Effect of injectable trace minerals on the humoral immune response to multivalent vaccine administration in beef calves[1]

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ABSTRACT: The objective of this experiment was to investigate the effects of injectable trace minerals on humoral responses of calves receiving a viral vaccination. Beef steer calves (n = 99; average BW = 316 ± 4.2 kg), seronegative for bovine herpesvirus-1 (BHV-1) and bovine viral diarrhea virus, genotypes 1 and 2 (BVDV-1 and BVDV-2), were sourced from 2 locations. All calves, except 15 non-vaccinated (sentinel) calves, received a single dose of a multivalent modified live vaccine (Titanium 5; AgriLabs, St. Joseph, MO) containing BHV-1, BVDV-1, BVDV-2, bovine parainfluenza virus type 3, and bovine respiratory syncytial virus. Among the vaccinated calves, 2 treatments were concurrently and randomly applied on the basis of initial serum Se status and BW, including 1) injectable trace mineral supplement (ITM; n = 42; 7 mL subcutaneous.; MultiMin, Fort Collins, CO) containing 15, 40, 10, and 5 mg/mL of Cu, Zn, Mn (all as disodium EDTA salts), and Se (as Na selenite) or 2) saline-injected control (Control; n = 42). As a measure of humoral immunity, neutralizing antibody titers were measured on d 0, 14, 30, 60, and 90, relative to vaccine administration. All calves were seronegative for each of the 3 viruses on d 0, and sentinel calves remained seronegative throughout the study. Serum mineral concentrations were evaluated on d 0 and 14. No differences ($P \geq 0.30$) in serum Cu, Zn, Mn, or Se were observed between treatments on d 0. Control steers experienced a decrease ($P < 0.001$) in serum Zn and Se, and ITM steers had an increase ($P = 0.007$) in serum Cu on d 14 relative to initial d 0 values. On d 14, serum Zn and Se concentrations were greater ($P < 0.01$) in ITM compared with Control steers. Vaccinated calves experienced marked increases in neutralizing antibody titers by d 30 following vaccine administration. Calves receiving ITM at the time of vaccination experienced greater ($P \leq 0.003$) neutralizing antibody titers to BHV-1 on d 14, 30, and 60 compared with Control. These results demonstrate that the injectable trace mineral formulation evaluated in this study, administered concurrently to viral vaccination, does not impair humoral immune responses in beef calves. Further, concurrent administration of ITM and BHV-1 vaccine may enhance the production of neutralizing antibodies to BHV-1 in previously naïve beef calves.

Key words: bovine herpesvirus, bovine viral diarrhea virus, injectable trace minerals, vaccination

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INTRODUCTION

The humoral immune response is mediated by the secretion of antigen-specific antibodies from B-lymphocytes (Tizard, 2004). This reaction is fundamental for actively acquired humoral immunity, which may result from the administration of vaccine or exposure to disease-causing pathogens. Morbidity and mortality of beef cattle, as a result of bovine respiratory disease (**BRD**), is a major issue affecting the economics of feedlot-finished beef cattle (Edwards, 1996; Duff and Galyean, 2007), and the presence of serum neutralizing antibodies against BRD-causing pathogens is evidence of a humoral immune response (Nobiron et al., 2003; Chase et al., 2004).

Trace minerals have been reported to be important for optimal immune function in livestock (Underwood

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and Suttle, 1999) and, in particular, the health and performance of stressed feeder cattle (Duff and Galyean, 2007). Although the benefits of injectable trace minerals (**ITM**) in ruminants have been previously evaluated in sheep (Cunningham, 1957), dairy cows (Dunkley et al. 1964), and beef cattle (Boila et al., 1984b), the few studies focusing on immune competence of feeder cattle have produced variable results (Droke and Loerch, 1989; Chirase et al., 1994; Clark et al., 2006). One potential influencing factor may be injection-site reactions, particularly with Cu-containing injectable supplements (Boila et al., 1984a), leading to activation of the pro-inflammatory acute phase response (Tothova et al., 2009) and reduced DMI (Chirase et al., 1994).

Interest in injectable trace minerals for stressed feeder calves is increasing (Berry et al., 2000, Clark et al., 2006; Richeson et al., 2009); therefore, the objective of the current study was to investigate advantages or disadvantages of a single injection of an EDTA-chelated source of Cu, Se, Mn, and Zn, administered concurrently with a modified live vaccine on measures of the humoral response, serum mineral status, and visual signs of injection site reactions in naïve beef calves.

MATERIALS AND METHODS

The study was conducted in cooperation with Heska (Des Moines, IA) at a USDA-APHIS, Veterinary Biologics-inspected animal research facility in Carlisle, IA. The protocol was reviewed and approved by the Heska Institutional Animal Care and Use Committee (Approved Protocol No. 08-03).

Animals, Experimental Design, and Sample Collection

Ninety-nine crossbred beef steers (10 to 12 mo of age; average BW = 316 ± 4.2 kg) were sourced from 2 herds and had no previous exposure to vaccines. Before commencement of the study, all steers were assessed and found to be seronegative for BHV-1, BVDV-1, and BVDV-2, on the basis of virus antibody titer. Eighty-four of the initial 99 steers were administered a 2-mL subcutaneous dose of a commercial modified live virus vaccine (Titanium 5; AgriLabs, St. Joseph, MO) containing BHV-1, BVDV-1, BVDV-2, bovine parainfluenza virus type 3, and bovine respiratory syncytial virus. Treatments were administered concurrently with vaccination following stratification by initial BW and serum Se concentrations (Ericson et al., 1986) assessed 21 d before the start of the study. Treatments consisted of (1) 7-mL subcutaneous injectable of trace minerals (**ITM**; MultiMin, Fort Collins, CO; n = 42) or (2) 7-mL subcutaneous injection of sterile saline (Control; n = 42). Administration of ITM provided 15, 40, and 10 mg/mL of Cu, Zn, and Mn (as disodium EDTA chelates) and 5 mg/mL of Se (as Na selenite). The remaining 15 steers received no vaccine or treatment and thus served as sentinel animals to verify that no field virus exposure occurred during the study. Treatments (ITM and Control) were administered in the neck region on the right side of the steer and vaccine was administered in the similar neck region on the left side. Steers were observed visually each day following the morning feeding by trained personnel, blinded to treatment, for visual signs of swelling at the injection site. In addition, the occurrence of palpable swellings at the injection sites was investigated at each of the bleeding days.

All steers were managed as a single group on a 14.3-ha perennial mixed grass pasture consisting of orchard grass, tall fescue, and smooth brome. Steers were also provided free-choice access to grass/alfalfa mixed hay and water. In addition, steers were fed a concentrate ration in a single bunk feeder, multiple times daily in amounts to ensure ad libitum consumption. The ration consisted of ground hay (35%), whole shelled corn (20%), dried distillers grains (43%), and a beef mineral premix (2%; New Providence Beef Formulator R1124; Prairie Land Cooperative, New Providence, IA). Hand grab samples (n = 5/sampling date) of concentrate ration and hay were collected on d 0, 14, 30, and 60 (and also d 90 for concentrate ration) and composited within and across days for a single pooled sample of both feed components. Pasture clipping samples were collected (n = 6/sampling date) on d 0, 14, 30, and 60 and also composited within and across days for a single pooled sample of pasture forage. Nutrient analysis of the pooled samples was conducted by the Olson Agricultural Analytical Services Laboratory at South Dakota State University (Brookings, SD) and are presented in Table 1. Briefly, Se concentration of feedstuffs was analyzed following the AOAC method 996.16 and all other minerals via the adapted European Committee for Standardization (CEN/TS 15621:2007). Nitrogen concentration was assessed following AOAC method 992.23 and presented as % CP by N × 6.25. A Tecator Fibertec (Tecator, Sweden) extraction method was used to assess ADF content following AOAC method 973.18. Calcium and P concentrations were determined via dry ashing (AOAC 968.08) and flame atomic absorption or colorimetry procedures for Ca and P, respectively (AOAC methods 935.13 and 931.01). Estimates of TDN were achieved via the following equation: 93.53 – (1.03 × ADF).

Blood was collected via jugular venipuncture on d 0 (immediately before vaccination and treatment administration), 14, 30, 60, and 90 using a 20-gauge × 3.8-cm needle (Vacutainer; BD Diagnostics, Franklin Lakes, NJ) into 2 individual 12.5-mL blood collection tubes (Monoject Corvac SST, Becton-Dickinson). Blood sam-

Table 1. Nutrient concentration of feed components (DM basis)[1]

Nutrient	Concentrate	Pasture	Hay	NRC requirement[2]	NRC maximum[3]
CP, %	16.5	20.4	13.4	-	-
ADF, %	26.4	27.4	40.7	-	-
TDN, %	66.4	69.4	55.2	-	-
Ca, %	0.77	0.48	0.62	-	-
P, %	0.55	0.38	0.34	-	-
Se, mg/kg	0.52	0.15	0.10	0.10	5.0
Mn, mg/kg	63	56	94	20	2000
Cu, mg/kg	16.0	10.1	8.7	10.0	40.0
Zn, mg/kg	57	26	23	30	500
Fe, mg/kg	550	306	912	50	500
S, %	0.49	0.23	0.15	0.15	0.40

[1]All steers (n = 99) were penned in a single 14.3-ha mixed grass pasture consisting of orchard grass, tall fescue, and smooth brome. Steers were also provided free-choice access to grass/alfalfa mixed hay and a concentrate ration consisting of ground hay (35%), whole shelled corn (20%), dried distillers grains (43%), and a beef mineral premix (2%).

[2] NRC (1996).

[3] NRC (2005).

ples were allowed to clot for 45 min at room temperature and then serum was harvested following centrifugation at 2,200 × *g* for 30 min at 4°C (Beckman Coulter TJ6 refrigerated centrifuge, Fullerton, CA). Serum was transferred into two 8-mL storage tube (Sarstetd Inc., Newton, NC) and stored at −20 ºC before analysis for serum neutralizing antibody titers (all collection dates) and serum mineral concentrations (d 0 and 14).

Serum neutralizing antibody titers were analyzed by the Biological Quality Control Laboratory of Diamond Animal Health, Inc. (Des Moines, IA) with analytical personnel blinded to treatment groups at all blood sampling dates, including d −21. Individual serum samples were analyzed for neutralizing antibody to BHV -1, BVDV-1, and BVDV-2 using a constant virus decreasing serum neutralization test in cell culture using 50-300 $TCID_{50}$ of virus reference. All titers were calculated according to Reed and Muench (1938). Briefly, samples were heat inactivated at 56ºC for 30 min, serially diluted (2-fold dilutions with media), dosed with virus, mixed, and let sit at room temperature for 1 h. Dilutions were then transferred to plates, pre-plated with 0.10 mL of virus-free bovine kidney target cell suspension and incubated for the number of days required.

Serum minerals were analyzed in randomly selected steers (15/treatment) by Michigan State University, Center for Population and Animal Health (East Lansing, MI) using an Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP/MS; Agilent Technologies Inc., Santa Clara, CA) via procedures previously described (Wahlen et al., 2005). Briefly, serum samples were diluted 20-fold with a solution containing 0.5% EDTA and Triton X-100, 1% ammonium hydroxide, 2% propanol, and 20 ng/g of scandium, rhodium, indium, and bismuth as internal standards. The ICP/MS was adjusted to yield a minimum of 7500 cps sensitivity for 1 n/g yttrium (mass 89), less than 1.0% oxide level as determined by the 156/140 mass ratio and less than 2.0% double charged ions as determined by the 70/140 mass ratio. Elemental concentrations were calibrated using a 4-point linear curve of the analyte-internal standard response ratio. The least detectable mineral concentrations were 0.1 μg/mL for Cu and Zn, 0.5 ng/mL for Mn, and 0.1 ng/mL for Se. No sample concentrations less than these points were reported. Standards were from Specpure (Alfa Aesar, Ward Hill, MA) a National Institute of Standards and Technology (Gaithersburg, MD) corn bran standard.

Antibody titer values were transformed with a base 10 scale. Histograms, normal probability plots, and the Shapiro-Wilk test were used to verify the normality assumption of the log-transformed titer data. A linear mixed effects model with repeated measurements was used to evaluate differences among treatments, days, and the interaction, when appropriate (SAS Inst. Inc., Cary, NC), and data were analyzed using calf (treatment) as the random variable. For all analyses, calf was the experimental unit. All *P*-values were 2-sided, with $P < 0.05$ indicating statistical significance. All results are reported as least squares means and were separated by LSD. Mean separations within days were only performed when treatment × day interactions were significant ($P < 0.05$).

RESULTS AND DISCUSSION

The average initial BW of steers did not differ ($P = 0.09$) among treatment groups (312, 312, and 330 kg for Control, ITM, and sentinel steers, respectively; SEM = 4.3). There were no tissue injection-site reactions observed, either visually or by palpable inspection of the injection site, in any calves following vaccination or treatment administration. Previous studies have reported variability in injection-site reactions among different preparations of injectable Cu supplements, with CuCa-EDTA causing the least and Cu glycinate causing the greatest tissue inflammation (Boila et al., 1984a) and the subcutaneous injection route causing less tissue irritation compared with the intramuscular injection route (Allcroft and Uvarov, 1959). The preparation used in the current study was an EDTA-mineral chelated complex, which appears to cause no visual signs of injection-site inflammation following subcutaneous administration.

The unvaccinated sentinel steers remained seronegative for the duration of the study and thus confirmed that no exposure to a field strain of BHV-1, BVDV-1, or BVDV-2 occurred and also indicates that vaccinated animals did not shed virus, leading to the seroconver-

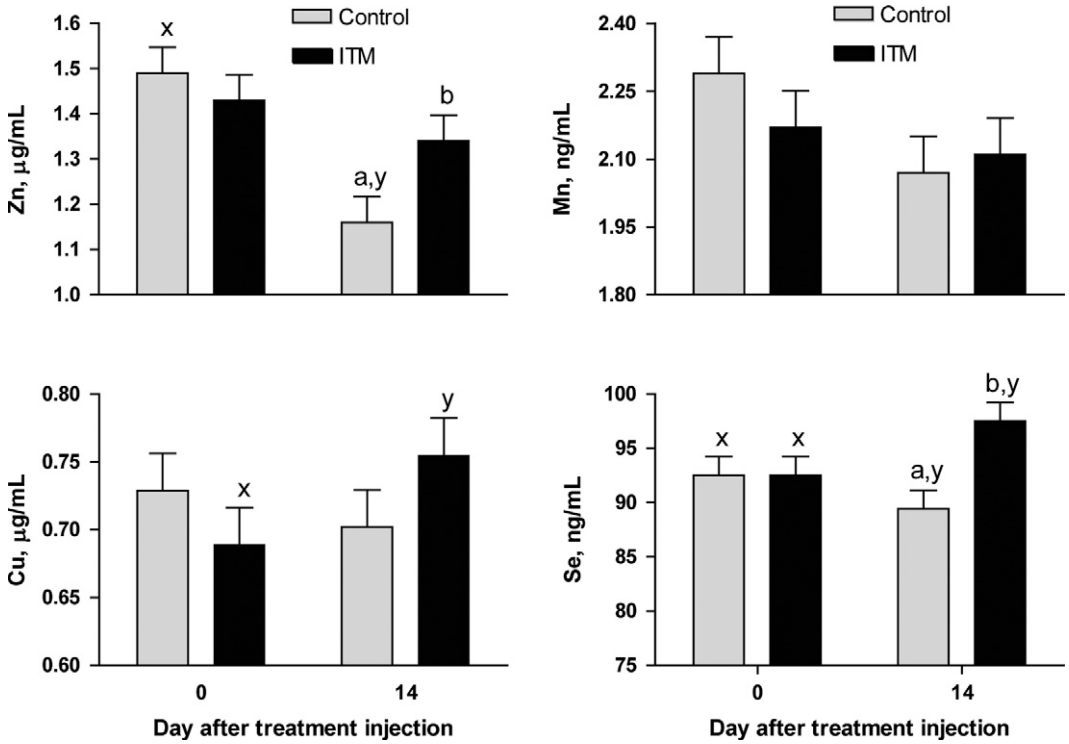

Figure 1. Serum mineral concentrations at d 0 and 14 relative to vaccination and treatment administration. Samples analyzed from a random sub-sample of steers in each treatment (n = 15 steers/treatment). Steers provided a 7-mL injection of trace minerals (ITM) or 7 mL of sterile saline (Control) on d 0. Treatment × time, $P \leq 0.05$, 0.33, 0.01, and 0.001 for Zn, Mn, Cu, and Se, respectively. Values with unlike letters differ ($P < 0.05$) between treatments within sampling days (a,b) and within treatments across sampling days (x,y).

sion of sentinel steers. No clinical signs of BRD were observed in any of the calves during the study.

On d 0, immediately before vaccination and treatment administration, there were no differences ($P \geq 0.30$) in serum Cu, Zn, Mn, or Se between treatments (Figure 1), and all values were within the sufficient range for cattle (Puls, 1994), suggesting that there were no pre-existing mineral deficiencies among the group of calves utilized in this study. By d 14, Control, but not ITM, steers experienced a decrease ($P < 0.001$) in serum Zn and Se concentrations (Figure 1). On d 14, serum Zn and Se concentrations were greater ($P < 0.01$) in ITM compared with Control steers (Figure 1). These findings suggest that the injectable ITM preparation used in this study was effective in increasing and/or abating decreases of trace mineral status (Cu, Se, and Zn, but not Mn) of vaccinated beef calves. Chirase et al. (1994) reported similar decreases in plasma Zn concentrations following respiratory virus challenge in beef steers, which is thought to be the result of increased glomerular filtration and urinary Zn excretion in calves inflicted with respiratory disease (Arthington et al., 1997). Other researchers have reported variable results in the change in serum Se concentrations following administration of a *Pasteurella hemolytica* vaccine (Droke and Loerch, 1989); however, the general tendency was for Se-injected calves to experience an increase or no change in serum Se compared with numeric declines in serum Se among calves not receiving injectable Se. The increase, or maintenance, of serum Se among Se-supplemented calves is likely the result of increased concentrations of glutathione peroxidase (Reffett Stabel et al., 1989), which is a Se-dependent antioxidant enzyme.

Steers provided ITM had increased ($P = 0.007$) serum Cu relative to initial d 0 values (Figure 1). Most viral vaccines, such as the one used in the current study, contain adjuvants that will assist in eliciting an immune response via activation of the pro-inflammatory reaction (Stokka et al., 1994). This reaction causes an increase in Cu liberation into the blood stream via the transport protein ceruloplasmin (Cousins, 1985).

Neutralizing antibody concentrations increased following vaccination and were greater ($P < 0.01$) than baseline for both treatments on d 14 for BHV-1 titers and d 30 for BVDV-1 and BVDV-2 titers (Figures 2 and 3) and remained greater on each of the subsequent sampling days. Steers provided ITM had greater ($P \leq 0.003$) neutralizing antibody titers against BHV-1 on d 14, 30, and 60 post-vaccination compared with Control (Figure 3). The biological value in increasing serum neutralizing antibodies against BHV-1 lies in the functions these antibodies have in limiting disease progression by inhibiting attachment of the virus to host cells, neutralizing extracellular virus, and collaborating with polymorphonuclear neutrophils to assist with antibody dependent cell cytotoxicity (Babiuk et al., 1987, 1996). Similar to the cur-

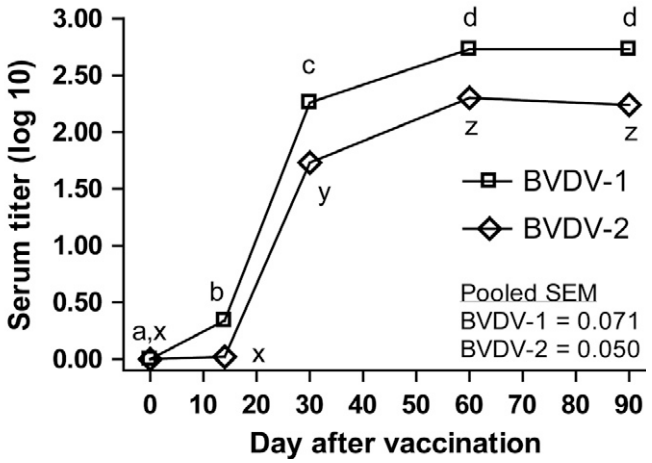

Figure 2. Pooled treatment means for bovine viral diarrhea virus (BVDV), genotypes 1, and 2 (BVDV-1 and BVDV-2) serum titers (log 10). Seronegative calves vaccinated on d 0. Values with unlike letters differ ($P < 0.05$) across sampling days (a-d = BVDV-1 and x-z = BVDV-2). Effect of day; $P < 0.001$ for both BVDV 1 and 2 and treatment × day; $P = 0.99$ and 0.81 for BVDV-1 and 2, respectively.

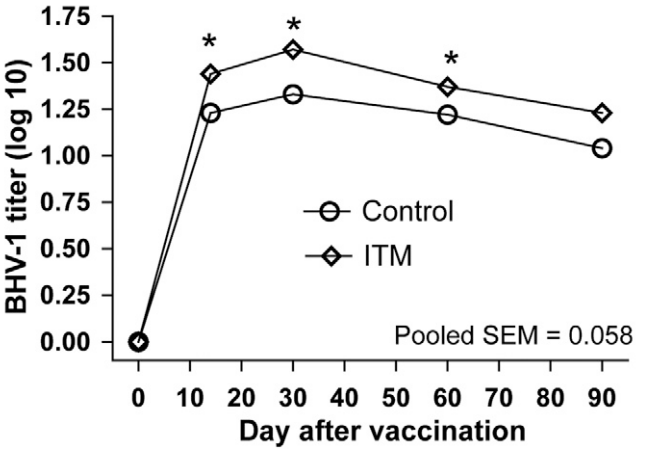

Figure 3. Bovine herpesvirus-1 (BHV-1) serum titers (log 10) of calves provided a 7-mL injection of trace minerals (ITM) or 7 mL of sterile saline (Control). Seronegative calves vaccinated on d 0. Treatment × day; $P < 0.001$. * = Values within day and between treatments differ; $P < 0.05$.

rent study, injectable Se, with or without vitamin E, has been shown to enhance humoral responses to antigens such as *E. coli* and *Mannheimia haemolytica* (Droke and Loerch, 1989; Panousis et al., 2001). Collectively, these findings suggest that injectable trace minerals may be beneficial to the humoral immune responses to pathogens common in beef production systems. In contrast, Chirase et al. (1994) investigated injectable Cu glycinate (36 mg Cu), in combination with oral chelated Cu, in BHV-1 challenged beef steers. Their results revealed a negative impact of injectable Cu on BW gain and feed DMI. The authors suggested that this response may have been due to the development of abscesses in 25% of the Cu-injected calves, which supports the importance of using ITM products which cause as little injection site reaction as possible.

Stressors, which often accompany feeder calves at the time of feedlot placement, such as vaccination, weaning, and transport, exacerbate trace mineral balances leading to special considerations relative to the trace mineral nutrition of these calves (NRC, 1996). Management systems focused on improving the trace mineral status of beef feeder calves have the potential for decreasing morbidity and improving performance. Previous studies have reported potential advantages of parenterally administered combinations of Zn, Cu, Mn, and Se, such as the formulation used in the current study, which incorporates disodium EDTA chelates (Cu, Zn, and Mn) and Se (as Na selenite). These benefits have been associated with improved feed efficiency (Clark et al., 2006), reduced treatments for illness (Berry et al., 2000), and overall reduced morbidity treatment costs (Richeson et al., 2009). The enhanced humoral immune response to BHV-1 among ITM-treated calves in the current study may be a contributing component of the positive health and performance effects reported in these previous studies.

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