Effect of Selenium Source on Production, Reproduction, and Immunity of Lactating Dairy Cows

Flavio T. Silvestre1, Heloisa M. Rutigliano2, William W. Thatcher1, Jose E-P Santos2 and Charles R. Staples1

1Department of Animal Sciences, University of Florida and 2Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare, CA

Introduction

A nutraceutical is defined as a product isolated or purified from feeds that is demonstrated to have a physiological benefit or provide protection against chronic disease. Indeed selenium (Se) is an essential nutrient in that Se deficiency is associated with increased incidences of retained fetal membranes, clinical mastitis, calf mortality and increased milk somatic cell counts. Selenium supplementation in Se deficient diets reduced the incidence of these clinical problems. Consequently, Se should be considered a nutraceutical which has an array of biological responses.

Cows fed Se deficient diets had reduced blood glutathione peroxidase (GPx) activity compared to those fed Se supplemented diets (Grasso et al., 1990; Gunter et al., 2003). Also, cows receiving injections containing sodium selenite had greater concentrations of Se in plasma and greater GPx activity than cows not injected with sodium selenite (Hogan et al., 1990). Glutathione peroxidase is one of the selenoenzymes (Se-GPX) capable of protecting the cell against oxidative injury. The Se-GPx catalyzes the reduction of hydrogen peroxide (H2O2) to water and organic hydroperoxides to alcohols while utilizing the peptide glutathione as a cofactor (Mezzetti et al., 1990). It is important for example that neutrophils provide a high oxidizing intracellular environment to kill phagocytized bacteria, but it is essential that neutrophils regulate the balance between reactive oxygen metabolites (superoxide [O2-] and hydrogen peroxide [H2O2]) in order not to damage the cell leading to it’s death. Iodothyronine deiodinase enzyme is a selenoenzyme that catalyzes the activation and inactivation of thyroid hormones that regulate metabolic processes and may contribute to production responses.

Basal feed ingredients and selenium yeast provide mostly selenoamino acids (i.e., selenomethionine and selenocysteine) and inorganic selenium supplements provide selenate and selenite. The basic pathways for selenium metabolism have been summarized by Weiss (2003) as depicted in Figure 1. Inorganic selenium sources undergo reduction to form selenide which leads to the formation of Se-Cysteine (i.e., the

---

1 Contact at: P.O. Box 110910, Department of Animal Sciences, University of Florida, Gainesville, FL 32611; (352) 392 5590 Phone; (352) 392 5595 Fax; thatcher@animal.ufl.edu

January 30-31, 2007 • Florida Ruminant Nutrition Symposium • Best Western Gateway Grand • Gainesville, FL
hydroxyl group of a serine molecule linked to a specific tRNA [UGA codon] is replaced with a selenol moiety to form SeCyst-tRNA that is inserted into selenoproteins; Figure 1). Thus various Se sources (both inorganic and organic) must first be converted to inorganic selenide before the synthesis of Se-Cysteine which contribute to the bioactive components of Selenoproteins. Following absorption of Se-Methionine from the intestinal tract, Se-methionine can be found in blood proteins and in the plasma methionine pool as it is transported to body tissues. For example the mammary gland extracts large quantities of methionine to synthesize milk proteins. This would account for the large amounts of selenium found in milk which may benefit the neonate or serve as a selenium source for human consumption.

Figure 1. Basic pathways for selenium metabolism in animals.

During the immediate postpartum period, the cow’s immune system is challenged severely (Goff, 2006), and the innate and humoral defense systems are reduced. The incidence of diseases and disorders can be high during this time period and have a negative impact on reproductive performance. For example the “risk” of pregnancy (odds ratio) was reduced if cows had retained fetal membranes (RFM) or lost one BCS (Loeffler, de Vries and Schukken, 1999). Reduction in adaptive and innate immunity at parturition increases the risk of health disorders such as RFM, metritis, and mastitis. Selenium has long been associated with immunity. Cattle supplemented with Se-yeast had an 18% increase of Se in plasma in comparison with those given sodium selenite in some studies (Weiss, 2003). The state of Florida, USA is a selenium-deficient area, and lactating dairy cows are exposed to a seasonal period of heat stress that impacts reproductive performance and health.
We have conducted a joint experiment between the laboratories of W.W. Thatcher at the University of Florida (Silvestre et al., 2006a; Silvestre et al., 2006b) and J.E.P. Santos at the University of California, Davis (Rutigliano et al., 2006) to evaluate a supplemental source of organic selenium on reproductive and immune responses by dairy cows. Objectives were to evaluate effects of organic Se on pregnancy rate (PR) at the first and second postpartum AI services, uterine health, and milk yield during the summer heat stress period. The concept of replicating the experiment at two sites was to have sufficient numbers of lactating dairy cows to test the effects of selenium supplements on pregnancy rates to first and second services.

Florida Site

Experimental Design:

Cows were assigned (23 ± 8 days prepartum) to diets of organic Se (Se-yeast [SY; Sel-Plex®, Alltech; n = 289] or inorganic sodium Se [SS; n = 285]) fed at 0.3 mg/kg (DM basis) for more than 81 days postpartum. Rectal temperature was recorded each morning for 10 days postpartum (dpp). Vaginoscopies were performed at 5 and 10 dpp. Cows within diet were assigned randomly to 2 reproductive management programs (Presynch-Ovsynch vs CIDR-Ovsynch [i.e., Ovsynch began 3 days after withdrawal of a 7 day-CIDR]). All cows were resynchronized for a second service with Ovsynch at 20 to 23 days after first service. An ultrasound pregnancy diagnosis was conducted at 27 to 30 days after first timed AI (TAI). Cows in estrus following Presynchs were AI up to the second TAI service. Strategic blood sampling determined anovulatory status at Ovsynch and ovulatory response after TAI to first service. The PR at second service was determined by rectal palpation at ~ 42 dpp.

Plasma Se and Immuno Responses:

Blood was sampled for Se (n = 20 cows/diet) at -25, 0, 7, 14, 21, and 37 dpp and measurements of immuno-status. Plasma Se increased in SY-fed cows (0.087 vs 0.069 ± 0.004 µg/ml; P < 0.01) and the patterns throughout the sampling period are depicted in Figure 2. It is clear that concentrations of plasma selenium were lower in cows of the SS group and that concentrations of 0.069 µg/ml are low even with SS supplementation. In contrast feeding SY caused a 1.26 fold increase in plasma selenium concentrations.

Innate immunity (i.e., neutrophil function) was determined by phagocytic and oxidative burst capacity of neutrophils in whole blood using a dual color flow cytometric method. Samples were collected from a subsample of 36 cows at -26 and 40 cows at 0, 7, 14, 21 and 37 dpp and analyzed for neutrophil function. Adaptive immunity (ability to induce an antibody response) was monitored by measuring IgG concentrations in blood in response to Ovalbumin (Ovalb) injections. Ovalb antigen (1 mg [i.m.]) was dissolved in an E. coli J5 endotoxemia preventive vaccine and injected at -60 and -22 ± 6 dpp (day of initiating SY [n = 38] and SS [n = 47] diets). Ovalb was dissolved in PBS with Quil-A adjuvant and injected again at parturition (day 0). Serum samples were collected on days of immunization and at 21 and 42 dpp.
Percentage of gated neutrophils that phagocytized E. coli and underwent oxidative burst did not differ between dietary groups at -26 dpp (44.6 ± 4.6%). For subsequent samples, SY improved neutrophil function in which phagocytosis and killing activity were increased. This is reflected in Figure 3; however the average response to SY was greater for primiparous animals than multiparous cows (Figure 3). This was due to a diet by parity by day interaction (P < 0.05); namely, SY improved neutrophil function at parturition in multiparous cows (42 ± 6.14% vs 24.3 ± 7.2%) and at 7, 14 and 37 dpp in primiparous cows (53.9 vs 30.7%, 58.6 vs 41.9%, and 53.4 vs 34.8%, respectively; pooled SE = 6.8%). Overall neutrophil function was suppressed in primiparous cows at the time of parturition and was not restored until 7 to 14 days postpartum. In contrast, multiparous cows did not have a restoration in neutrophil function until between 14 to 21 days postpartum. Organic Se improved phagocytosis and killing activity of neutrophils in both multiparous and primiparous cows. However, the primiparous cows seemed to be more responsive in that SY stimulated neutrophil function throughout 0 to 21 dpp whereas, SY stimulation in multiparous cows was evident on only the day of parturition. In most of our postpartum experiments, we detect distinct differences between primiparous and multiparous cows for a multiplicity of physiological and biochemical responses.
Anti-IgG to Ovalb did not differ between dietary groups at -60 and -22 dpp (0.18 ± 0.01 and 0.97 ± 0.04 OD). Concentrations of Anti-IgG to Ovalb were higher (P< 0.01) in SY multiparous cows at 21 and 42 dpp (Figure 4). However, Anti-IgG to Ovalb did not differ between selenium supplements for primiparous cows (1.40 ± 0.08 OD). Thus measurement of adaptive immunity was improved in multiparous dairy cows in response to SY but not in primiparous cows. Our findings indicated that feeding Se as organic Se (Se-yeast, Sel-Plex®), beginning at 26 days prepartum, elevated plasma Se concentrations, increased neutrophil function at the time of parturition, and improved immuno-responsiveness in multiparous cows.

**Uterine Health and Pregnancy Rates:**

Frequencies of retained fetal membrane (9.7%), mastitis (14.4%), anovulation (17.7%), and synchronized ovulation after TAI (82.5%) were not affected by diets or reproductive program. Selenium yeast (SY) reduced the frequency of multiparous cows detected with more than 1 event of fever (rectal temperature > 39.5°C; SY, 13.3% [25/188] vs SS, 25.5% [46/181]; P < 0.05), but the SY effect was not observed in primiparous cows which had a much higher frequency of fever (40.5%). Cervical discharge scores measured at 5 and 10 dpp were better for the SY group (Table 1).
Figure 4. Abundance (optical density) of serum anti-ovalbumin antibody in multiparous and primiparous cows immunized with ovalbumin and fed either Selenium Yeast (Sel-Plex) or control (sodium selenite).

Table 1. Overall frequencies of cervical discharge scores measured at 5 and 10 dpp in cows fed selenium yeast (Sel-Plex) or control (sodium selenite).

<table>
<thead>
<tr>
<th>Diet</th>
<th>C (%) (n)</th>
<th>M (%) (n)</th>
<th>P (%) (n)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35% (153)</td>
<td>47% (209)</td>
<td>17% (75)</td>
<td></td>
</tr>
<tr>
<td>Sel-Plex</td>
<td>47% (217)</td>
<td>43% (200)</td>
<td>9.3% (43)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

1 C (clean mucous, lochia or no discharge), M (mucopurulent) and P (purulent). Parity (P<0.01); Day (P<0.02); Calving Difficulty (P<0.01); RFM (P<0.01)
The frequency of cows with a purulent-fetid discharge was reduced and proportion of cows with a clean discharge was increased. This is additional support that feeding the organic selenium (i.e., SY) improved immunocompetence and this was associated with improved uterine health measured at 5 and 10 days postpartum.

Diet failed to alter first service PR at ~ 30 days post AI (SY, 24.9% [62/249] vs SS, 23.6% [62/262]) or pregnancy losses between ~ 30 and ~ 55 days post AI (SY, 39.3% vs SS, 37.1%.) These low pregnancy rates and high embryonic losses are typical of cows managed during the summer heat stress period of Florida. Diet did indeed alter second service PR [SY, 17% (34/199) vs SS, 11.3% (24/211); P < 0.05]. The benefit of SY on second service pregnancy rate is very interesting (Figure 5). We hypothesize that cows of the SY group were better able to reestablish an embryotrophic environment at second service following either early or late embryonic losses. For example, cows presented for second service may not have been pregnant to the first service by 30 days at the ultrasound diagnosis or were pregnant and underwent embryonic lost and required a second service. Indeed pregnancy rate to the second service for cows that had lost an embryo was 22.7% for the SY versus 4.2% in the control or SS group (Figure 5).

Figure 5. Second service pregnancy rates determined by rectal palpation (~42d) for cows fed Selenium Yeast (Sel-Plex) or control (sodium selenite) and for cows that were open or lost a pregnancy to first service.
The product Mu-Se ® (Schering-Plough Animal Health Corp, NJ), that contains inorganic selenium (50 mg) and Vitamin E (500 mg), increased pregnancy rate to 2nd service (i.e., no effect at first service), reduced services per conception, and decreased the interval from calving to conception (Arechiga et al., 1998). This is somewhat similar to the results detected for organic selenium above in that fertility was increased in cattle that did not become pregnant at first service.

**Milk Production and Somatic Cells:**

Mean milk yield and milk composition determined from monthly samples for cows receiving supplemental inorganic selenium (sodium selenite, SS) and organic selenium (selenium yeast, SY; Sel-Plex) are presented in Table 2. Both monthly milk yield and fat-corrected milk were greater for cows receiving Sel-Plex. A series of analyses were conducted to determine when the differences in milk production occurred. Milk production was monitored on a daily basis for the first 81 days of lactation and mean daily milk yield did not differ between treatments (35.6 kg/d). Additional analyses were conducted with the monthly estimates of milk production presented in Figure 6. Monthly milk yields were elevated in the Sel-Plex group in later stages of lactation between 6-8 months of lactation. The increase in milk production in later stages of lactation occurred in primiparous cows but not in multiparous cows (Figure 7).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Se-Inorganic</th>
<th>Se-Methionine</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk (Kg)</td>
<td>36.41</td>
<td>37.14</td>
<td>= 0.03</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>3.37</td>
<td>3.39</td>
<td>NS</td>
</tr>
<tr>
<td>FCM (Kg)</td>
<td>35.33</td>
<td>36.20</td>
<td>= 0.05</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>2.88</td>
<td>2.89</td>
<td>NS</td>
</tr>
<tr>
<td>Total Protein (g)</td>
<td>1040</td>
<td>1060</td>
<td>= 0.03</td>
</tr>
<tr>
<td>SCC (X1000)</td>
<td>286.61</td>
<td>283.24</td>
<td>NS</td>
</tr>
<tr>
<td>Log SCC</td>
<td>4.88</td>
<td>4.86</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure 6. Mean monthly milk yield (kg) for cows fed Selenium Yeast (Sel-Plex) or sodium selenite (control).

Figure 7. Mean monthly milk yield for primiparous and multiparous cows fed Selenium Yeast (Sel-Plex) or sodium selenite (control). Diet*Parity*Month Interaction, P < 0.05.
The slight increase in milk production due to SY may be due to a carry-over effect of SY since supplementation ceased at 81 days of lactation. The monthly profiles of somatic cells in milk (Figure 8) indicated that concentrations of somatic cells were lower in SY-treated cows during the later stages of lactation (i.e., months 6-8). Perhaps this represented a lower loss of alveolar epithelial cells that possibly accounted for the subtle increase in milk yield. Milk somatic cells were lower in primiparous cows than multiparous cows between 3 to 8 months of lactation.

**Figure 8.** Mean monthly concentrations of milk somatic cells (Log 10 Milk SCC [x1000]) for primiparous and multiparous cows fed Selenium Yeast (Sel-Plex) or sodium selenite (control). Diet x Parity x Month Interaction P<0.05.

**California Site**

The objectives in the California study (Rutigliano et al., 2006) were comparable to those of the Florida site experiment to determine the effects of source of supplemental Se on lactation, postpartum health, immune responses, and Se status of periparturient Holstein cows.

Treatments were sodium selenite (SS, n = 285) or Se-yeast (SY, n = 281) supplemented at 0.3 mg/kg from 25 d prior to calving to 80 d in milk. In Experiment 1, health of dairy cows was monitored daily in the first 80 d in milk, and lactation performance was followed for the first 90 d postpartum. In experiment 2, 37 cows fed
SS and 35 cows fed SY were evaluated for cellular and humoral immune responses. Concentrations of Se in plasma were determined at -45, 0, 21, 42 and 63 d relative to calving. Glutathione peroxidase activity in plasma, neutrophil phagocytic and bactericidal activity, and its oxidative metabolism were evaluated on the day of calving and 42 postpartum. Cows received i.m. injections of ovalbumin and anti-ovalbumin IgG concentrations were measured in serum. A sample of colostrum was analyzed for total IgG concentrations.

Incidence of retained placenta, fever, mastitis, clinical ketosis, and displacement of abomasum, and proportion of cows that left the study before 80 d postpartum were not affected by source of supplemental Se. Cows receiving SY produced more ($P = 0.02$) 3.5% FCM and milk fat than those fed SS (Table 3). Estimates of daily production of 3.5% fat corrected milk (FCM), determined from monthly estimates of milk production for the first 3-month period of lactation, indicated that cows fed SY had a 5% increase in daily production of 3.5% FCM.

Table 3. Effect of sodium selenite (SS) and selenium yeast (SY) on lactation performance

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SS</th>
<th>SY</th>
<th>SEM</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk, kg/d</td>
<td>40.1</td>
<td>41.1</td>
<td>0.47</td>
<td>0.12</td>
</tr>
<tr>
<td>3.5% FCM, kg/d</td>
<td>38.5</td>
<td>40.4</td>
<td>0.56</td>
<td>0.02</td>
</tr>
<tr>
<td>Milk fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>3.40</td>
<td>3.50</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Kg/d</td>
<td>1.35</td>
<td>1.42</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>True protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>2.80</td>
<td>2.79</td>
<td>0.01</td>
<td>0.58</td>
</tr>
<tr>
<td>Kg/d</td>
<td>1.12</td>
<td>1.14</td>
<td>0.01</td>
<td>0.31</td>
</tr>
<tr>
<td>SCC, x 10$^3$/mL</td>
<td>132</td>
<td>120</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Linear SCC</td>
<td>1.77</td>
<td>1.80</td>
<td>0.08</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Concentration of Se and glutathione peroxidase activity in plasma were similar for SS and SY groups throughout the study. Concentration of IgG in colostrum was similar for SS and SY groups. Phagocytic and killing activities of neutrophils were influenced ($P < 0.01$) by day postpartum, but not by source of Se. Similarly, the ability of neutrophils to reduce nitroblue tetrazolium was not influenced by source of Se in stimulated and nonstimulated neutrophils. Pregnancy on d 28 after first AI was not influenced by source of Se or method of presynchronization. Similarly, pregnancy loss from 28 to 56 d of gestation was not influenced by source of Se or method of presynchronization. Source
of Se and method of presynchronization did not affect pregnancy rates or embryonic survival in dairy cows.

Replacing SS with SY did not improve health or immunological status of periparturient dairy cows, but increased yields of 3.5% fat-corrected milk and fat, and concentration of fat in milk. The lack of treatment effects, other than on milk yield, during the first 90 days of lactation is perplexing. One of the potential selenoproteins induced by organic supplementation of selenium is iodothyronine deiodinase that may increase the availability of triiodothyronine to enhance milk production.

**General Discussion and Summary of Site Differences**

Weiss (2003) pointed out that in many of the clinical and experimental efforts, most control diets would be considered deficient in selenium and the companion diets contained either 0.1 or 0.3 mg/Kg of supplemental selenium. Supplementation of selenium to deficient diets often elicits a positive response whereas additional supplementation of Se-adequate diets would not be expected to produce additional clinical benefits. This appears to be the case when comparing the efficacy of selenium yeast (SY) in the present studies in which supplemental SY had distinctive effects on immune responses, uterine health, plasma Se concentrations, and second service pregnancy rates on cows in Florida. Basically, there were no SY effects within the California study other than a significant increase in milk production. Both studies were conducted during the summer heat stress period. Analyses of the composite feed samples for both experiments identified clear differences in dietary Se concentrations. Selenium concentrations in the prepartum diets differed substantially between the Florida (Control of 0.44 and SY of 0.49 mg/kg of dry matter) and California (Control of 0.60 and SY of 0.74 mg/kg of dry matter) experiments. Differences in selenium concentrations (mg/kg of dry matter) for the postpartum diets were even greater; that is, Florida with 0.36 for control and 0.36 for SY and California with 0.71 for control and 0.60 for SY. Based upon the higher overall Se concentrations (i.e., supplemental + background Se levels) in the California experiment, it was not surprising that clinical and immuno-responses did not differ. In contrast, Florida was essentially in a Se-deficient environment in which the basic Se source was via the two supplements. Under these conditions, selenium yeast fed in the ration (Sel-Plex®; 0.33 mg/Kg), beginning at 26 days prepartum, elevated plasma Se concentrations, increased neutrophil function at the time of parturition, improved immuno-responsiveness in multiparous cows, improved uterine health, and increased second service PR during summer in an environment that is Se deficient. Such effects were not observed when Se supplementation of a Se-adequate diet was in place. It is noteworthy that in the California study, Se supplementation did result in a substantial increase in milk production.
References


