Effects of supplement type and selenium source on measures of growth and selenium status in yearling beef steers

J. D. Arthington

Institute of Food and Agricultural Sciences, Range Cattle Research and Education Center, University of Florida, Ona 33865

ABSTRACT: Sugarcane molasses is a widely used animal feed by-product, but is concentrated in S (approximately 1%, DM basis) and has been shown to reduce the Cu status of cattle. Dietary S may also antagonize Se; therefore, two 90-d studies were conducted with forage-fed, yearling steers (12 pens; 2 steers/pen for each study) to investigate the impact of molasses supplementation on measures of Se status. In Exp. 1, steers were assigned isonitrogenous supplements with equivalent amounts of TDN from 2 sources (molasses or corn). Supplemental Se was provided (3.0 mg of Se/d; Na selenite) to both treatments. After 90 d of supplementation, steers provided corn diets had greater (P = 0.02) liver Se concentrations and tended (P = 0.07) to have greater ADG compared with steers supplemented with molasses. Irrespective of treatment (P ≥ 0.54), plasma Se concentrations decreased (P < 0.001) and plasma glutathione peroxidase activity increased (P < 0.001) from d 0 to 90. In Exp. 2, sources of supplemental Se (2.5 mg/d), fed within molasses supplements, were compared. Treatments included 1) Na selenite, 2) Se-yeast (Selplex, Alltech, Nicholasville, KY), or 3) no Se (control). Cattle provided supplemental Se, irrespective of source, had greater (P ≤ 0.01) liver and plasma Se concentrations and greater (P ≤ 0.01) plasma glutathione peroxidase activity compared with control steers on d 60 and 90. Measures of Se status did not differ among steers supplemented with Na selenite and Se-yeast. These data suggest that dietary S, derived from sugarcane molasses, may antagonize liver tissue accumulation of Se in cattle. The Se status of cattle consuming sugarcane molasses was similar when provided 2.5 mg of supplemental Se/d from Na selenite or Se-yeast sources.

Key words: corn, glutathione peroxidase, molasses, selenium, steer

INTRODUCTION

Sulfur is found naturally in nearly all feedstuffs. The form of S varies widely from multiple inorganic salt compounds to organic S-containing amino acids. Sulfur is recognized as an essential nutrient for beef cattle, although minimum requirements for optimal growth and development are not well defined (NRC, 1996). In addition, dietary S excesses are recognized as important contributors to reduced utilization of Cu (Underwood and Suttle, 1999) and Se (Ivancic and Weiss, 2001) in cattle consuming forage-based diets and to polioencephalomalacia in finishing cattle fed concentrate diets (Gould, 1998). There are multiple sources of excessive dietary S, including 1) S-containing fertilizers (Arthington et al., 2002), high-sulfate water (Weeth and Capps, 1972; Gould, 1998), and high S-containing feedstuffs (Arthington and Pate, 2002).

Sugarcane molasses is a by-product of the production of food-grade sugar. It is an important base component of cattle liquid feed supplements (Pate, 1983), which are fed to mature beef cows (Pate and Kunkle, 1989; Kalmbacher et al., 1995), yearling stockers (Brown, 1993), and finishing cattle (Heimann and Hanks, 1977). Although sugarcane molasses can antagonize Cu utilization in cattle (Arthington et al., 2003), the influence of molasses-derived S on Se utilization in cattle has not been investigated.

Therefore, the objectives of these studies were to 1) investigate the influence of molasses- vs. corn-based supplements on measures of Se status in yearling steers, and 2) investigate the influence of Se source (organic vs. inorganic), offered in molasses-based supplements, on measures of Se status in yearling steers.


1Appreciation is expressed to the Liquid Feed Committee of the American Feed Industry Association (Arlington, VA) and to Alltech, Inc. (Nicholasville, KY) for their partial financial support of this research. Appreciation is also expressed to L. R. McDowell, N. Wilkinson, and T. Wood for their technical assistance and academic support during the conduct of these experiments.

2Corresponding author: jarth@ufl.edu
Received October 22, 2007.
Accepted January 31, 2008.
Table 1. Analyzed nutrient content of the hay and supplement, Exp. 1 and 2

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Exp. 1 Hay</th>
<th>Molasses</th>
<th>Corn</th>
<th>CSM2</th>
<th>Exp. 2 Hay</th>
<th>Molasses</th>
<th>CSM2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>89.3</td>
<td>71.5</td>
<td>88.7</td>
<td>90.7</td>
<td>90.2</td>
<td>72.1</td>
<td>89.3</td>
</tr>
<tr>
<td>ADF</td>
<td>44.4</td>
<td>NA</td>
<td>6.2</td>
<td>18.8</td>
<td>42.3</td>
<td>NA</td>
<td>18.7</td>
</tr>
<tr>
<td>CP</td>
<td>6.5</td>
<td>12.4</td>
<td>11.6</td>
<td>45.6</td>
<td>6.2</td>
<td>10.4</td>
<td>45.5</td>
</tr>
<tr>
<td>Ca</td>
<td>0.28</td>
<td>1.10</td>
<td>0.60</td>
<td>0.27</td>
<td>0.40</td>
<td>1.23</td>
<td>0.24</td>
</tr>
<tr>
<td>P</td>
<td>0.28</td>
<td>0.22</td>
<td>0.91</td>
<td>1.44</td>
<td>0.15</td>
<td>0.13</td>
<td>1.26</td>
</tr>
<tr>
<td>Na</td>
<td>0.01</td>
<td>0.20</td>
<td>0.34</td>
<td>0.17</td>
<td>0.05</td>
<td>0.25</td>
<td>0.07</td>
</tr>
<tr>
<td>S</td>
<td>0.14</td>
<td>0.91</td>
<td>0.16</td>
<td>0.45</td>
<td>0.26</td>
<td>1.08</td>
<td>0.50</td>
</tr>
<tr>
<td>Cu</td>
<td>6.0</td>
<td>19.7</td>
<td>6.9</td>
<td>9.0</td>
<td>5.7</td>
<td>22.7</td>
<td>12.0</td>
</tr>
<tr>
<td>Zn</td>
<td>28.5</td>
<td>40.5</td>
<td>52.1</td>
<td>67.0</td>
<td>40.3</td>
<td>20.3</td>
<td>66.6</td>
</tr>
<tr>
<td>Mn</td>
<td>40.5</td>
<td>30.7</td>
<td>44.5</td>
<td>26.0</td>
<td>59.5</td>
<td>19.9</td>
<td>29.3</td>
</tr>
<tr>
<td>Se</td>
<td>0.008</td>
<td>0.13</td>
<td>0.07</td>
<td>0.28</td>
<td>&lt;0.08</td>
<td>0.13</td>
<td>0.17</td>
</tr>
</tbody>
</table>

1Dry matter (Shreve et al., 2006); CP (AOAC, 1996; method 976.06); ADF (Ankom Technology, 2003); Ca and Na (AOAC, 1996; method 968.08); P (AOAC, 1996; method 965.17); S, Cu, Zn, Mn (AOAC, 1996; method 985.01); and Se (AOAC, 1996; method 996.16).
2CSM = nonpelleted cottonseed meal.

Table 2. Ingredient formulation of the mineral supplements, Exp. 1 and 2

<table>
<thead>
<tr>
<th>Mineral1</th>
<th>Exp. 12</th>
<th>Exp. 23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>11.7</td>
<td>6.1</td>
</tr>
<tr>
<td>P</td>
<td>11.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Na</td>
<td>7.7</td>
<td>9.8</td>
</tr>
<tr>
<td>Cu</td>
<td>0.15</td>
<td>0.25</td>
</tr>
<tr>
<td>Zn</td>
<td>0.30</td>
<td>1.0</td>
</tr>
<tr>
<td>Mn</td>
<td>0.10</td>
<td>0.39</td>
</tr>
<tr>
<td>Se</td>
<td>0.0052</td>
<td>Varied4</td>
</tr>
</tbody>
</table>

1Mineral sources included Ca(H2PO4), NaCl, CuSO4·5H2O, KCl, MgO, CaCO3, ZnSO4·H2O, MnO, and Na2SeO3.
2Exp. 1, fed at a rate of 58 g/steer daily.
3Exp. 2, fed at a rate of 40 g/steer daily.
4Inclusion varied by Se content of Se-source treatment ingredient.

MATERIALS AND METHODS

Animal Care, Handling, and Diet

Animals used in these experiments were cared for by acceptable practices (FASS, 1999), and the protocols were approved by the University of Florida Institutional Animal Care and Use Committee. The experiments were conducted at the University of Florida, Institute of Food and Agricultural Sciences, Range Cattle Research and Education Center, Ona.

In Exp. 1, 24 crossbred (Brahman × British; approximately 13 mo of age; average initial BW = 296 ± 10.8 kg) steers were stratified by BW and assigned randomly to dry-lot pens of equal size (114 m2, 2 steers/pen). Two supplement treatments (6 pens/treatment) were formulated by using ground corn and nonpelleted cottonseed meal or molasses and nonpelleted cottonseed meal. Each supplement was formulated to provide 1.5 kg of TDN and 0.3 kg of CP daily (DM basis; Table 1) and included a mineral premix (Table 2). Supplemental Se (from Na selenite) was provided at a rate of 3.0 mg of Se/steer daily. Supplement components were weighed individually, handmixed, and delivered to the pens 3 times weekly (3.5 and 0.7 kg of TDN and CP/steer, provided on Monday, Wednesday, and Friday; DM basis) in individual rubber-based feeding pans. Steers were provided free-choice access to long-stem stargrass hay (Cynodon spp.; Table 1). To assess the effect of supplement composition on steer Se status, jugular blood and liver biopsy samples were collected on d 0, 30, 60, and 90. Individual steer ADG was determined by the difference between final and initial steer BW after a 16-h withdrawal from hay and supplement, but not water.

In Exp. 2, 24 (Brahman × British; approximately 13 mo of age; average initial BW = 348 ± 11.9 kg) crossbred steers were stratified by BW and assigned randomly to pens of equal size (114 m2, 2 steers/pen). Three Se treatments (4 pens/treatment) were assigned randomly to pens and were provided within a molasses and nonpelleted cottonseed meal slurry. Each supplement was formulated to provide 1.5 kg of TDN and 0.3 kg of CP/steer (DM basis; Table 1) and included a mineral premix (Table 2). Selenium treatments consisted of 1) no Se (negative control), 2) Na selenite, or 3) Se-yeast (Sel-Plex, Alltech, Nicholasville, KY). Treatments provided 2.5 mg of supplemental Se/steer daily. Supplement components were weighed individually, hand-mixed, and delivered to pens 3 times weekly (3.5 and 0.7 kg of TDN and CP/steer, provided on Monday, Wednesday, and Friday; DM basis) in individual rubber-based feeding pans. Steers were provided free-choice access to long-stem stargrass hay (Table 1). To assess the effect of supplement composition on steer Se status, jugular blood, and liver biopsy samples were collected on d 0, 30, 60, and 90. Individual steer ADG was determined by the difference between final and initial steer BW.
after a 16-h withdrawal from hay and supplement, but not water.

**Feed, Liver, and Plasma Analysis**

The nutrient contents of supplement components and hay were analyzed by a commercial laboratory (SDK Laboratories, Hutchinson, KS; Table 1). Total digestible nutrients of corn and cottonseed meal were estimated by the equation 83.0 – [1.12 × ADF (% DM basis)] (Ankom Technology, 2003) and of molasses by the manufacturer’s label guarantee (United States Sugar Corporation, Clewiston, FL). Liver biopsy samples were collected by using techniques described previously (Arthington and Corah, 1995) and were analyzed for Se concentration (SDK Laboratories) by using inductively coupled plasma-atomic emission spectroscopy (AOAC, 1996; method 996.16). Blood was collected by jugular venipuncture into heparin-coated, evacuated tubes (Becton Dickinson, Franklin Lakes, NJ). Plasma was harvested from blood after centrifugation at 2,000 × g for 20 min and then was frozen at −20°C until analyzed for Se concentration by using the modified fluorometric procedure, as described previously (Whetter and Ulrey, 1978), and for glutathione peroxidase (GPX3) activity with a commercial GPX3 assay kit (catalog no. 703102, Cayman Chemical, Ann Arbor, MI), which measures GPX3 activity indirectly by a coupled reaction with glutathione reductase (Paglia and Valentine, 1967).

**Statistical Analysis**

Statistical analyses of all variables for both experiments were achieved by ANOVA for a repeated measures experiment within a completely randomized design by using PROC MIXED (SAS Inst. Inc., Cary, NC), with pen × treatment included as the random effect. For analysis of liver and plasma Se concentration and GPX3 activity, the model statement contained the effects of treatment and time and the interaction for treatment × time. For all measures of Se status, when the initial values (d 0) differed (P < 0.05) among groups, they were used as a covariate in the model to test subsequent sampling dates. For overall change in liver and plasma Se concentrations and ADG, the model statement contained the effect of treatment. Pen was the experimental unit for both experiments.

When treatment or treatment × day interactions were significant, differences among treatments were compared by using PDIFF in Exp. 1 and single-df orthogonal contrasts in Exp. 2. Contrasts included 1) Na selenite vs. Se-yeast and 2) control vs. Na selenite and Se-yeast. All values are reported as least squares means.

**RESULTS AND DISCUSSION**

Although similar intakes of supplemental TDN and CP among treatments were achieved in Exp. 1, steers fed corn-based supplements tended (P = 0.07) to have a greater ADG compared with steers fed molasses-based supplements (0.24 vs. 0.10 kg/d; SEM = 0.05). A similar response was reported in yearling Brahman × British heifers supplemented 3 times weekly (Cooke et al., 2007b). In that study, heifers provided dry feed supplements (citrus pulp based) had greater (P < 0.05) ADG over a 115-d period compared with heifers provided molasses-based supplements similar to those used in the current study. In contrast, 2 other studies, also using yearling, forage-fed cattle with similar age and breeding as in the current study, reported no differences in ADG among isocaloric and isonitrogenous supplements composed of dry by-products (wheat middlings and citrus pulp) vs. liquid molasses (Arthington et al., 2004; Cooke et al., 2007a).

Second to the kidney, the liver is a substantial reservoir for Se deposit in the body (Sunde, 1997), making it a logical sampling tissue for estimating Se status. In Exp. 1, a sampling day × supplement source interaction was detected for liver Se concentration (P < 0.01; Table 3). Steers fed molasses-based supplements had greater initial liver Se concentrations than steers fed corn-based supplements; therefore, d 0 values were used as a covariate to test mean comparisons in subsequent sampling days (Table 3). Over the 90-d supplementation period, steers fed corn-based supplements had a greater (P = 0.02) overall increase in liver Se accumulation compared with steers fed molasses-based supplements (1.18 vs. 0.69 ± 0.12 mg/kg). As a consequence, on d 90, steers fed corn-based supplements had greater (P = 0.02) liver Se concentrations compared with steers fed molasses-based supplements (Table 3). Steers were individually offered equivalent amounts of supplemental Se (3.0 mg/d) and the Se supplied by the base feedstuffs differed only slightly (0.19 vs. 0.33 mg/d for corn- and molasses-based supplements, respectively); there-
fore, differences in liver Se accumulation cannot be attributed to differences in dietary Se intake. One exception may be the contribution of Se from forage. Because forage DMI was not measured in this study, the potential for differences in consumption of forage-derived Se among treatments cannot be ruled out.

No sampling day × supplement source interaction was observed for plasma Se concentrations \((P > 0.54); \text{ Table 4}\); however, molasses-supplemented steers tended \((P < 0.06)\) to have greater plasma Se concentrations, averaged over all sampling days, compared with steers fed corn-based supplements \((0.046 \pm 0.002 \text{ mg/L, respectively})\). Similarly, no supplement or supplement × day interactions were detected for plasma GPX3 activity \((P = 0.59)\). However, GPX3 activity increased \((P < 0.001)\) over time, irrespective of supplement source (Figure 1). This increase in GPX3 activity appeared to follow increases in liver Se concentration. The minimum liver Se concentration required for maintaining GPX3 activity in the bovine is unknown; however, it appears likely that the bioavailability of dietary Se was adequate in both treatments to maintain similar GPX3 activity values. The S component of the 2 supplement sources was the most significant nutritional difference that we can attribute to the liver Se status differences reported in Exp. 1 (Table 4). Other researchers have also reported reduced Se status in cows provided S-fortified diets (Ivancic and Weiss, 2001) or in steers grazing forages that had received an S-containing fertilizer (Murphy and Quirke, 1997).

In Exp. 2, steer BW gain did not differ \((P = 0.96)\) among treatments \((0.30, 0.33, \text{ and } 0.31 \pm 0.14 \text{ kg/d for control, Se-yeast-, and Na selenite-supplemented steers, respectively})\). Unlike Exp. 1, the supplement source (sugarcane molasses + cottonseed meal) was identical across all treatments. The difference included only the source of Se. Other researchers (Gunter et al., 2003) have also reported no effects on BW gain of cows and calves when Na selenite and Se-yeast supplements were compared with each other or with a control treatment that provided no supplemental Se.

In Exp. 2, a sampling day × supplement source interaction was detected for liver Se concentration \((P < 0.01)\). Steers assigned to the control treatment had a lesser \((P < 0.05)\) initial liver Se concentration compared with steers assigned to the Na selenite and Se-yeast treatments (Table 4); therefore, d 0 values were used as a covariate to test subsequent sampling day. Liver Se concentrations were greater \((P \leq 0.03)\) in Se-supplemented steers compared with control steers on d 30, 60, and 90, irrespective of Se source (Table 4). In addition, liver Se concentration from d 0 to 90 was reduced \((P = 0.02)\) in control steers, tended to be reduced \((P = 0.08)\) in steers supplemented with Na selenite, but did not change \((P = 0.51)\) in steers provided Se-yeast.

Similar to liver Se measures, plasma Se concentrations (Table 4) were greater \((P < 0.01)\) in Se-supplemented steers compared with steers that did not receive a Se supplement on d 30, 60, and 90; however, source of Se had no impact on plasma Se concentrations at any of the sampling days \((P > 0.30); \text{ Table 4}\). Other research has reported increased serum Se concentrations as a result of increased dietary Se offered to Holstein calves \((0.40 \text{ vs. } 0.73 \mu g/g; \text{ Awadeh et al., 1998})\). A further outcome of that study revealed that Se-yeast-supplemented cows had similar serum Se concentrations but greater whole blood Se concentrations compared with cows receiving an equivalent amount of Se from Na selenite. Similarly, Ortman and Pehrsone (1999) reported greater whole blood but similar plasma Se concentrations in dairy cows provided supplemental Se-yeast vs. Na selenite. Differences among these studies, compared with the current study, may be partially explained by the varied use of whole blood Se vs. plasma (or serum) Se as indicators of Se status. Whole blood Se accounts for the Se present in the erythrocyte, typically a component of erythrocyte-bound GPX3 and non-

### Table 4. Effect of supplemental Se source on liver and plasma Se concentrations in growing steers (least squares means, Exp. 2)

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment¹</th>
<th>Contrast²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Se-yeast</td>
</tr>
<tr>
<td>Liver Se</td>
<td>mg/kg</td>
<td></td>
</tr>
<tr>
<td>0²</td>
<td>1.77</td>
<td>2.62</td>
</tr>
<tr>
<td>30</td>
<td>1.11</td>
<td>2.09</td>
</tr>
<tr>
<td>60</td>
<td>0.80</td>
<td>2.24</td>
</tr>
<tr>
<td>90</td>
<td>0.97</td>
<td>2.30</td>
</tr>
<tr>
<td>Plasma Se</td>
<td>mg/L</td>
<td></td>
</tr>
<tr>
<td>0²</td>
<td>0.015</td>
<td>0.021</td>
</tr>
<tr>
<td>30</td>
<td>0.019</td>
<td>0.054</td>
</tr>
<tr>
<td>60</td>
<td>0.020</td>
<td>0.051</td>
</tr>
<tr>
<td>90</td>
<td>0.016</td>
<td>0.053</td>
</tr>
</tbody>
</table>

¹Supplemental Se provided by the designated source \((2.5 \text{ mg/steer daily})\) within molasses-based supplements. Control steers received no supplemental Se.

²Contrasts: 1) Na Selenite vs. Se-yeast, and 2) control vs. the average of Na selenite and Se-yeast.

³Values on d 0 were used as a covariate to test subsequent sampling day.
specific hemoglobin-associated Se (Butler et al., 1991), in addition to the Se present in plasma (Awadeh et al., 1998). Use of plasma Se as an indicator of Se status in supplementation studies with treatment periods less than the average life span of the erythrocyte (60 to 120 d) may underestimate the true Se status of the subject because these 2 pools accumulate Se differently depending on the dietary form of Se being consumed (Whanger, 1986; Butler et al., 1991).

In the current study, plasma GPX3 activity increased sharply in Se-supplemented steers, was greater \( (P < 0.01) \) on d 60 and 90, and tended \( (P = 0.09) \) to be greater on d 30 compared with steers receiving no supplemental Se (Figure 1). Steers provided Se-yeast tended to have greater GPX3 activity on d 30 \( (P = 0.08) \), but not on d 60 and 90 \( (P > 0.60) \), compared with steers provided Na selenite (Figure 1). Similar to the current study, other researchers have reported increased erythrocyte GPX activity, plasma GPX3 activity, or both in Se-fortified diets fed to mice (Zhu et al., 1993), swine (Mahan et al., 1999; Yoon and McMillan, 2006), steers (Hintze et al., 2002), and cows (Ortman and Pehrson, 1999; Gunter et al., 2003). However, blood GPX3 activity appears to be similar when supplemental Se is provided via Na selenite or Se-yeast (Mahan et al., 1999; Ortman and Pehrson, 1999; Gunter et al., 2003).

These results imply that liver accumulation of Se is reduced in forage-fed steers consuming sugarcane molasses-based vs. corn-based supplements. Additionally, steers provided supplemental Se, irrespective of source (Na selenite vs. Se-yeast), blended into sugarcane molasses-based supplements have greater indices of Se status compared with control steers provided the same sugarcane molasses supplement with no supplemental Se.

**Figure 1.** Plasma glutathione peroxidase activity in growing, yearling steers. A unit of activity is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of NADPH to NADP+ per min at 25°C. In Exp. 1, steers were supplemented with Na selenite (2.5 mg of Se/d) within corn- or sugarcane molasses-based supplements. Day 30 values are missing. In Exp. 2, 2 sources of Se, Na selenite and Se-yeast (each at 2.5 mg of Se/d), or the control (0 mg of Se/d) were provided in sugarcane molasses-based supplements. Treatment × day interaction: \( P = 0.59 \) and 0.01 for Exp. 1 and 2, respectively; SEM = 0.43 and 0.33 for Exp. 1 and 2, respectively. Mean contrasts involving the average of Na selenite and Se-yeast vs. control differ \( (P < 0.01) \) on d 60 and 90 and tend to differ \( (P = 0.09) \) on d 30 compared with control. Mean contrasts of Na selenite vs. Se-yeast tend to differ \( (P = 0.08) \) on d 30 but not d 60 and 90 \( (P > 0.60) \).

**LITERATURE CITED**


