An Injectable Trace Mineral Supplement in Yearling Bulls Causes a Short-Term Increase in Circulating Trace Mineral Levels But Does Not Improve Sperm Quality

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Abstract
Proper trace mineral supplementation is necessary for reproductive development and function. Supplementation with various trace minerals has been shown to improve overall sperm quality and morphology and increase the percentage of live sperm. When developing beef bulls, it is necessary to meet trace mineral requirements to ensure proper reproductive success. An injectable trace mineral product has been made commercially available for use in cattle as a supplemental form of chelated selenium, copper, zinc, and manganese. Considering the role of trace minerals in bull reproductive function, we posed the question of whether using an injectable trace mineral product beyond dietary supplementation could improve sperm quality and percentage of bulls passing yearling breeding soundness exams. The objectives of our study were to compare serum trace mineral concentrations of bulls before and after administration of an injection of trace minerals or saline and to compare semen trace mineral concentrations after treatment. To determine if an injectable trace mineral product could be of benefit, we compared serum and semen trace mineral concentrations as well as semen quality and percentage passing a yearling breeding soundness examination in treated and untreated bulls.

Keywords
injectable trace mineral, sperm quality, yearling bulls

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Introduction
Proper trace mineral supplementation is necessary for reproductive development and function. Supplementation with various trace minerals has been shown to improve overall sperm quality and morphology and increase the percentage of live sperm. When developing beef bulls, it is necessary to meet trace mineral requirements to ensure proper reproductive success. An injectable trace mineral product has been made commercially available for use in cattle as a supplemental form of chelated selenium, copper, zinc, and manganese. Considering the role of trace minerals in bull reproductive function, we posed the question of whether using an injectable trace mineral product beyond dietary supplementation could improve sperm quality and percentage of bulls passing yearling breeding soundness exams. The objectives of our study were to compare serum trace mineral concentrations of bulls before and after administration of an injection of trace minerals or saline and to compare semen trace mineral concentrations after treatment. To determine if an injectable trace mineral product could be of benefit, we compared serum and semen trace mineral concentrations as well as semen quality and percentage passing a yearling breeding soundness examination in treated and untreated bulls.

Experimental Procedures
Data for this project were collected from 90 yearling bulls sourced from the Kansas State University Purebred Beef Unit. At the beginning of the trial, bulls were 276 ± 20 days of age and 829 ± 21 lb. Bulls were representative of Hereford, Angus, and Simmental breeds. Bulls received one of two treatments. Half of the bulls (n = 45) received a subcutaneous injection at 1 ml/100 lb of body weight of an injectable trace mineral product (Multimin 90; Multimin USA, Fort Collins, CO; 15 mg Cu/mL, 60 mg Zn/mL, 10 Mg/mL, 5 mg Se/mL). The control group (n = 45) received an injection of sterilized saline at the same dose. All bulls were maintained in a dry lot and fed a grower ration designed to attain an average daily gain of 2.64 lb/day. The ration contained 70% concentrate and 30% roughage and included dietary trace mineral supplementation at levels recommended by the National Research Council.
Blood samples from all bulls were collected immediately before treatment (hour 0) to determine pretreatment serum concentrations of Cu, Mn, Se, and Zn. To assess post-treatment serum concentrations of Cu, Mn, Se, and Zn, blood samples were collected at 8 and 24 hours posttreatment. Body weights and scrotal circumferences were measured on the day of treatment administration (day 0) and days 20, 42, 59, and 91 posttreatment.

Semen was collected via electroejaculation on days 42 and 91 posttreatment. On day 91, when bulls averaged 362 ± 25 days of age, a complete breeding soundness examination was performed. Bulls passed their breeding soundness exam if scrotal circumference was at least 32 cm, semen contained 70% normal morphology and 30% progressive motility, and there was no evidence of reproductive unsoundness. Serum and semen samples collected on days 42 and 91 from a subset of 26 bulls per treatment group were analyzed for concentrations of Cu, Mn, Se, and Zn.

All data were analyzed in SAS (SAS Institute, Cary, NC) with bull as the experimental unit. Scrotal circumference, body weight, trace mineral concentrations, and sperm parameters were evaluated using the MIXED model, with fixed effects of treatment, time of sampling, and time × treatment. Breed was the random effect. Breeding soundness examination data were analyzed using GLIMMIX, with treatment as a fixed effect and breed as the random variable.

**Results and Discussion**

Body weights and scrotal circumference increased \((P < 0.0001)\) throughout the duration of the trial but did not differ between treatments \((P ≥ 0.89)\). At the time of breeding soundness examinations (day 91), bulls weighed 1,137 ± 34.64 lb and had a scrotal circumference of 36.9 ± 0.394 cm. A time × treatment interaction effect \((P ≤ 0.003)\) was observed for serum trace mineral concentrations, with treatment bulls having greater trace mineral concentrations at 8 hours posttreatment. A peak in concentration for all minerals at 8 hours was followed by a decline approaching baseline for Cu and Zn (Figures 1 and 2). No differences were observed in semen trace mineral concentrations between treatment groups at day 42 or day 91 \((P ≥ 0.25; \text{Figures 1 and 2})\). Treatment groups did not differ in sperm parameters on day 42 or day 91, although treated bulls tended \((P = 0.06)\) to have greater sperm concentrations at day 42 (Table 1). Treatment groups also did not differ \((P = 0.9362)\) in percentage of bulls passing a yearling breeding soundness exam (Figure 3).

Semen concentrations of Cu, Zn, Se, and Mn all were within range of previously reported semen mineral concentrations. Overall, the percentage of bulls, both treated and untreated, passing a breeding soundness exam at day 91 was relatively low. Bulls that failed to pass were mostly hindered by semen parameters characteristic of immaturity. Both proximal and distal droplets, along with poor morphology, were primary causes of failure. Bulls were retested two weeks later, prior to sale, and the percentage passing improved.
Implications
Use of an injectable trace mineral product in yearling beef bulls when dietary trace mineral levels are adequate causes a short-term increase in circulating trace mineral concentrations but does not alter semen trace mineral content, improve semen quality, or increase percentage of bulls passing a yearling breeding soundness examination.

Acknowledgements
Thank you to Ryan Breiner, the Kansas State Purebred Beef Unit, the Kansas Artificial Breeding Unit, and various graduate students for the many hours of assistance in completing this trial.

Table 1. Sperm concentration, morphology, and motility in bulls 42 or 91 days after administering a saline placebo (control) or injectable trace mineral

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Trace mineral</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 42</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm concentration, million/mL</td>
<td>90.49 ± 39</td>
<td>151.51 ± 39</td>
<td>0.06</td>
</tr>
<tr>
<td>Normal sperm, %</td>
<td>29.40 ± 4.5</td>
<td>35.47 ± 4.5</td>
<td>0.34</td>
</tr>
<tr>
<td>Progressive sperm, %</td>
<td>38.33 ± 2.6</td>
<td>39.19 ± 2.6</td>
<td>0.82</td>
</tr>
<tr>
<td><strong>Day 91</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm concentration, million/mL</td>
<td>219.04 ± 29</td>
<td>226.66 ± 28</td>
<td>0.83</td>
</tr>
<tr>
<td>Normal sperm, %</td>
<td>59.09 ± 5.5</td>
<td>58.26 ± 5.4</td>
<td>0.89</td>
</tr>
<tr>
<td>Progressive sperm, %</td>
<td>44.43 ± 2.6</td>
<td>42.33 ± 2.5</td>
<td>0.56</td>
</tr>
</tbody>
</table>
Figure 1. Serum manganese and selenium concentrations of control and trace mineral bulls at 0, 8, and 24 hours posttreatment.
Time × treatment, $P < 0.0001$; * Treatment $P < 0.0001$.

Figure 2. Serum copper and zinc concentrations of control and trace mineral bulls 0, 8, and 24 hours posttreatment.
Time × treatment, $P \leq 0.003$; * Treatment, $P < 0.0001$
Figure 3. Percentage control and trace mineral bulls passing a yearling breeding soundness exam (BSE).

* Treatment, $P = 0.93$. 

- Control: 68.2%
- Trace mineral: 67.4%