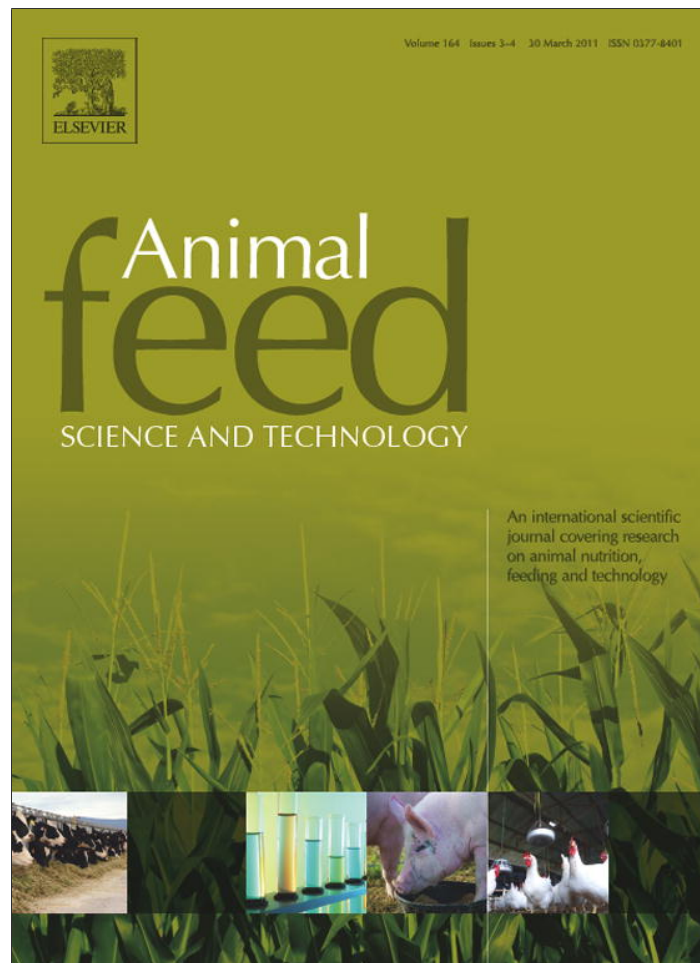


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Agronomic biofortification with selenium: Effects on whole blood selenium and humoral immunity in beef cattle[☆]

J.A. Hall^{a,*}, A.M. Harwell^a, R.J. Van Saun^b, W.R. Vorachek^a, W.C. Stewart^c, M.L. Galbraith^a, K.J. Hooper^a, J.K. Hunter^c, W.D. Mosher^c, G.J. Pirelli^c

^a Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University, Dryden Hall 206, Corvallis, OR 97331, USA

^b Department of Veterinary & Biomedical Sciences, College of Agricultural Sciences, Pennsylvania State University, University Park, PA 16802, USA

^c Department of Animal Sciences, College of Agriculture, Oregon State University, Corvallis, OR 97331, USA

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ABSTRACT

The purpose of this study was to evaluate Se supplementation strategies in mature beef cattle by measuring changes in whole-blood Se (WB-Se) status and humoral immune response to vaccination. Mature beef cows ($n=45$) were balanced by age and randomly assigned to 1 of 3 supplementation groups that received different chemical forms of Se or Se dosages compared to a standard (control) Se treatment. Supplementation treatment groups were provided limited access (6 weeks) to either sodium selenite (200 mg/kg Se; LSe) or Se-fertilized forage (FSe) and subsequently had no additional Se in their mineral supplement for the study duration. The LSe group cows grazed non-Se-fertilized forage. The control group grazed non-Se-fertilized forage and received continuous Se supplementation (CSe) from a free-choice mineral supplement (120 mg/kg Se from sodium selenite). Cows were bled pre and post grazing and then every 4 weeks thereafter for approximately 5 months to assess WB-Se concentration. All cows were immunized with J-5 *Escherichia coli* bacterin at the end of the 6-week supplementation period, and serum was collected for antibody titers 2 and 4 weeks after the third immunization. Covariate adjusted WB-Se concentrations were influenced ($P<0.0001$) by group, time and their interaction. Cows in the FSe group had higher ($P<0.0001$) WB-Se concentration (186 ± 5 ng/mL) immediately post-grazing (42 days) compared to LSe (117 ± 5 ng/mL) and CSe cows (130 ± 5 ng/mL). WB-Se concentration in FSe cows remained higher ($P=0.02$ to $P<0.0001$) over the next 4 (CSe) and 5 (LSe) months. Higher ($P<0.05$) WB-Se concentrations were observed in CSe compared to LSe cows over the last 4 months of the study. Treatment group ($P=0.036$) and time post vaccination ($P<0.0001$) influenced J-5 *E. coli* antibody titers, with FSe cows having higher titers than LSe cows ($P=0.01$), although FSe and CSe cows were not different. Short-term exposure of cattle to Se-fertilized forage elevates WB-Se concentrations within several weeks and this exposure is sufficient to maintain adequate concentrations throughout grazing periods when there is limited access to Se supplements. Short term exposure to higher levels of inorganic Se supplementation is not equivalent to ongoing inorganic Se supplementation at lower rates.

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Abbreviations: CSe, continuous Se supplementation; FDA, Food and Drug Administration; FSe, Se-fertilized forage; ICP-MS, inductively coupled argon plasma emission spectroscopy; LSe, limited Se supplementation; NRC, National Research Council; T-PBS, phosphate-buffered saline solution containing 4.5 mM Tween-20.

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* Corresponding author. Tel.: +1 541 7376537; fax: +1 541 7372730.

E-mail address: Jean.Hall@oregonstate.edu (J.A. Hall).

1. Introduction

Selenium is an essential trace element with recognized nutrient-specific deficiency and toxicity diseases. Many parts of the world, including Oregon, USA, are known to have soil conditions conducive to deficient forage Se content, potentially leading to clinical signs of Se deficiency in livestock grazing or fed crops raised on them (Stevens et al., 1985). Although the essentiality of Se has been known for five decades, the most effective method of Se delivery to cattle is unclear. Selenium may be administered as an injection or in feed and mineral supplements, with Se provided as inorganic sodium selenite or selenate. One limitation of supplementing with inorganic Se in salt or feed is the apparent short duration of Se storage in the animal (Surai, 2006a, 2006b).

Natural Se sources in plants are organic forms, e.g., selenomethionine, selenocysteine, or Se-methylselenocysteine. When inorganic Se is fed to animals, selenocysteine is the main seleno-compound formed (Rayman et al., 2008). Following ingestion of organic Se, protein-bound selenomethionine is also formed from the non-specific incorporation of plant-derived selenomethionine in place of methionine. Thus, Se accumulates in muscle by non-specific incorporation into proteins, and subsequently becomes available with protein catabolism. Both organic and inorganic forms of Se appear to be utilized in the body to produce specific selenoproteins, but Se enters at different points in cellular metabolism depending upon its chemical form (Rayman et al., 2008). With organic Se, it is possible to build Se reserves in the body, which are subsequently available in stress conditions when the Se requirement is increased but feed consumption is decreased (Surai, 2006b).

Agronomic biofortification is defined as increasing the bioavailable concentrations of essential elements in edible portions of crop plants through the use of fertilizers. The potential for using Se-containing fertilizers to increase forage Se concentrations and, thus, dietary Se intake has been demonstrated in Finland, New Zealand, and Australia where it has proven to be both effective and safe (reviewed in Broadley et al., 2006; Makela et al., 1993; Whelan, 1989; Whelan et al., 1994a, 1994b). We have recently shown in sheep that short-term exposure to Se-fertilized forage results in whole-body Se status sufficient to maintain adequate whole-blood Se (WB-Se) concentrations throughout grazing periods when there is limited access to Se supplements (Hall et al., 2009).

Selenium's role in animal health is based on the functions of selenoproteins, many of which have antioxidant activities (Fairweather-Tait et al., 2010). Although reactive oxygen species and free radicals are a natural result of the body's normal metabolic activity, excessive stress as a result of disease, environmental extremes, and nutritional imbalances can lead to over production of free radicals. Therefore, it is imperative that micronutrients involved in antioxidant functions be present in tissues to provide oxidant-antioxidant balance. Selenium enhances the ability of lymphocytes to respond to the cytokine IL-2 by increasing the expression of IL-2 receptors on lymphocytes (Rooke et al., 2004). Enhancement of these interactions leads to increased numbers of lymphocytes, increased cytotoxicity of killer cells, and increased antibody production by B cells (Rooke et al., 2004). The objectives of the current study were to evaluate changes in WB-Se status and humoral immune response to vaccination in beef cattle that received different Se supplementation programs.

2. Materials and methods

2.1. Animals and study design

This was a prospective, randomized block clinical trial of approximately 6-months duration involving 45 mature beef cows, primarily Angus, ranging in age from 5-to-11-years and originating from the Oregon State University Beef Ranch, USA. Body weights ranged from 661 to 782 kg (729 ± 16 kg, mean \pm SEM); body condition scores ranged from 7-to-8 (1-9 scale). The experimental protocol was reviewed and approved by the Oregon State University, USA, Animal Care and Use Committee.

Cows were blocked by age groups and randomly assigned to one of three treatment groups of 15 cows each, balanced by age. Ear tags were used to identify the cows. One group of cows ($n = 15$) grazed Se-fertilized forage (FSe) for 6 weeks and had no additional Se in their mineral supplement. The mineral supplement (dry-matter basis) contained 57.0–64.0 g/kg calcium; 30.0 g/kg phosphorus; 503–553 g/kg salt (NaCl); 50.0 g/kg magnesium; 50 mg/kg cobalt; 2500 mg/kg copper; 200 mg/kg manganese; 200 mg/kg iodine; 6500 mg/kg zinc (Wilbur-Ellis Company, Clackamas, OR). Estimated intake of the mineral supplement over the 6-week period for the 15 FSe cows was 22.5 g/day. A second limited Se supplementation group (LSe; $n = 15$) had free-choice access for 6 weeks to a custom-made mineral supplement that contained 200 mg/kg Se from sodium selenite. Other components of the mineral supplement were identical to the mineral supplement offered to the FSe cows (Wilbur-Ellis Company, Clackamas, OR). Average intake of the mineral supplement over the 6-week period for the 15 LSe cows was 24.0 g/day, thus providing 4.8 mg Se/day. These cows grazed on an adjacent, identical pasture that was not Se-fertilized. Following the 6-week treatment period, FSe and LSe cow groups were combined and grazed a non-Se-fertilized pasture with no Se in their mineral supplement. The third group of cows (controls) grazed non-Se-fertilized forage of similar composition, but had continuous Se supplementation (CSe; $n = 15$) provided by access to a free-choice mineral supplement containing 120 mg/kg Se from sodium selenite. Other components of the mineral supplement were identical to the mineral supplement offered to the FSe cows (Wilbur-Ellis Company, Clackamas, OR). Average mineral intake per cow in the CSe group was 26.4 g/day, thus providing 3.2 mg Se/day. After the 6 week treatment period all cows grazed non-Se-fertilized pasture until the forage dried up and then they were fed hay in addition to pasture (over the last 2 months of the study) that had been previously harvested from the same type of non-Se-fertilized pasture. The National Research Council beef cattle

Table 1

Pasture nutrient composition (dry matter basis) of composted samples collected prior to the grazing period.

Nutrient	Pasture
Dry matter (g/kg)	451
Crude protein (CP) (g/kg)	119
Soluble protein (g/kg CP)	321
Acid detergent fiber (g/kg)	321
Neutral detergent fiber (g/kg)	532
Nonfiber carbohydrates (g/kg) ^a	241
Ash (g/kg)	87
Calcium (g/kg)	4.6
Phosphorus (g/kg)	3.5
Magnesium (g/kg)	1.8
Potassium (g/kg)	30
Sodium (g/kg)	0.64
Copper (mg/kg)	8
Iron (mg/kg)	172
Manganese (mg/kg)	101
Zinc (mg/kg)	34

^a Nonfiber carbohydrates calculated by difference.

software (NRC, 2000) was used to generate estimated dry matter intake values for the cows using averaged body weight and measured forage analysis, with adjustment for neutral detergent fiber intake capacity. Estimated dry matter intake ranged between 14.8 and 16.4 kg/cow/day. Routine farm management practices were not altered.

2.2. Fertilized-forage pasture

The pasture type was primarily subclover-tall fescue sward. Nitrogen was applied in the form of urea-sol fertilizer at a rate of 89.6 kg of actual nitrogen per hectare to 5.2 hectares in early spring season. Sodium selenate (Selcote Ultra[®]; 10 g Se/kg as sodium selenate; Terralink, Vancouver, British Columbia, Canada) was mixed with the nitrogen fertilizer and applied at a rate of 3.4 kg/ha (34 g Se/ha). Pasture forage samples were obtained for Se analysis prior to fertilization and on days 1 and 42 relative to the grazing period, using a systematic grid pattern with one sample generated from 25 subsamples. Forage pasture samples were submitted frozen to commercial laboratories for routine nutrient analyses (Table 1; Cumberland Analytical Services, Maugansville, MD) and Se analysis (Center for Nutrition, Diagnostic Center for Population and Animal Health, Michigan State University, E. Lansing, MI) by inductively coupled argon plasma emission spectroscopy (ICP-MS). All samples were analyzed on an Agilent 7500ce ionized coupled plasma mass spectrometer. Selenium, at mass 78, was analyzed in hydrogen mode to reduce spectral interference. The dry matter determination was completed at a temperature of 105 °C for 12–14 h in a forced draught oven. Methods for crude protein (990.03), acid detergent fiber (973.18), ash (942.05), and minerals (985.01) were performed according to AOAC (2000). Neutral detergent fiber was determined according to Van Soest et al. (1991) with the addition of sodium sulfite and α -amylase. Soluble protein was determined according to Krishnamoorthy et al. (1982).

2.3. Whole-blood selenium assay

All cows were bled 7 days prior to study initiation (covariate value), at the end of the 6 week treatment period, and then every 4 weeks thereafter for approximately 5 months to collect whole blood for Se analysis. A subset of FSe cows ($n=5$) were also bled weekly during the treatment grazing period. Blood was collected into evacuated EDTA tubes and shipped on ice to a commercial laboratory (Center for Nutrition, Diagnostic Center for Population and Animal Health, Michigan State University, E. Lansing, MI) where WB-Se concentrations were determined using an ICP-MS method. Each whole-blood sample was diluted 1:20 with a solution containing EDTA, Triton X-100, ammonia hydroxide, and propanol with 20 μ g/kg of scandium, rhodium, indium and bismuth as internal standards. All samples were analyzed on an Agilent 7500ce ionized coupled plasma mass spectrometer. The instrument was tuned to yield approximately 5500 counts/ μ g/kg of Se at each of the analyzed mass. Selenium, at mass 78, was analyzed in hydrogen mode to reduce spectral interference.

2.4. J-5 *Escherichia coli* antibody titer

The humoral immune response was assessed by measuring antibody titers to a commercially available vaccine (UPJOHN J-5 BACTERIN[™] *E. coli* Bacterin J-5 Strain; Pharmacia and Upjohn Company, Division of Pfizer Inc., NY, USA). Cows were immunized with bacterin at the end of the 6 week supplementation period. Five-mL bacterin was administered subcutaneously in a three-dose regimen, each dose two-weeks apart, as per the manufacturer's instructions. Blood was collected prior to immunization and 2- and 4-weeks after the third immunization. Serum was harvested for J-5 *E. coli* antibody titers, which were measured by an indirect ELISA procedure described by Chaiyotwittayakun et al. (2004) with minor modification. We used the reporter compound TMB (3,3',5,5'-Tetramethyl-benzidine; Sigma, St. Louis, MO) to react with horseradish per-

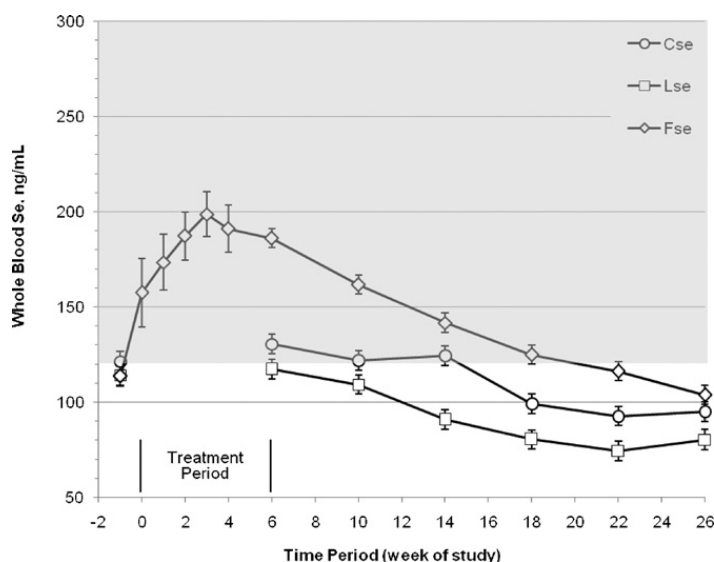


Fig. 1. Comparison of whole-blood Se concentrations in cows consuming Se-fertilized forage (FSe) with no Se in mineral supplement ($n = 15$), or limited Se (LSe, 200 mg/kg as selenite) in a mineral supplement ($n = 15$) for 6 weeks. After 6 weeks the FSe and LSe cow groups were combined and grazed non-Se-fertilized forage. A control group of cows ($n = 15$) received a continuous source of Se (CSe) from mineral supplement (120 mg/kg as selenite) throughout the study. Treatment periods began in May 2009 and lasted for 6 weeks (bordered by bars). The normal reference interval for WB-Se concentrations of adult cows is shaded (120–300 ng/mL).

oxidase conjugated to sheep-anti-bovine-IgG1 (Bethyl Laboratories Inc., Montgomery, TX). After 100 μ L of TMB was added to all wells, plates were incubated at room temperature in the dark in an Ultramark microplate reader (BioRad, Hercules, CA) until an absorbance of 0.6 was reached at 650 nm. Then 100 μ L of 1 N H_2SO_4 was added and absorbance at 450 nm was determined using the Ultramark microplate reader. The background was subtracted and final results were expressed as the difference in absorbance between post-immunization and pre-immunization.

2.5. Statistical analyses

Whole-blood Se concentrations were evaluated, after testing for normality, using an ANOVA method (Proc Mixed) for repeated measures accounting for covariance structure between time measurements (Statistical Analysis Software [SAS], ver. 9.1, Cary, NC). Cows in the CSe group tended ($P = 0.11$) to have higher WB-Se concentrations at study initiation (135 ng/mL) compared to the other cows (107 ng/mL, FSe; 108 ng/mL, LSe) therefore, initial WB-Se concentrations were used as a covariate in all models. Model main effects were block (age group), treatment group, time, and their interactions. Age group was found not to be significant, thus the block effect was removed from the model and included in the random error term. For main effects found to be significant, mean differences were determined by pairwise differences or probability values for differences of the least-squares means (PDIF) for preplanned comparisons.

Antibody titer data were log transformed to meet normality criteria. Mean transformed antibody titer data were evaluated by ANOVA for repeated measures (Proc Mixed, SAS, ver. 9.1, Cary, NC) with main model effects of treatment, time, and their interaction. Change in titer over time points and titer fold changes were evaluated by ANOVA (Proc GLM, SAS, ver. 9.1, Cary, NC). Data are reported as least square means \pm SEM. Significance was accepted at $P \leq 0.05$, unless otherwise specified.

3. Results and discussion

Forage Se concentration pre-fertilization was 0.11 mg/kg (DM basis). Forage Se concentration collected 62 days following fertilization, the day cows began grazing the pasture, was 1.52 mg/kg, and 42 days later, the day cows were removed from the pasture, it was 1.06 mg/kg. We reported a similar increase in Se concentration of forage (1.52 mg/kg) in response to application of the same rate of Selcote Ultra[®] in a previous study (Hall et al., 2009). Others have shown that Selcote Ultra[®] (10 g Se/kg as 1:3 sodium selenate:barium selenate) increased crop Se concentration from 0.067 to 0.187 mg/kg and 0.220 mg/kg at 5 and 10 g Se/ha, respectively (Gupta, 1995). A comparison across a limited number of studies that had varying amounts of Se applied per hectare demonstrates that there is a seemingly consistent linear response to Se dosage (Hall et al., 2009), although more studies are required to validate this relationship especially in light of known effects of soil pH, iron content, and other factors influencing Se availability to plant tissues (NRC, 1983).

Whole-blood Se concentrations were influenced ($P < 0.0001$) by supplementation group, time and their interaction (Fig. 1). Cows in the FSe group had higher ($P < 0.0001$) WB-Se concentration (186 ± 5 ng/mL) immediately post-grazing (6 weeks) compared to cows in the LSe group (117 ± 5 ng/mL) and CSe group (130 ± 5 ng/mL). Whole-blood Se in FSe cows remained higher ($P = 0.02$ to $P < 0.0001$) over the next 4 (CSe) and 5 (LSe) months. Higher ($P < 0.05$) WB-Se concentrations were observed

in CSe cows compared to LSe cows over the last 4 months of the study. Within the subset of FSe cows, WB-Se concentrations were higher ($P < 0.0001$) by 1 week of grazing compared to initial values and reached a peak within 4 weeks of initiating supplementation.

Based on average body weights and estimated dry matter intake, cows grazing Se-fertilized forage received approximately 15.6–24.9 mg organic Se/day. This is noticeably higher than the other groups. (LSe cows received 4.8 mg inorganic Se/day and CSe cows received 3.2 mg inorganic Se/day.) Inorganic Se intake for the LSe and CSe cow groups was based on calculated averages, because even though all cows had equal access to the salt-mineral mixtures, typical of free-choice mineral products, there potentially is marked individual variation in intake. Although Se intake was higher for FSe cows, at no time in our study were clinical signs of Se toxicosis observed, and whole-blood Se concentrations did not exceed the laboratory's normal range. Nevertheless, the goal of this study was not to match Se intake between treatment groups, but rather to show that cows fed high-Se fertilized forage by itself (with no additional Se in their mineral supplement) benefited from higher Se intake compared with variable Se intake of cows receiving typical selenized salt-mineral mixtures. Indeed, WB-Se concentrations were higher, albeit within the normal reference interval, for a longer period of time in FSe cows that received no Se containing salt-mineral supplement compared with LSe cows that received the salt-mineral supplement containing Se. (The normal reference interval for WB-Se of adult cows at the Michigan State University diagnostic laboratory is 120–300 ng/mL [RVS, personal communication].) Short-term exposure of cattle to Se-fertilized forage elevated WB-Se within several weeks and levels were sufficient to maintain adequate concentrations throughout grazing periods when there would be limited access to Se supplements. Short-term exposure to higher levels of inorganic Se supplementation (200 mg/kg) was not equivalent to ongoing inorganic Se supplementation at lower rates (120 mg/kg).

The salt-mineral supplement containing 200 mg/kg Se as sodium selenite is normally diluted with salt to provide 120 mg/kg Se as per Food and Drug Administration (FDA, 2009) regulations for a free-choice salt-mineral mixture, and consumed at a rate not to exceed an intake of 3 mg/head/day. In the United States, the FDA (2009) also regulates Se supplementation to ruminant diets at a level of 0.3 mg/kg Se from either sodium selenite or selenate. An organic source of supplemental Se was cleared by the FDA for dairy and beef cattle in 2003; again, dietary inclusion was set at 0.3 mg/kg. Although the use of feedstuffs naturally high in Se to deliver supranutritional levels of Se is not regulated, Se fertilization is not permitted in the United States, with one exception. In Oregon, USA, similar to in Finland, New Zealand, and Australia (reviewed in Broadley et al., 2006; Makela et al., 1993; Whelan, 1989; Whelan et al., 1994a, 1994b), the Department of Agriculture does not control the use of Se as a plant fertilizer; therefore, it is possible to produce feedstuffs with increased Se concentrations by applying Se as a fertilizer.

In general, organic forms of Se are absorbed and retained more readily by ruminants than inorganic forms (Qin et al., 2007). Selenomethionine, the major dietary form of organic Se, can undergo several different metabolic fates. Cells do not distinguish between methionine and selenomethionine during protein synthesis, so selenomethionine is incorporated into general body proteins in place of methionine depending on the pool size of methionine and the number of methionine residues in protein (Shiobara et al., 1998). Selenomethionine incorporated into proteins in this manner is not regulated and reflects dietary intake of selenomethionine. On the other hand, inorganic selenite is rapidly taken up by red blood cells (RBC), and then released into plasma after reduction to hydrogen selenide (H_2Se), which is the key central molecular form of Se in regulated Se-metabolic pathways (Fairweather-Tait et al., 2010). The H_2Se is converted to selenophosphate ($HSePO_3^{2-}$), which then reacts with tRNA-bound-serinyl residues to produce selenocysteine-bound-tRNA from which selenocysteine is inserted co-translationally at loci encoded by specific UGA codons to produce selenoproteins (Rayman et al., 2008). There are 25 known selenoprotein genes in humans that encode selenoproteins with a variety of functions, including many with antioxidant activities or with involvement in regulation of intracellular redox state (Fairweather-Tait et al., 2010). The pathways leading to H_2Se and subsequently to the disposal of H_2Se are well regulated, and the concentration of Se in the body is maintained through homeostatic control mechanisms within a narrow range (Shiobara et al., 1998). Organic selenomethionine also functions as a source of Se for the synthesis of selenoproteins (after being metabolized to H_2Se), but because of its interchangeability with methionine during protein synthesis, a major difference between organic and inorganic Se supplements is that the half-life of Se as selenomethionine, at least in humans, is twice that of Se from sodium selenite (Swanson et al., 1991). Also, with organic Se, it is possible to build Se reserves in the body, which are subsequently available in stress conditions when the Se requirement is increased, but feed consumption is decreased (reviewed in Surai, 2006b).

Treatment group ($P = 0.036$) and time post vaccination ($P < 0.0001$) influenced J-5 *E. coli* antibody titers, with FSe cows having higher titers than LSe cows ($P = 0.01$), although FSe and CSe cows were not different (Fig. 2). There was no group by time interaction ($P = 0.52$). Across time, the 2 and 4 week titers were similar and higher than baseline; there was no difference among groups at baseline (time 0). At 2 weeks post the third vaccination, the FSe group titers were higher than those for the LSe group ($P = 0.0013$) and tended to be higher than the CSe group of cows ($P = 0.075$). The LSe and CSe groups were not different ($P = 0.11$). At 4 weeks post the third vaccination, the FSe group titers were higher than those of the LSe group ($P = 0.012$), but not of the CSe group of cows ($P = 0.13$). Again, titers for the LSe and CSe groups were not different ($P = 0.29$). There were no differences among the groups for percent increase in titer for any time period. The change in titer over time (slope) was different ($P = 0.014$) from baseline to 2 weeks depending on treatment group. The FSe group of cows had a more rapid rise in titer than both the LSe group ($P = 0.0046$), and the CSe group ($P = 0.37$). There was no difference in slope between the LSe and CSe groups. There were also no differences between groups for change in titer (slope) from baseline to 4 week or from 2 to 4 weeks.

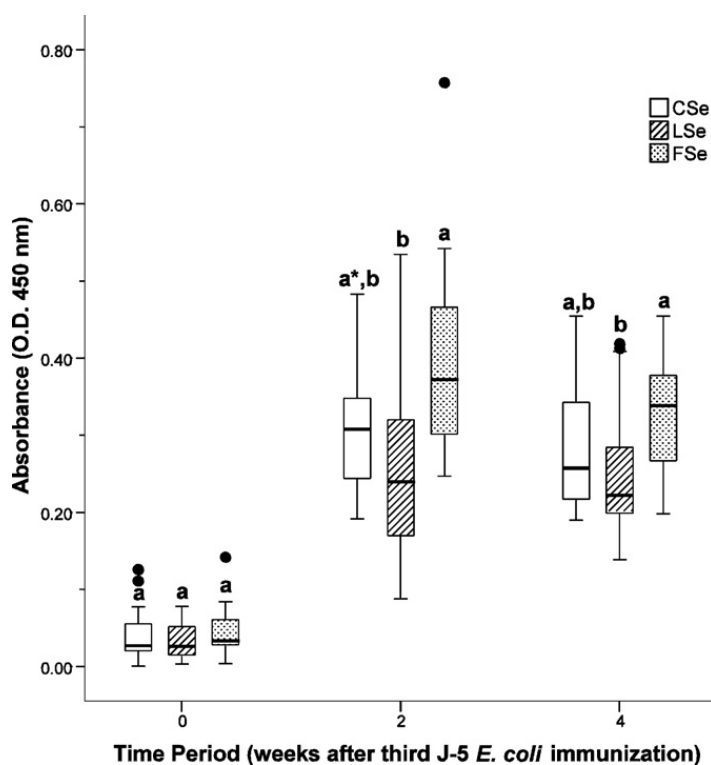


Fig. 2. Comparison of J-5 *E. coli* antibody titers (measured in absorbance) in cows consuming Se-fertilized forage (FSe) with no Se in mineral supplement ($n = 15$), or limited Se (LSe, 200 mg/kg as selenite) in a mineral supplement ($n = 15$) for 6 weeks. After 6 weeks the FSe and LSe cow groups were combined and grazed non-Se-fertilized forage. A control group of cows ($n = 15$) received a continuous source of Se (CSe) from mineral supplement (120 mg/kg) throughout the study. Cows were immunized with J-5 *E. coli* bacterin at the end of the 6 week supplementation period. Each box represents the middle 50% of the data, with the median indicated by a horizontal line in the box. The whiskers extend 1.5 times the interquartile range above and below the 75th and 25th percentiles. Values above or below the whiskers are outliers. Means with different superscripts within month are different (^{a,b} $P < 0.05$; * $P = 0.075$).

Selenium deficiency has been shown to result in immunosuppression and decreased antibody production, whereas administration of Se increases antibody production (Kiremidjian-Schumacher and Stotzky, 1987). For example, in rodents and cattle, Se deficiency results in lower antibody titers to vaccination, impaired lymphocyte proliferation, and impaired ability of neutrophils to destroy phagocytized bacteria (Sheffy and Schultz, 1979; Boyne and Arthur, 1979; Parnham et al., 1983; Gyang et al., 1984). In calves inoculated with infectious bovine rhinotracheitis virus, Se-treated calves had higher antibody titers than Se-deficient calves (Reffett et al., 1988). In other studies, calves deficient in Se had lower antibody titers than Se-treated calves (Swecker et al., 1989, 1995). However, it is unknown whether Se-supplementation enhances antibody production in Se-replete cattle. We are interested in supranutritional levels of Se, to determine if supplementing Se at concentrations above those currently recommended for cows (supranutritional) can modulate the immune response in a way that reduces the severity and/or improves recovery from a disease process. Immunoenhancing effects of Se may be achieved at supplementation levels much higher than what the NRC currently recommends.

The goal of enhancing immunity is to increase resistance to disease. A decreased incidence of metritis in Se-treated dairy cows provides a good example of an association between Se deficiency and decreased disease resistance (Suttle and Jones, 1989). Numerous studies have linked low Se to increased susceptibility of dairy cows to mastitis (reviewed in Surai, 2006b). It has been observed in cattle herds, with long-standing annual problems with foot rot and pinkeye, that there is a markedly reduced incidence (seasonal) of these diseases once exposed to continuous Se supplementation (Koller et al., 1983).

4. Conclusion

Short-term exposure of cattle to Se-fertilized forage elevates WB-Se within several weeks and levels are sufficient to maintain adequate concentrations throughout grazing periods when there is limited access to Se supplements. Short term exposure to higher levels of inorganic Se supplementation is not equivalent to ongoing inorganic Se supplementation at lower rates. Although WB-Se levels were only modestly elevated in the FSe group, it was enough to produce a statistical difference in J-5 *E. coli* antibody titers in the FSe group compared to the LSe group.

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References

- AOAC, 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Arlington, VA, USA.
- Boyne, R., Arthur, J.R., 1979. Alterations of neutrophil function in selenium-deficient cattle. *J. Comp. Pathol.* 89, 151–158.
- Broadley, M.R., White, P.J., Bryson, R.J., Meacham, M.C., Bowen, H.C., Johnson, S.E., Hawkesford, M.J., McGrath, S.P., Zhao, F.J., Breward, N., Harriman, M., Tucker, M., 2006. Biofortification of UK food crops with selenium. *Proc. Nutr. Soc.* 65, 169–181.
- Chaiyotwittayakun, A., Burton, J.L., Weber, P.S., Kizilkaya, K., Cardoso, F.F., Erskine, R.J., 2004. Hyperimmunization of steers with J5 *Escherichia coli* bacterin: effects on isotype-specific serum antibody responses and cross reactivity with heterogeneous gram-negative bacteria. *J. Dairy Sci.* 87, 3375–3385.
- Fairweather-Tait, S.J., Collings, R., Hurst, R., 2010. Selenium bioavailability: current knowledge and future research requirements. *Am. J. Clin. Nutr.* 91, 1484S–1491S.
- FDA, 2009. Title 21. Food and Drugs: Food Additives Permitted in Feed and Drinking Water of Animals, <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=573.920> (accessed 03.06.10.).
- Gupta, U.C., 1995. Effects of Selcote® ultra and sodium selenate (laboratory versus commercial grade) on selenium concentration in feed crops. *J. Plant Nutr.* 18, 1629–1636.
- Gyang, E.O., Stevens, J.B., Olson, W.G., Tsitsamis, S.D., Usenik, E.A., 1984. Effects of selenium-vitamin E injection on bovine polymorphonucleated leukocytes phagocytosis and killing of *Staphylococcus aureus*. *Am. J. Vet. Res.* 45, 175–177.
- Hall, J.A., Van Saun, R.J., Nichols, T., Mosher, W., Pirelli, G., 2009. Comparison of Se status in sheep after short-term exposure to high-Se-fertilized forage or mineral supplement. *Small Ruminant Res.* 82, 40–45.
- Kiremidjian-Schumacher, L., Stotzky, G., 1987. Selenium and immune responses. *Environ. Res.* 42, 277–303.
- Krishnamoorthy, U., Muscato, T.V., Sniffen, C.J., Van Soest, P.J., 1982. Nitrogen fractions in selected feedstuffs. *J. Dairy Sci.* 65, 217–225.
- Koller, L.D., South, P.J., Exon, J.H., Whitbeck, G.A., 1983. Selenium deficiency of beef cattle in Idaho and Washington and a practical means of prevention. *Cornell Vet.* 73, 323–332.
- Makela, A.L., Nanto, V., Makela, P., Wang, W., 1993. The effect of nationwide selenium enrichment of fertilizers on selenium status of healthy Finnish medical students living in south western Finland. *Biol. Trace Elem. Res.* 36, 151–157.
- National Research Council, 1983. Selenium in Nutrition, Revised Edition. National Academy Press, Washington, DC.
- National Research Council, 2000. Nutrient Requirements of Beef Cattle, 6th rev edition 2000 update. National Academy Press, Washington, DC.
- Parnham, M.J., Winkelmann, J., Leyck, S., 1983. Macrophage, lymphocyte and chronic inflammatory responses in selenium deficient rodents. Association with decreased glutathione peroxidase activity. *Int. J. Immunopharmacol.* 5, 455–461.
- Qin, S., Gao, J., Huang, K., 2007. Effects of different selenium sources on tissue selenium concentrations, blood GSH-Px activities and plasma interleukin levels in finishing lambs. *Biol. Trace Elem. Res.* 116, 91–102.
- Rayman, M.P., Infante, H.G., Sargent, M., 2008. Food-chain selenium and human health: spotlight on speciation. *Br. J. Nutr.* 100, 238–253.
- Reffett, J.K., Spears, J.W., Brown, T.T., 1988. Effect of dietary selenium on the primary and secondary immune response in calves challenged with infectious bovine rhinotracheitis virus. *J. Nutr.* 118, 229–235.
- Rooke, J.A., Robinson, J.J., Arthur, J.R., 2004. Effects of vitamin E and selenium on the performance and immune status of ewes and lambs. *J. Agric. Sci.* 142, 253–262.
- Sheffy, B.E., Schultz, R.D., 1979. Influence of vitamin E and selenium on immune response mechanisms. *Fed. Proc.* 38, 2139–2143.
- Shiobara, Y., Yoshida, T., Suzuki, K.T., 1998. Effects of dietary selenium species on Se concentrations in hair, blood, and urine. *Toxicol. Appl. Pharmacol.* 152, 309–314.
- Stevens, J.B., Olson, W.G., Kraemer, R., Archambeau, J., 1985. Serum selenium concentrations and glutathione peroxidase activities in cattle grazing forages of various selenium concentrations. *Am. J. Vet. Res.* 46, 1556–1560.
- Surai, P.F., 2006a. Selenium in food and feed: selenomethionine and beyond. In: *Selenium in Nutrition and Health*. Nottingham University Press, Nottingham, United Kingdom, pp. 151–212.
- Surai, P.F., 2006b. Selenium in ruminant nutrition. In: *Selenium in Nutrition and Health*. Nottingham University Press, Nottingham, United Kingdom, pp. 487–587.
- Suttle, N.F., Jones, D.G., 1989. Recent developments in trace element metabolism and function: trace elements, disease resistance and immune responsiveness in ruminants. *J. Nutr.* 119, 1055–1061.
- Swanson, C.A., Patterson, B.H., Levander, O.A., Veillon, C., Taylor, P.R., Helzlsouer, K., McAdam, P.A., Zech, L.A., 1991. Human [⁷⁴Se]selenomethionine metabolism: a kinetic model. *Am. J. Clin. Nutr.* 54, 917–926.
- Swecker, W.S., Eversole, D.E., Thatcher, C.D., Blodgett, D.J., Schurig, G.G., Meldrum, J.B., 1989. Influence of supplemental selenium on humoral immune responses in weaned beef calves. *Am. J. Vet. Res.* 50, 1760–1763.
- Swecker, W.S., Thatcher, C.D., Eversole, D.E., Blodgett, D.J., Schurig, G.G., 1995. Effect of selenium supplementation on colostral IgG concentration in cows grazing selenium-deficient pastures and on postsuckle serum IgG concentration in their calves. *Am. J. Vet. Res.* 56, 450–453.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597.
- Whelan, B.R., 1989. Uptake of selenite fertilizer by subterranean clover pasture in Western Australia. *Aust. J. Exp. Agric.* 29, 517–522.
- Whelan, B.R., Barrow, N.J., Peter, D.W., 1994a. Selenium fertilizers for pastures grazed by sheep. 1. Selenium concentrations in whole-blood and plasma. *Aust. J. Agric. Res.* 45, 863–875.
- Whelan, B.R., Barrow, N.J., Peter, D.W., 1994b. Selenium fertilizers for pastures grazed by sheep. 2. Wool and liveweight responses to selenium. *Aust. J. Agric. Res.* 45, 877–887.