionine, selenocysteine, selenite, and selenate. The Se status can be manipulated by strategically using Se injections or by varying dietary sources of Se. The mechanism of intestinal absorption is different for inorganic Se and organic Se; factors that affect absorption of inorganic Se such as sulfate are less likely to influence absorption of organic Se (6). The metabolism of inorganic Se and organic Se within a cell also differs. Inorganic Se is used exclusively in the synthesis of selenocysteine, the precursor of seleno-specific enzymes like GSH-Px; selenomethionine is not directly used in the synthesis of those enzymes, being incorporated into any protein that contains methionine (7).
Reduced feed intake is the major nutritional problem in newly received beef cattle. Feed intake by stressed calves is low (8), averaging approximately 1.5% of BW during the first 2 wk after arrival of lightweight beef cattle (9). Low feed, and thereby low Se, intake makes correction of nutritional deficiencies difficult especially in Se-deficient animals. This could further compromise the animal’s immune function (8) and potentially lead to infectious diarrhea. Stowe et al (10) showed that serum Se increased within 1 mo following dietary Se supplementation. Selenium injection can restore the animal’s Se status during the first weeks after arrival at the feedlot until the animal regains its full Se status (11). Therefore the objectives of this study were to measure changes in GSH-Px and Se in the blood of beef heifers fed organic or inorganic Se and to compare changes in GSH-Px and Se in the effect of subcutaneous Se injection in beef heifers fed organic or inorganic Se.

### Materials and methods

#### Animals

The study was conducted at the Centre de Recherche en Sciences Animales de Deschambault (CRSAD) of the Institut de Recherche et de Développement en Agroenvironnement (IRDA) in Deschambault, Quebec during the summer of 2006. Animal care procedures followed the guidelines of the Canadian Council on Animal Care (11) and the protocol was approved by the Animal Care Committee of the CRSAD-IRDA. One hundred and twenty auction-market-derived Charolais-Simental crossbred heifers [7 mo of age; 289 ± 51 kg body weight (BW)] were purchased in central Quebec and used in this trial. At the beginning of the experiment, the heifers were weighed, blood was taken, and the animals were divided into 2 equal groups based on their body weight. Heifers in 1 group were given a subcutaneous injection of DL alpha-tocopherol acetate and sodium selenite combination (Dystosel; Pfizer Canada Animal Health Group, London, Ontario), 3.02 IU/kg BW and 0.13 mg/kg BW, respectively. The Se status of the animals was determined by measuring serum Se at admission (week 0). The mean heifers’ serum Se concentration (0.40 ± 0.02 μmol/L) was less than 0.68 μmol/L, which is considered deficient according to reference values (12).

#### Diets

Each group was randomly distributed into 12 pens (5 animals per pen) at week 0 based on body weight. All heifers were fed the same diet for 2 wk consisting of 50% of an adaptation diet (Table 1) and 50% hay. For this period the diet provided 1.25 ppm Se/animal per day. After this period and until the end of the experiment (12 wk), mineral supplements with organic Se yeast Sel-plex (Altech, Nicholasville, Kentucky, USA) or inorganic Se (sodium selenite) were added to the total mixed rations to provide approximately 3 mg of Se/animal per day. Dry matter content of the total mixed rations was determined by drying at 100°C for 48 h. Crude protein determination was done by the Kjeldahl method (13). Neutral detergent fiber component was measured according to the nonsequential procedures of Van Soest et al (14). Colorimetry was used to determine phosphorus (P) and atomic absorption spectroscopy was used to determine calcium (Ca), magnesium (Mg), potassium (K), and Se according to the methods 965.17 and 968.08 of the AOAC (13). Heifers were allowed access to the diet ad libitum, and the amounts of feed offered and refused were periodically recorded to determine dry matter intake.

#### Biochemical analyses

Every 2 wk and before the daily feeding, approximately 7 mL of blood were collected from the jugular vein using a 20-gauge (G) needle (Becton Dickinson, Rutherford, New Jersey, USA), and placed into 7-mL vacuum tubes with sodium heparin (Vacutainer; Becton Dickinson, Rutherford, New Jersey, USA). All samples were divided into 2 subsamples for whole blood and plasma analyses. Subsamples for plasma were centrifuged within 24 h at 1200 × g for 20 min. The individual whole blood and plasma samples from the same pen were pooled and maintained at −20°C until analysis. Based on the method described by Paglia and Valentine (15), enzymatic activities of GSH-Px in plasma and whole blood were measured by a kinetic-enzymatic technique using Randox commercial kits (Randox Laboratories Canada, Mississauga, Ontario) on a Beckman-Synchron CX5 autoanalyzer (Beckman Instruments; Fullerton, California, USA). Plasma and whole blood Se were measured by high performance liquid chromatography (HPLC) using a modified method of Hawkes and Kuthnik (16). Serum vitamin E was evaluated by HPLC according to Gueguen et al (17).

### Table 1. Ingredients and chemical composition of the total mixed ration

<table>
<thead>
<tr>
<th>Item</th>
<th>Adaptation</th>
<th>Organic Se</th>
<th>Inorganic Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass silage</td>
<td>24.8</td>
<td>23.3</td>
<td>23.3</td>
</tr>
<tr>
<td>Corn silage</td>
<td>21.9</td>
<td>23.6</td>
<td>23.6</td>
</tr>
<tr>
<td>Ground corn</td>
<td>47.0</td>
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<td>46.7</td>
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<tr>
<td>Soya meal</td>
<td>4.6</td>
<td>4.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Urea</td>
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<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Mineral supplement&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Chemical composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, % of DM</td>
<td>55.6</td>
<td>52.2</td>
<td>51.7</td>
</tr>
<tr>
<td>CP, % of DM</td>
<td>12.4</td>
<td>14.2</td>
<td>13.4</td>
</tr>
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<td>NDF, % of DM</td>
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<td>32.6</td>
<td>33.0</td>
</tr>
<tr>
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<td>0.52</td>
<td>0.59</td>
</tr>
<tr>
<td>P, % of DM</td>
<td>0.36</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>Mg, % of DM</td>
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<td>0.19</td>
<td>0.20</td>
</tr>
<tr>
<td>K, % of DM</td>
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<td>1.29</td>
<td>1.32</td>
</tr>
<tr>
<td>Se, mg/kg of DM</td>
<td>0.25</td>
<td>0.36</td>
<td>0.31</td>
</tr>
<tr>
<td>NE&lt;sub&gt;V&lt;/sub&gt;, Mcal/kg of DM&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.76</td>
<td>1.74</td>
<td>1.74</td>
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<tr>
<td>NE&lt;sub&gt;C&lt;/sub&gt;, Mcal/kg of DM&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.13</td>
<td>1.13</td>
<td>1.13</td>
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</tbody>
</table>

<sup>a</sup> Adaptation diet: 8% Ca; 3.0% P; 5% Mg; 4.4% sodium (Na); 12.0% K; 3.0% sulfur (S); 500 mg/kg copper (Cu); 3150 mg/kg manganese (Mn); 5000 mg/kg zinc (Zn); 30 mg/kg cobalt (Co); 50 mg/kg I; 200 000 IU vitamin A; 20 000 IU vitamin D-3; and 1 300 IU vitamin E. Se diets: 24% Ca; 1.3% Mg; 10.0% Na; 1.5% K; 1.5% sulfur (S); 1000 mg/kg Cu; 1400 mg/kg Mn; 3000 mg/kg Zn; 50 mg/kg Co; 100 mg/kg I; 400 mg/kg vitamin A; 400 IU vitamin D-3; and 1500 IU vitamin E.

<sup>b</sup> Calculated using published values of feed ingredients (40).
Statistical analysis
Mixed Procedure (SAS statistical software version 8; SAS Institute, Cary, North Carolina, USA) was used to evaluate treatment effects arranged as a $2 \times 2$ factorial with 2 levels of Se injections (with and without Se injections) and 2 dietary sources of Se supplementation (inorganic and organic Se). The model used to analyze pen’s averaged weights, Se, GSH-Px and vitamin E concentrations contained the fixed effects of Se injection, dietary Se source, Se injection $\times$ dietary Se source interaction, repeated effect of time and associated interactions of time with the main effects. Appropriate orthogonal contrasts were used to estimate differences between treatments least square means at a specific sampling time or to compare differences between treatments at different sampling times. On the other hand, time was the model's covariable in the model used to analyze treatment effect on pen's averaged weights. Slopes of the regression lines of time interactions with the main effects were designated as pen’s averaged weight gain coefficients. The main effects on weight gain were then estimated by testing heterogeneity of slopes between treatments. A $P$-value $\leq 0.05$ was considered indicative of significance. The mixed model analysis estimation method was restricted maximum likelihood (REML). All mean values are reported with standard error of the mean ($\pm S\bar{E}$).

Results
Dry matter intake and weight gain
Selenium source did not significantly affect dry matter intakes that averaged 9.4 $\pm$ 0.88 kg dry matter (DM)/day for heifers fed inorganic Se and 8.9 $\pm$ 0.81 kg DM/day for heifers fed organic Se.

Mean weight gain was 1.34 kg/d. Neither the injection of sodium selenite at the beginning of the experiment nor the source of Se supplementation had significant effects on heifers' weight gain ($P > 0.05$). No interactions were found between the effect of injection and Se source.

Plasma and whole blood GSH-Px
After 2 wk, heifers that received a sodium-selenite injection showed significantly higher plasma GSH-Px ($P < 0.001$) than those that did not (Figure 1A). No treatment effects were detected for the other weeks. Results of week 8 were not reported because plasma samples were inadvertently lost. Sources of Se intake did not significantly change plasma GSH-Px values until week 12 ($P < 0.05$) (Figure 1B). There were no interaction effects between Se injection and Se sources ($P > 0.90$).

Whole blood GSH-Px was not affected by sodium-selenite injections as shown in Figure 1C. Starting from week 4, whole blood GSH-Px concentrations increased significantly ($P < 0.001$) with both Se sources (Figure 1D) but the interaction between time and Se source showed that the increase was significantly higher ($P = 0.004$) with organic than with inorganic Se.

Plasma and whole blood Se
At the beginning of the experiment (week 0), serum Se concentrations for the 2 groups of heifers (injected and non-injected)
were 0.41 ± 0.03 μmol/L and 0.38 ± 0.02 μmol/L, respectively; these were not significantly different. Subcutaneous Se injections had a significant effect \((P < 0.01)\) on plasma and whole blood Se during the first 2 wk after injection (Figures 2A, 2B) and no treatment effects were detected for the other weeks.

Effects of Se source on plasma and whole blood Se were greater than those observed with GSH-Px. Starting on week 4 or week 6, for plasma or whole blood Se, respectively, concentrations were significantly higher \((P < 0.0001)\) with organic than with inorganic Se (Figures 2C, 2D).

**Serum vitamin E**

Serum vitamin E concentrations showed no significant variations throughout the experiment. The average concentrations were 6.63 ± 0.46 μmol/L and 5.75 ± 0.41 μmol/L, respectively for injected and non-injected groups \((P > 0.10)\) and 6.14 ± 0.06 μmol/L and 6.26 ± 0.49 μmol/L, respectively for the groups receiving organic Se and inorganic Se. There was no significant interaction between serum vitamin E and Se or GSH-Px levels over time \((P > 0.10)\).

**Discussion**

Dry matter intakes were not significantly affected regardless of the Se source. This result is in agreement with previous reports that showed no effect of Se source on feed intake in growing beef heifers (18) or lambs (19).

Injection of sodium selenite at the beginning of the experiment had no significant effect on heifers’ weight gain, in agreement with previous studies (20–23). However, in some reports more than 1 injection of sodium selenite resulted in significant weight gains (22,24). In our 10-week study, heifers’ weight gain was not significantly affected by the source of Se supplementation as reported by Gunter et al (23).

Selenium functions in the antioxidant system as an essential component of a family of glutathione peroxidase enzymes. The most well-known are cellular GSH-Px (mainly found in whole blood, and plasma) and extracellular GSH-Px (25). Both enzymes prevent oxidative damage in extra- and intra-cellular milieu. In our study, heifers injected with sodium-selenite showed significantly higher plasma GSH-Px after 2 wk. In 9-month-old calves, Thompson et al (26) observed a similar increase in serum GSH-Px (equivalent to plasma GSH-Px) following injection of Se at 0.1 and 0.2 mg/kg BW. Whole blood GSH-Px was not affected by sodium-selenite injection as reported by Rowntree et al (27). Concentrations of plasma GSH-Px are supposed to reflect short time changes in Se intake (26) and the high concentrations of GSH-Px in whole blood (100-fold plasma GSH-Px) might conceal small variations.

Plasma GSH-Px values were affected by the sources of Se intake at week 12. This is much later than has been reported in the literature. Indeed, Thompson et al (28) demonstrated an increase of plasma GSH-Px in 5-month-old calves after 30 d of their transfer to a property where the pasture Se concentration was considerably higher (average level = 0.113 mg/kg DM). Similarly, during the first 40 d of the experiment inorganic Se significantly enhanced plasma GSH-Px compared to selenomethionine (29).

![Figure 2. Concentrations of Se in plasma (2A, 2C) and whole blood (2B, 2D) in heifers with (Se+) or without (Se−) Se administered by subcutaneous injection and fed organic (org Se) or inorganic Se (inorg Se). The data points correspond to the mean of 12 pens (± S). **Indicates a significant difference \((P < 0.01)\) and *** indicates a highly significant difference \((P < 0.001)\).](image-url)
Both Se sources increased concentrations of whole blood GSH-Px from week 4 until the end of the experiment. The interaction between time and Se source showed that this increase was greater with organic than with inorganic Se, as reported in previous studies (30,31). Furthermore, some reports demonstrated a positive effect of organic Se on whole blood GSH-Px compared to inorganic Se (32,33).

Subcutaneous Se injections enhanced plasma and whole blood Se during the first 2 wk after injection but this effect decreased markedly at week 3 as previously reported by Van Vleet et al (34) after Se injection of 0.0825 mg/kg BW. This decline is less marked than that noticed by Thompson et al (26) at week 4 after Se injection of 0.1 mg/kg in calves grazing on a pasture deficient in Se (0.018 mg/kg BW). This difference is mainly due to Se content in the diet in our experiment. In heifers receiving Se injections, mean concentration of serum Se observed at week 2 (0.772 ± 0.087 μmol/L) (data not shown) was in the range of normal values for calves of 30 to 300 days of age (12).

In this experiment, selenomethionine, the major selenium-containing compound in organic Se yeast (35), is incorporated into general body proteins (36) with little effect on GSH-Px concentration. The balance between protein synthesis and catabolism may explain the gradual increase of Se concentrations that reach a plateau in plasma earlier than in whole blood. Ortman et al (31) reported a gradual increase of whole blood Se concentration after submitting 17-month-old heifers to a daily supplement (2 mg) of organic and inorganic Se. The authors noticed an increase of Se concentrations in both plasma and whole blood but only plasma Se reached a plateau after 11 weeks of supplementation. In beef cattle, Gunter et al (23) and Nicholson et al (32) obtained similar results.

Even though organic Se supplementation was more efficient than inorganic Se in enhancing blood Se, both Se sources induced adequate blood concentrations according to reference values (12). Throughout the experiment, vitamin E concentrations were also adequate compared to reference values (37) and they did not show significant effects on Se or GSH-Px status as previously demonstrated in heifers and dairy cows (38,39).

This study showed that Se injection introduced a short-term increase in plasma GSH-Px activity and plasma and whole blood Se concentrations. Also, feeding Se yeast versus sodium selenite-induced higher plasma GSH-Px activity after 12 wk and higher plasma or whole blood Se concentrations after 4 wk. Overall, Se injection to deficient calves prior to dietary Se supplementation can give a normal Se and GSH-Px status to beef heifers especially during the starting period in the feedlot.

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References


