Effects of Pre- or Postpartum Selenium Supplementation on Selenium Status in Beef Cows and Their Calves

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ABSTRACT: The effect of Se supplementation before or after calving on Se status in deficient cows and their calves was studied using 72 beef cows in two experiments. In Exp. 1, cows calving in February or March 1997 were supplemented orally for 15 d in late pregnancy with 13.0, 32.5, or 45.5 mg of Se/d as sodium selenite. Glutathione peroxidase (GSH-Px) activities were measured in red blood cells (RBC) or plasma of cows and calves at d 15 and between d 17 and 88 after calving. In Exp. 2, cows calving in January 1997 were supplemented orally with 0, 13.0, or 32.5 mg of Se/d for 15 d postpartum, and calves were injected with 1.38 mg of Se when 2 d old and at an average age of 49 d. The GSH-Px activities were measured in 30-d-old calves and in cows and calves between d 77 and 115 after calving. In both experiments, Se supplementation resulted in adequate Se status for the dams. The increase in RBC GSH-Px activity was faster with 45.5 mg of Se/d, and GSH-Px activities remained high for up to 98 d after the end of supplementation. The improvement in Se status in calves as a result of maternal supplementation was greater in Exp. 1 than in Exp. 2, suggesting that the placental transfer of Se is more efficient than milk transfer. Prepartum oral Se supplementation of deficient beef cows with 13.0 mg of Se/d for 15 d allowed adequate Se status of dams and calves, and 45.5 mg of Se/d resulted in a faster improvement of Se status. Parenteral administration of 1.38 mg of Se to newborn calves did not sustain normal Se status in calves issued from deficient cows.

Key Words: Selenium, Glutathione Peroxidase, Cows, Calves

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Introduction

Nutritional muscular dystrophy that is due to Se deficiency is a common disease in calves in many areas in the world. Moreover, Se deficiency has been shown to reduce reproductive performance in cows by increasing the incidence of retained placenta, metritis, or cystic ovaries (Harrison et al., 1984) and to impair immune response (Droke and Loerch, 1989; Stabel et al., 1989). Prevention can be performed via oral Se supplementation, and a wide range of values have been reported for Se absorption, from 10 to 16% (Koenig et al., 1991) to 51% (Harrison and Conrad, 1984). Because Se efficiently passes through the placental barrier in cattle (Van Saun et al., 1989), prevention of deficiency in calves can be expected via maternal supplementation before calving.

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Among criteria used for assessment of Se status, whole blood, plasma (Smith et al., 1988), or liver (Van Saun et al., 1989) Se concentrations have been measured in cows and in calves. Glutathione peroxidase (GSH-Px) activities have been proposed as the best estimate of Se status because the enzyme is a physiologically functional form of Se (Smith et al., 1988). Because 98% of GSH-Px activity in blood is associated with erythrocytes (Scholtz and Hutchinson, 1979) and because GSH-Px is incorporated into red blood cells (RBC) during their formation, the analysis of RBC for GSH-Px activity should be effective for assessment of long-term Se status, and plasma GSH-Px concentrations should reflect short-term Se supply (Gerloff, 1992).

The aim of this study was to determine the effects of peroral Se supplementation on RBC and plasma GSH-Px activities in beef cows and their calves. Three levels of supplementation were used for 15 d, providing approximately the same amount of Se as a diet containing 2 ppm of Se, as required by beef cows (NRC, 1984), during 100, 250, and 350 d.
Experimental Procedures

**Cows and Diets.** All procedures for this study complied with recognized standards for the humane care and treatments of animals. Seventy-two multiparous Salers beef cows (average BW 600 kg, average body condition score 3.5 on a 5-point scale; Petit and Agabriel, 1993) were used in two experiments. Hay was given in a limited amount to pregnant cows (10 kg DM/d) and was provided ad libitum until calving. Additionally, after calving, cows received rolled barley (1 kg/d) and a mineral-vitamin mix (100 g/d). The Se content of feedstuffs was .024, .10, and 2.48 mg/kg DM for hay, barley, and mineral-vitamin mix, respectively.

In Exp. 1, 36 cows with expected calving dates in February or March 1997 were divided into three groups with identical expected mean calving dates and assigned to receive 13.0, 32.5, or 45.5 mg of Se as sodium selenite daily. Supplemental Se was given orally, after mixing with 50 g of barley, for 15 consecutive days preceding calving, from January 2 to January 16. Five cows were excluded from the experiment because of death, disease, abortion, or calf mortality. Cows were tested for RBC GSH-Px activity just before the beginning of the experiment. Cows and calves were tested for RBC GSH-Px activity 15 d after calving and for RBC and plasma GSH-Px activities on April 24, between 17 and 88 d after calving (average 54 d).

In Exp. 2, 36 cows with expected calving dates in January 1997 were divided into three groups and assigned to receive either .0, 13.0, or 32.5 mg of Se as sodium selenite daily. Supplemental Se was given orally for 15 d from d 2 to 16 postpartum. Additionally, calves received i.m. 1.38 mg of Se as sodium selenite (Selepherol ND, Vetoquinol SA, Lure, France) when 2 d old and on March 3 (average 49 d of age). Five cows were excluded from the experiment. Cows were tested for RBC GSH-Px activity just before the beginning of the experiment. Cows and calves were tested for RBC GSH-Px activity 15 d after calving and for RBC and plasma GSH-Px activities on April 24, 217 and 88 d after calving (average 54 d).

**Sample Processing and Analysis.** Blood samples were collected from the jugular vein into 10-mL heparinized tubes (5,000 USP units of heparin/L of whole blood) and kept at 4°C. Less than 15 h after sampling, the tubes were centrifuged at 3,000 · g for 15 min at 4°C. The RBC were washed three times in an isotonic saline solution. Determination of GSH-Px was performed using a commercial kit (Ransel ND, Randox Laboratories, Crumlin, U.K.), based on the method of Paglia and Valentine (1967). Activity of GSH-Px was expressed in enzyme units (1 u = 1 μmol of NADPH oxidized per minute). For RBC GSH-Px determination, results were expressed as units per gram of hemoglobin (Hb), assuming a concentration of 328 g of Hb/L of RBC.

**Statistical Analysis.** Validity of the differences was analyzed using the general linear model of SYSTAT (Version 5.03 for Windows, SYSTAT, Evanston, IL). For data from samples taken before experiments or at a fixed interval from the beginning of supplementation (i.e., at a fixed date in Exp. 1 or at a fixed age in Exp. 2), the model used was

\[ Y_{ij} = + L_i + e_{ij} \]

where \( Y_{ij} \) is the dependent variable, \( L_i \) is mean effect of Se level as a categorical variable, and \( e \) is random residual.

For data from samples taken at a date or age varying among animals (i.e., for samples taken 15 d postpartum in Exp. 1 and for samples taken 77 to 115 d postpartum in Exp. 2), the model used was

\[ Y_{ij} = + L_i + C V_j + (L \times C V)_{ij} + e_{ij} \]

where \( CV_j \) is the continuous variable (interval between the beginning of supplementation and sampling (ISS) in Exp. 1, and ISS minus 100 d (ISS100) in Exp. 2), and \((L \times CV)_{ij}\) is the interaction of Se level \( \times CV_j \).

When the effect of Se level was significant, the analysis was followed by a Tukey pairwise comparison test (Winer et al., 1991). When the interaction of Se level \( \times ISS \) or \( \times ISS100 \) was significant, differences between slopes representing the variation of the dependent variable against ISS for the three Se levels were tested with contrasts.

For comparison between results of Exp. 1 and 2, the model used was

\[ Y_{ij} = + E_i + A_j + (E \times A)_{ij} + e_{ij} \]

where \( E_i \) is the experiment, \( A_j \) is the supplemental amount of Se given to cows as a continuous variable, and \((E \times A)_{ij}\) is the interaction of experiment \( \times Se \) amount.

Statistical significance was declared at \( P < .05 \) for all comparisons.

**Results**

**Experiment 1.** The RBC GSH-Px activities were not different between groups before supplementation (3.1 ± 1.14 u/g of Hb). The Se supplementation had important effects on RBC GSH-Px activities 15 d postpartum; 45.5 mg of Se/d gave significantly greater values than did 13.0 mg (Table 1). Statistical analysis of all data did not show any effect of ISS or interaction between ISS and Se level. However, graphic examination of data shows that RBC GSH-Px...
activities increased while ISS was below 90 d (Figure 1). Statistical analysis of data from cows with ISS shorter than 90 d (10, 7, and 7 cows for 13.0, 32.5, and 45.5 mg of Se/d, respectively) showed an interaction between Se level and ISS (P < .05). The slopes representing the increase of RBC GSH-Px activities for 1-d ISS were 2.71 and 3.44 u/g of Hb with 13.0 and 32.5 mg Se/d, respectively. The slope was significantly lower (.06 u/g of Hb) with 45.5 mg Se/d. Between 17 and 88 d postpartum (98 d after the end of Se supplementation), RBC GSH-Px activities were slightly modified compared with values 15 d postpartum, and 32.5 mg of Se/d gave significantly greater values than 13.0 mg, but differences were not significant between 13.0 and 45.5 mg of Se/d, in contrast to what was observed 15 d postpartum. Plasma GSH-Px activities did not differ among Se levels.

The RBC GSH-Px activities in 15-d-old calves were greater than values of cows. The effect of ISS was significant, and slopes representing the variation of RBC GSH-Px for 1-d ISS (Figure 1) were 2.97, 1.44, and -9 u/g of Hb for 13.0, 32.5, and 45.5 mg of Se/d, respectively, with a significant difference between 13.0 and 45.5 mg. The RBC GSH-Px activities of 15-d-old calves (u/g of Hb) were significantly correlated with values of their dams at the same date:

Calf RBC GSH-Px = 59.8
+ 1.26Maternal RBC GSH-Px
(r² = .52; P < .001)

Between 17 and 88 d of age, RBC GSH-Px activities in calves were lower than at 15 d of age, particularly with 13.0 or 45.5 mg of Se/d, and were no longer correlated with maternal values (r² = .08; P > .05). Supplementation with 32.5 or 45.5 mg of Se/d gave significantly greater values than 13.0 mg for RBC and plasma GSH-Px activities.

Experiment 2. Between 77 and 115 d postpartum, unsupplemented cows had much lower RBC GSH-Px activities than supplemented cows, but greater than before the experiment (Table 2). The effect of ISS100 was significant (Figure 2) without interaction between Se level and ISS100. The mean daily increase of RBC GSH-Px activity was 2.57 u/g of Hb. However, no effect of Se level could be observed on plasma GSH-Px activities. In 30-d-old calves, 32.5 mg of Se/d gave much greater RBC GSH-Px activities than did .0 mg. Differences between Se levels were no longer observed between 77 and 115 d of age. The effect of ISS100 was significant, with a mean 2.77 u/g of Hb increase of RBC GSH-Px activities for 1-d ISS, and a trend (P = .077) toward faster increases in calves from supplemented cows (Figure 2). The RBC GSH-Px activities in cows and their calves (u/g of Hb) were significantly correlated 77 to 115 d after calving:

Calf RBC GSH-Px = 78.6
+ .60Maternal RBC GSH-Px
(r² = .44; P < .001)

Comparison of Results from Experiments 1 and 2. The RBC or plasma GSH-Px activities did not differ between Exp. 1 and 2, neither in cows on April 24, nor in calves at 15 or 30 d of age or on April 24. The effect of Se amount was significant on all values except plasma GSH-Px activities in cows on April 24. Increases of RBC GSH-Px activities for each supplemental milligram of Se were 1.86 and 2.13 u/g of Hb for the cows and calves on April 24, respectively. A significant interaction between experiment and the Se amount was observed for RBC GSH-Px activities in the young calves, the slopes were 6.52 and 2.28 u/g of Hb for each daily supplemental milligram of Se in Exp. 1 and 2, respectively.

Discussion

Different maximum tolerable levels of Se have been proposed, from 2 ppm (NRC, 1984) to 4 or 5 ppm...
(Underwood, 1977). With 5 ppm of Se in the diet, signs of toxicity take weeks, or even months, to appear (Underwood, 1977), so that concentrations below 5 ppm used in our experiment for 15 d should not be expected to produce toxic effects.

Because the variations of GSH-Px results between laboratories are important, several values have been proposed as a normal range. Erskine (1993) recommended activities in the range of 45 to 85 u/g of Hb. A lower limit of 50 u/g of Hb can be calculated from concentrations of plasma Se indicative of adequate Se status (more than .07 μg/mL; Smith et al., 1988) and the relation between plasma Se (μg/mL) and the RBC GSH-Px activity (nanomoles of NADPH oxidized per minute per milligram of Hb) established by Stevens et al. (1985) for cattle (RBC GSH-Px = 216 Plasma Se + 35.6; r² = .90; P < .001).

Selenium Status of Cows. According to these recommendations, initial activities in cows in both experiments were very low. Unsupplemented cows in Exp. 2 had much lower RBC GSH-Px activities 77 to 115 d postpartum than did treated cows. However, despite a mean activity (67.2 u/g of Hb) in a normal range, 5 of the 10 unsupplemented cows had low or borderline values (50 u/g of Hb or below). The diet contained approximately .024 ppm Se before calving and .05 ppm Se after calving, well below the .2 ppm recommended by the NRC (1984). Plasma GSH-Px activities were not lower in unsupplemented cows than in supplemented cows. This measure has already been reported to be poorly related with Se status (Podoll et al., 1992) or Se intake (Weiss et al., 1990).

The improvement of Se status after oral supplementation was important in both experiments. This suggests a good availability of Se selenite, despite the lower absorption and retention observed in sheep by Koenig et al. (1997) with a forage based-diet compared with a concentrate-based diet. In Exp. 1, 15 d

![Graphs of RBC GSH-Px for Cows and Calves](image1)

Figure 1. Experiment 1: individual values and regression between red blood cell glutathione peroxidase (RBC GSH-Px) activities of cows and calves 15 d after calving, and interval from beginning of a 15-d Se supplementation of pregnant cows (■ = 13.0, ⋄ = 32.5, ▲ = 45.5 mg of Se/d).

![Graphs of RBC GSH-Px for Cows and Calves](image2)

Figure 2. Experiment 2: individual values and regression between red blood cell glutathione peroxidase (RBC GSH-Px) activities of cows and calves 77 to 115 d after calving, and interval from beginning of a 15-d Se supplementation of cows postpartum (■ = 0, ⋄ = 13.0, ▲ = 32.5 mg of Se/d).
postpartum (i.e., 39 to 110 d after the beginning of oral supplementation), all Se levels produced normal RBC GSH-Px activities. This increase in RBC GSH-Px activity was consistent with the bovine RBC life span of approximately 120 d (Schalm et al., 1975) and the lack of incorporation of Se into GSH-Px by mature RBC (Hafeman et al., 1974). In cows, improvement in RBC GSH-Px activity has previously been observed 30 d after the beginning of an oral supplementation (Hogan et al., 1990) but with greater initial values than observed in our experiment (more than 50 u/g of Hb). In our experiment, the increase in RBC GSH-Px activities in cows was rapid and continued up to 90 d after the beginning of an oral supplementation when 13.0 or 32.5 mg of Se/d were given. However, no time effect could be observed with 45.5 mg of Se/d, owing to high-RBC GSH-Px activities even in cows with low ISS. Philippo et al. (1987) suggested that, in deficient animals, Se supplementation was first used for repletion of tissue reserves and then for synthesis of RBC GSH-Px. Consequently, the time necessary for tissue repletion can be expected to be shorter with high amounts of supplemental Se, and this could explain the faster increase in RBC GSH-Px activities observed with 45.5 mg of Se daily.

As expected from the life span of bovine RBC, the effect of 15 d of oral Se supplementation on RBC GSH-Px in cows persisted for 98 and 61 to 99 d after the end of supplementation for Exp. 1 and 2, respectively.

**Selenium Status of Calves.** Placental transfer of Se has already been demonstrated in cattle because the maternal supplementation of cows in late gestation increases Se reserves in the liver of the fetus or newborn (Van Saun et al., 1989; Abdelrahman and Kincaid, 1995). In Exp. 1, as observed in dams, a shorter ISS was necessary to reach high-RBC GSH-Px activity when Se supply consisted of 45.5 mg/d vs 13.0 or 32.5 mg/d. The correlation between RBC GSH-Px activities in cows and young calves observed 15 d after calving in Exp. 1 could also be due to colostral transfer of Se, because 20 to 30% increases in Se content in the colostrum of cows receiving 2 to 3 mg of supplemental Se daily have been reported (Stowe et al., 1988; Abdelrahman and Kincaid, 1995). The RBC GSH-Px activities of calves were greater than maternal values, as previously observed by House and Bell (1994) on liver Se, and by Van Saun et al. (1989) on whole-blood GSH-Px activity. In Exp. 1, at 17 to 88 d of age, the Se status in calves was still satisfactory, with greater RBC GSH-Px activities when prepartum supplementation of cows consisted of 32.5 or 45.5 mg of Se daily compared with 13.0 mg daily. This persistency of effects is consistent with the results of Pehrson and Johnsson (1985) who showed that two oral administrations of 30 mg of Se during late pregnancy maintained a normal Se status in calves for 2 to 3 mo.

Conflicting results have been reported about the effects that Se supplementation has on milk Se content. The increase of Se concentration in milk after Se supplementation has been shown to be of very little amplitude 7 d postpartum when serum Se concentrations suggest a deficient Se status, even in treated cows (Stowe et al., 1988). However, milk Se concentration has been shown to increase linearly with Se intake between 2 and 6 mg/d in dairy cows with normal plasma Se values (Maus et al., 1980), and a 28% increase in milk content has been reported 18 d postpartum in ewes after Se injection (Norton and McCarthy, 1986). In our experiment, the significant effect of postpartum maternal Se supplementation on Se status in calves and the significant correlation between maternal and calve RBC GSH-Px activities at 77 to 115 d of age in Exp. 2 suggested transfer of Se from the dams to the milk. However, this transfer was less efficient than placental transfer because the RBC GSH-Px activities in calves increased much more rapidly as a function of Se amount in Exp. 1 with prepartum supplementation than in Exp. 2 with postpartum supplementation. Various concentrations of Se in milk from cows receiving supplemental Se have been reported. Ammerman et al. (1980) ob-
served for beef cows values decreasing from approximately .015 mg/L 2 wk after calving to .010 8 wk after calving, and Maus et al. (1980) reported values between .029 and .064 mg/L in midlactation dairy cows. Comparable values (.04 to .06 mg/L) have been reported in colostrum (Abdelrahman and Kincaid, 1995). Such values are below or close to the usual .3-ppm requirement for young calves (NRC, 1988), so that relying on milk to provide adequate Se to calves is risky. The decrease in calves’ RBC GSH-Px activities observed in Exp. 1 between 15 and 17 to 88 d of age is consistent with this hypothesis of a poorly efficient milk transfer of maternal Se.

One i.m. injection of 1.38 mg of Se 2 d postpartum without maternal supplementation could not sustain a normal Se status in young calves because, when 30 d old, 7 of the 10 calves from unsupplemented cows in Exp. 2 had individual RBC GSH-Px activities lower than 55 ug of Hb. The lack of increase in blood GSH-Px activity when newborn calves are Se deficient has already been observed 50 to 120 d after s.c. administration of 6 mg of Se (Phillippo et al., 1987). The little effect of i.m. Se in our experiment cannot be explained by the short interval between injection and sampling (28 d) because RBC GSH-Px activities increased at the same age when dams where supplemented postpartum. It can be due to slow incorporation of Se into RBC GSH-Px, as already discussed for cows receiving low levels of supplemental Se in Exp. 1, or to the low amount of injected Se compared with amounts reported in the literature: .078 mg/kg body weight (Weiss et al., 1984) or 6 mg (Phillippo et al., 1987). The Se status in calves was improved by a second injection (average 49 d of age) as attested by high RBC GSH-Px activities 62 d later.

Implications

In this experiment, parenteral supplementation of newborn beef calves with 1.38 mg of Selenium (Se) did not result in normal Se status 1 mo later when dams were Se-deficient and remained unsupplemented postpartum. However, this study demonstrated that adding 13.0 to 45.5 mg of dietary Se daily to beef cows for 15 d in late pregnancy produced satisfactory Se status in cows and their calves, even 3 mo after the end of supplementation. Because 45.5 mg of Se daily produced faster effects, this higher amount can be proposed when calving is to occur soon after the period of supplementation.

Literature Cited


