Livestock Science 251 (2021) 104665

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Comparison of injectable trace minerals vs. adjuvant on measures of innate and humoral immune responses of beef heifers

L.S. Caramalac [a], P. Moriel [a,*], J. Ranches [b], G.M. Silva [a], J.D. Arthington [c]

[a] Range Cattle Research and Education Center, University of Florida/IFAS, Ona, FL 33865, United States
[b] Eastern Oregon Agricultural Research Center, Oregon State University, Burns, OR 97720, United States
[c] Department of Animal Sciences, University of Florida/IFAS, Gainesville, FL 32611, United States

ARTICLE INFO

Keywords:
Acute-phase proteins, Acute-phase response
Antibody titers
Calves
Innate immune system
Injectable trace minerals

ABSTRACT

Previous studies reported heightened innate and humoral immune responses among cattle administered injectable trace minerals (ITM) concurrent to immunogen challenge, thus suggesting a potential adjuvant-like activity of ITM. To pursue this concept further, a two-year study was conducted at the Mountain Research Station (North Carolina State University, Waynesville) to evaluate immunological responses of Angus heifers (n = 22, 235 ± 18 kg of BW and 225 ± 4 d of age in yr 1; n = 36, 237 ± 6 kg of BW and 247 ± 5 d of age in yr 2). On d 0 (30 days after weaning), heifers were administered two 5-mL i.m. injections of porcine red blood cells (PRBC; Lampire Biological Laboratories, Pipersville, PA) solution (25% PRBC and 75% sterile PBS; Amresco, Solon, OH), concurrent to either a single 2.5-mL s.c. injection of sterile saline (SAL; 0.9% NaCl), a squalene-based oil-in-water adjuvant (ADJ; AddaVax; InvivoGen, Inc. San Diego, CA) that specifically induces a humoral immune response, or injectable trace minerals (ITM; MultiMin 90; MultiMin USA, Inc. Fort Collins, CO; 60, 10, 15 and 5 mg/mL of Zn, Mn, Cu and Se). During the 28-d study, heifers were offered tall fescue hay (*Lolium arundinacea*) and concentrate at 1.2 and 1.0% of BW (DM basis), respectively, blended with 114 g daily of a complete mineral mix. Shrunk BW was recorded on d 0 and 28 following 12 h of feed and water withdrawal. Blood samples were collected on d 0, 2, 7, 15, and 21 for determination of antibody titers against PRBC and plasma concentrations of ceruloplasmin and haptoglobin. Liver biopsy samples were collected 1 mo prior to weaning and on d 15 after weaning for determination of trace mineral status. No differences were detected ($P \geq 0.13$) for BW change, ADG, G:F, or DMI. Liver Se concentrations on d 15 were greater ($P \leq 0.01$) for heifers receiving ITM compared to SAL and ADJ treatments. Plasma haptoglobin concentrations on d 2 were greatest for ADJ ($P < 0.0001$) and did not differ between SAL and ITM heifers ($P \geq 0.46$). Plasma ceruloplasmin concentrations on d 2 were greatest for ADJ ($P \leq 0.04$) and did not differ between SAL and ITM heifers ($P = 0.39$). On d 7, plasma concentrations of ceruloplasmin did not differ ($P = 0.37$) between ADJ and ITM, were less for SAL vs. ADJ ($P = 0.005$), and tended to differ ($P = 0.064$) between ITM and SAL heifers. Serum PRBC titers were greatest for ADJ ($P \leq 0.03$) vs. SAL on d 7, 15, and 21 and ITM on d 7 ($P < 0.001$). Serum PRBC titers did not differ between SAL and ITM on d 7 and 15 ($P \geq 0.23$), but were greater in ITM vs. SAL on d 21 ($P = 0.03$). In summary, ITM prolonged the antibody titers against PRBC on d 21 compared to saline injection in mineral-adequate beef calves, but not as quickly or to the extent of ADJ injection.

The authors are thankful to Multimin USA, Fort Collins, CO, for their partial financial support of this research.

* Corresponding author.
E-mail address: pmoriel@ufl.edu (P. Moriel).

https://doi.org/10.1016/j.livsci.2021.104665
Received 11 December 2020; Received in revised form 3 August 2021; Accepted 5 August 2021
Available online 8 August 2021

1. Introduction

Injectable trace minerals (ITM) offer the advantage of delivering specific amounts of trace minerals without the variability associated with fluctuations in voluntary intake of free-choice mineral mixes (Arthington and Swenson, 2004). Multiple studies have built upon our knowledge of a link between certain trace minerals and immune function in cattle (Spears, 2000). An increasing number of studies have associated ITM with improvements in antibody titers when administered at the time of vaccination (Arthington and Havenga, 2012; Arthington et al., 2014; Roberts et al., 2016; Bittar et al., 2020). In addition to heightened production of neutralizing antibodies, ITM administration produces a pro-inflammatory acute phase reaction in beef cattle (Arthington et al., 2014) similar to the acute phase reaction induced by vaccination (Arthington et al., 2013). This acute phase reaction from vaccines is variable and often related to the adjuvant included in the vaccine preparation. Commercial vaccines typically contain adjuvants to elicit heightened adaptive immune responses with the goal of creating greater immune protection to the target antigen (Coffman et al., 2010). The question has arisen, based upon results of current studies, whether this positive antigen × antibody response, with concurrent vaccine and ITM administration, is the result of a direct nutritional impact of supplemental trace minerals or an adjuvant-like response elicited from the injection of minerals. It is likely that the answer is not an either/or solution, but rather a multifaceted response impacting both the innate and humoral immune system. As a start, this study was designed to compare the acute phase protein reaction and neutralizing antibody titers to a novel immunogen in weaned beef heifers receiving a commercially available adjuvant, ITM, or saline control. The calves used in this study were purposely managed to be trace mineral adequate to support the study of our hypothesis, which was, both ITM and adjuvant treatments will produce similar increases in the acute phase protein response (innate immunity) and antibody production to PRBC challenge (humoral immunity) compared to saline-injected control calves.

2. Materials and methods

The Institutional Animal Care and Use Committee of North Carolina State University (protocol 14-157-A) approved all procedures for the two-yr experiment conducted at the Mountain Research Station (Waynesville; 35.48° N, 82.99° W; elevation = 659 m) from October to November 2014 and August to September 2015.

2.1. Animals, diets and sample collection

On d 0 (approximately 30 days after weaning), 58 Angus heifers (n = 22, 235 ± 18 kg of BW and 225 ± 4 d of age in yr 1; n = 36, 237 ± 6 kg of BW and 247 ± 5 d of age in yr 2) were stratified by BW and age, and randomly assigned to receive a single 2.5-mL s.c. injection of sterile saline (**SAL**; 0.9% NaCl), a squalene-based oil-in-water adjuvant (**ADJ**; AddaVax; InvivoGen, Inc. San Diego, CA) that specifically induces a humoral immune response, or **ITM** (MultiMin 90; Multimin USA, Inc. Fort Collins, CO; 60, 10, 15 and 5 mg/mL of Zn, Mn, Cu and Se). The ITM treatment was delivered at approximately ½ the manufacturer recommended dosage rate (1 mL/45 kg BW). This dosage was used for two reasons: (1) calves were adequate in these selected trace minerals at the time of weaning; and (2) the primary focus of the study was to investigate an adjuvant-like activity of the ITM and not necessarily the ability to increase mineral status in mineral-adequate calves. All treatments were administered subcutaneously on the right side of the neck. Immediately following treatment administration (d 0), heifers were immunologically challenged with two 5-mL i.m. injections of porcine red blood cells (PRBC; Lampire Biological Laboratories, Pipersville, PA) solution (25% PRBC and 75% sterile PBS; Amresco, Solon, OH) administered simultaneously on each side of the neck. Thereafter, heifers were stratified by BW and randomly allocated to concrete floor pens (18 × 4 m) in a half-covered feedlot facility (2 pens/treatment and 3 to 4 heifers/pen in yr 1; 4 pens/treatment and 3 heifers/pen in yr 2).

Heifers were offered ground tall fescue hay (*Lolium arundinaceum*) at 1.2% of BW (DM basis) and concentrate (50:50; ground corn/cottonseed meal) at 1.0% of BW (DM basis) from d 0 to 28. Concentrate and hay were offered separately in the same feed bunk once daily at 0800 h. In addition, all heifers were limit-fed 114g/d of a complete mineral mix (RU-MIN 1600, Southern States, Richmond, VA; DM basis: 18.2% Ca, 0.72% K, 0.88% Mg, 0.76% S, 7.0% Na, 10.8% Cl, 2.9 % P, 29 mg/kg Co, 1,220 mg/kg Cu, 2,130 mg/kg Mn, 29 mg/kg Se and 2,530 mg/kg Zn) that was hand-mixed daily into the concentrate.

Heifers consumed all concentrate within 1 h after supplementation. Hay DM offered and refused were obtained daily for each pen by drying samples of hay offered and refused in a forced-air oven at 56°C for 48 h. Daily DMI was determined by subtracting the daily hay DM refused from the daily hay DM offered. Although total and hay DMI were determined daily, both measurements were pooled by week (1 to 4) to simplify the statistical analyses and data interpretation. Samples of hay, concentrate, and mineral mix offered were collected weekly and sent in duplicate to a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) for wet chemistry analysis of all nutrients (Table 1). Samples were analyzed for concentrations of CP (method 984.13; AOAC, 2006), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp., Fairport, NY; AOAC, 2006) and NDF (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp.). Concentrations of TDN were calculated as proposed by Weiss et al. (1992), whereas NEm and NEg were calculated using equations from NRC (2000).

Shrunk BW was measured on d 0 and 28 following 12 h of feed and water withdrawal. Blood samples (10 mL) were collected via jugular venipuncture into sodium-heparin (158 USP) containing tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) for plasma harvest on d 0,

Table 1
Average nutritional composition of tall fescue hay and concentrate offered to heifers in yr 1 and 2.

Ingredients[1]	Year 1 Tall fescue hay	Concentrate[2]	Year 2 Tall fescue hay	Concentrate[2]
	DM basis			
DM, %	91.4	93.0	92.3	88.5
TDN[3], %	56.0	80.0	56.5	80.0
NEm[4], Mcal/kg	1.08	1.98	1.09	1.96
NEg[4], Mcal/kg	0.53	1.34	0.53	1.32
CP, %	16.8	25.3	11.5	29.1
ADF, %	43.8	14.0	42.5	15.7
NDF, %	63.9	21.1	65.4	23.1
Ca, %	0.73	1.47	0.44	0.83
Co, mg/kg	1.03	1.68	0.43	1.56
Cu, mg/kg	12.0	82	9.0	88
Fe, mg/kg	1660	82	572	265
K, %	2.02	1.22	2.15	0.97
Mg, %	0.51	0.42	0.25	0.39
Mn, mg/kg	108	151	99	109
Mo, mg/kg	1.03	0.60	0.60	0.38
Na, %	0.02	0.57	0.03	0.36
P, %	0.33	0.89	0.29	0.83
S, %	0.30	0.34	0.19	0.33
Se, mg/kg	0.06	1.59	0.07	1.17
Zn, mg/kg	46	204	33	142

[1] Samples of hay and concentrate were pooled by week and analyzed in duplicate at a commercial laboratory (Dairy One Laboratory, Ithaca, NY).
[2] Concentrate consisted of 48.1% ground corn, 48.1% cottonseed meal and 3.8% commercial mineral mix (Rumin 1600, Southern States, Richmond, VA; DM basis: 18.2% Ca, 0.72% K, 0.88% Mg, 0.76% S, 7.0% Na, 10.8% Cl, 2.9 % P, 29 mg/kg Co, 1,220 mg/kg Cu, 2,130 mg/kg Mn, 29 mg/kg Se and 2,530 mg/kg Zn) that was hand-mixed daily with concentrate.
[3] Calculated as described by Weiss et al. (1992).
[4] Calculated using equations from NRC (2000).

2, 7, 15, and 21 to determine the plasma concentrations of ceruloplasmin and haptoglobin. Additional blood samples (10 mL) from jugular vein were collected into tube containing no additives (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) for serum harvest on d 0, 2, 7, 15, and 21 to evaluate serum antibody titers against PRBC. Blood samples were placed on ice immediately following collection, and then centrifuged at 1,200 × g for 25 min at 4°C. Plasma and serum samples were stored frozen at -20°C until later laboratory analysis.

Liver biopsy samples were collected on d -32 and -27 in yr 1 and 2, respectively, to determine the baseline liver trace mineral concentrations using needle biopsy procedures (Arthington and Corah, 1995). Samples were assessed for trace mineral concentrations at Michigan State University Diagnostic Center for Population & Animal Health (Braselton et al., 1997). Following this initial collection, all heifers were managed as a single group on a 22-ha tall fescue pasture with free-choice access to water and a white stock salt without trace mineral fortification until d 0. This approach was used to avoid the liver biopsy-induced inflammatory response that could either mask or hasten the acute phase response induced by PRBC and treatment injections on d 0. In both years, a second liver sample was collected on d 15, relative to treatment and PRBC injections.

2.2. Laboratory analyses

Plasma concentrations of haptoglobin were determined in duplicate samples using a biochemical assay measuring haptoglobin-hemoglobin complex by the estimation of differences in peroxidase activity (Cooke and Arthington, 2013). Inter- and intra-assay CV of haptoglobin assays using the biochemical procedure were 2.77 and 2.64%, respectively. Plasma ceruloplasmin oxidase activity was measured in duplicate samples by using the colorimetric procedures described by Demetriou et al. (1974) and expressed as mg/dL, as described by King (1965). Inter- and intra-assay CV for ceruloplasmin assays were 5.2 and 9.5%, respectively.

Hemagglutination to PRBC was determined by the procedure described by Engle et al. (1999) based on previously established methods (Ferket and Qureshi, 1992). Results were recorded as $\log_2$ PRBC titers, corresponding to the total anti-PRBC immunoglobulin titers.

2.3. Statistical analyses

All data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA, version 9.3) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Pen was the experimental unit and pen(treatment × yr) as the random variable for the analysis of feed efficiency, weekly hay and total DMI. Heifer was considered the experimental unit and heifer(treatment × yr) as the random variable for the analyses of ADG, serum PRBC titers, liver trace mineral concentrations, and plasma concentrations of haptoglobin and ceruloplasmin. Fixed effects of all analyses included treatment, yr, day of blood or tissue collection (when necessary) and resulting interactions. Plasma and serum measurements, and liver trace mineral concentrations were analyzed as a repeated measures with heifer(treatment × yr) as the subject, whereas weekly hay and total DMI were analyzed as a repeated measures with pen(treatment × yr) as the subject. Compound symmetry covariance structure was used for all statistical analysis once it generated the lowest Akaike information criterion. Heifer age was considered a non-significant covariate in all statistical analyses ($P \geq 0.32$) and removed from the model, whereas BW on d 0 was included in the fixed model ($P < 0.0001$) to covariate adjust BW on d 28. All results are reported as least-squares means. Data were separated using PDIFF if a significant preliminary F-test was detected. Significance was set at $P \leq 0.05$, and tendencies if $P > 0.05$ and ≤ 0.10.

3. Results and discussion

3.1. Post-weaning DM intake and BW change

Body weight on d 0 did not differ among treatments ($P \geq 0.98$), but was included as a covariate in the BW analysis. Effects of treatment × year and treatment were not detected ($P \geq 0.13$) for BW on d 28 or ADG and G:F from d 0 to 28 (Table 2). Additionally, there were no differences in overall voluntary DMI. Heifers were limit-fed concentrate at a rate of 1% BW with no refusal. Hay was offered in amounts to ensure free-choice consumption. Although voluntary hay intake was greater ($P = 0.001$) in yr 2 vs. yr 1 (average intake = 3.04 vs. 2.78 kg/heifer daily; SEM = 0.071), no treatment ($P = 0.92$) or treatment × yr effects ($P = 0.94$) were observed. This intake pattern resulted in no differences ($P \geq 0.87$) among treatments for total trace mineral intake over the 28-d post-weaning evaluation (Table 3).

The effects of ITM on beef calf performance has been variable. Similar to the current study, Genther-Schroeder and Hansen (2015) did not detect differences on final BW or ADG during a 28-d preconditioning phase of steers receiving either ITM or saline injections (1 mL/45.4 kg of BW). However, Arthington et al. (2014) reported greater ADG for heifers receiving ITM when compared to heifers receiving saline (2.5 mL/head in 3 applications); although that study was conducted in grazing conditions over a longer period of time (177 d) and supplemental trace minerals were withdrawn from both ITM and Control. In another study, Richeson and Kegley (2011) reported greater final BW, ADG, and G:F, when compared to negative control heifers, on a 55 d study where heifers received ITM (1 mL/45 kg) at the beginning of the study.

3.2. Liver Mineral Concentrations

Effects of treatment × day × yr and treatment × yr were not detected ($P \geq 0.22$) for liver trace mineral concentrations. There was a treatment × day interaction ($P \leq 0.01$) for liver Se concentrations, but not for the remaining trace minerals ($P \geq 0.20$). Liver Se concentrations did not differ among treatments on d -32 and -27 ($P \geq 0.50$), but were greater ($P < 0.0001$) for ITM vs. SAL and ADJ heifers on d 15 (Table 4). Liver trace mineral concentrations were adequate at the beginning of the study (Herdt and Hoff, 2011; Puls, 1988). A 2.5-mL injection of ITM promoted an increase in liver Se concentrations from d 0 to 15, but no changes in liver concentrations of Cu, Mn, and Zn. Although liver Se concentrations increased as a result of ITM treatment, initial values and post-injection values (range = 1.52 to 2.58 mg/kg DM) were adequate as suggested by Puls (1988; ≤ 0.17 mg/kg WW or 0.61 mg/kg DM-converted) and Michigan State University Diagnostic Center for Population and Animal Health (< 0.70 mg/kg DM; 2015 Reference Ranges [https://www.dcpah.msu.edu/ sections/Nutrition/ (Accessed 16 February 2017).]). Pogge

Table 2
Growth performance and DMI of heifers receiving a 2.5-mL s.c. injection of sterile saline (SAL), a squalene-based oil-in-water adjuvant (ADJ; AddaVax), or MultiMin 90 (ITM; 60, 10, 15 and 5 mg/mL of Zn, Mn, Cu and Se) on d 0.

	Treatment[1]				*P*-value	
Item	SAL	ADJ	ITM	SEM	Trt	Trt × yr
BW, kg						
d 0	237	236	236	7	0.99	0.99
d 28[2]	271	271	270	2	0.90	0.19
d 0 to 28						
ADG, kg/d	1.25	1.35	1.20	0.07	0.25	0.64
Total DMI, kg/d	5.49	5.43	5.51	0.14	0.90	0.95
Hay DMI, kg/d	2.98	2.95	2.99	0.08	0.92	0.94
G:F[3]	0.23	0.25	0.22	0.01	0.13	0.42

[1] Heifers were provided ground tall fescue hay at 1.2% of BW (DM basis) and supplemented with concentrate at 1% of BW (DM basis) from d 0 to 28.
[2] Covariate-adjusted to BW on d 0.
[3] Calculated as total BW gain divided by total DMI from d 0 to 28.

Table 3
Calculated trace mineral intake of heifers receiving a 2.5-mL s.c. injection of sterile saline (SAL), a squalene-based oil-in-water adjuvant (ADJ; AddaVax), or MultiMin 90 (ITM; 60, 10, 15 and 5 mg/mL of Zn, Mn, Cu and Se) on d 0.

	Treatment[1]				*P*-value	
Item[2]	SAL	ADJ	ITM	SEM	Trt	Trt × yr
	Intake, mg/d					
Co	6.22	6.12	6.20	0.16	0.91	0.97
Cu	247	242	245	7	0.88	0.94
Fe	3,661	3,633	3,673	96	0.96	0.98
Mn	636	626	634	16	0.90	0.96
Mo	3.62	3.58	3.62	0.09	0.93	0.98
Se	3.64	3.57	3.62	0.10	0.87	0.96
Zn	553	544	550	14	0.89	0.96

[1] Heifers were provided ground tall fescue hay at 1.2% of BW (DM basis) and supplemented with concentrate at 1% of BW (DM basis) from d 0 to 28. Values represent the sum of trace mineral intake from forage and trace mineral-fortified supplement.

Table 4
Average liver trace mineral concentrations of beef heifers receiving a 2.5-mL s.c. injection of sterile saline (SAL), a squalene-based oil-in-water adjuvant (ADJ; AddaVax), or MultiMin 90 (ITM; 60, 10, 15 and 5 mg/mL of Zn, Mn, Cu and Se) on d 0.

	Treatment[1]				*P*-value	
Item[3]	SAL	ADJ	ITM	SEM	Trt	Trt × day
	mg/kg DM					
Co	0.27	0.30	0.28	0.01	0.22	0.55
Cu	259	291	295	22	0.41	0.75
Fe	365	395	369	18	0.42	0.20
Mn	10.7	11.6	11.3	0.4	0.27	0.84
Mo	3.70	3.63	3.71	0.18	0.84	0.56
Se						
d 0	1.87[a]	2.02[a]	1.88[a]	0.17	0.01	<0.0001
d 15	1.53[a]	1.52[a]	2.58[b]			
Zn	146	140	145	8	0.85	0.84

[a-b]Within a row, means without a common superscript differ ($P \leq 0.05$).
[1]Effects of treatment × day × yr and treatment × yr were not detected for any liver trace mineral ($P \geq 0.22$). Liver biopsy samples were collected on d -32 and -27 in yr 1 and 2, respectively, and then again on d 15 in yr 1 and 2.

et al. (2012) reported greater liver Cu, Se, and Zn concentrations among steers provided ITM vs. saline treatments (1mL/45 kg). Different results were also reported by the current investigators in another study investigating ITM (Arthington et al., 2014). In that study, freshly-weaned heifers receiving ITM immediately following transport experienced a 13-d post-injection increase in liver Se, Cu, and Zn concentrations compared to saline injected heifers. One important difference between these studies and the current study is the dosage rate of ITM. The same commercial product was used, but dosed at 5.0 vs. 2.5 mL/heifer in the former and current study, respectively. Since calf BW was similar in both studies (average BW = 230 and 241 kg for the current study and comparison study, respectively), it is reasonable to expect that a greater ITM dosage would impact liver tissue accumulation of these trace elements. Further, heifers in the current study were anticipated to be trace mineral adequate at the start of the study. The lower dosage rate was chosen to focus more directly on the potential impact of ITM on innate and humoral immune responses vs. increases in mineral status of mineral-adequate calves. The experimental approach attempted to mimic the model outlined by Arthington et al. (2014), where 2.5 mL ITM was dosed 3X over a 127-d period preceding the same PRBC challenge as used in the current study.

3.3. Plasma Acute Phase Protein Response

Haptoglobin and ceruloplasmin are major acute phase proteins in the bovine. These proteins are synthesized by the liver parenchymal cells and released in the bloodstream as part of the normal acute phase reaction, which is a component of the innate body defense mechanism (Baumann and Gauldie, 1994; Suffredini et al., 1999). In cattle, the acute phase reaction, and subsequent production and release of acute phase proteins, can be elicited by a variety of conditions, such as illness (Heegaard et al., 2000; Peterson et al., 2004), transportation (Arthington et al., 2003), diet restriction and change (Capellozza et al., 2011; Gozho et al., 2005), and vaccination (Arthington et al., 2013; Stokka et al., 1994).

Effects of treatment × day × year, treatment × year, and year were not tested for plasma haptoglobin concentrations. Reasons for this approach were explained below. In yr 1, plasma haptoglobin concentrations were below detection limits (< 0.003 mg/mL) for all ITM and SAL heifers. In contrast, ADJ heifers experienced a rapid increase ($P <$ 0.0001) from non-detectable concentrations on d 0 to 0.73 ± 0.052 mg/mL on d 2 of yr 1. Seven of the 8 heifers receiving ADJ in yr 1 experienced a rise in haptoglobin on d 2 with all heifers back to non-detectable concentrations by d 7. Unlike ceruloplasmin, haptoglobin concentrations are often undetectable in unstressed cattle (Makimura and Suzuki, 1982) making it a uniquely reliable indicator of pro-inflammatory distress. In yr 2, all heifers experienced a measurable plasma haptoglobin response following weaning and treatment administration (Fig. 1A). Heifers receiving ADJ had a rapid increase in plasma haptoglobin concentrations from d 0 to 2 with concentrations greater than both ITM and SAL ($P < 0.001$). Although, ITM and SAL did not differ from each other on any sampling day, SAL heifers experienced an increase ($P = 0.03$) in plasma haptoglobin concentrations from d 0 to 7, with heifers receiving ITM tending ($P = 0.062$) to increase over this same sampling interval (Fig. 1A).

Commercial vaccines usually contain adjuvants to stimulate adaptive immune responses, leading to greater immune protection (Coffman et al., 2010). Therefore, the increase in plasma haptoglobin concentrations on d 2 among ADJ heifers was expected and likely explained as a normal innate immune response to adjuvant administration. Increased plasma haptoglobin concentrations in response to vaccination have been reported previously (Stokka et al., 1994; Arthington et al., 2013). In the latter study, peaks in plasma haptoglobin concentrations occurred on differing days depending on the type of vaccine administered (d 3 and d 5, respectively for *Mannheimia haemolytica* and *Clostridium*), suggesting a modulation of the pro-inflammatory response caused by vaccine preparation.

In the current study, there were no differences in plasma haptoglobin concentrations between heifers receiving ITM or SAL treatments. Previous research by our group (Arthington et al, 2014) showed greater plasma haptogobin concentrations at 7 d after ITM administration when compared to heifers receiving saline. It is important to note that in the previous study calves received nearly 2X the ITM dose of the current study and were transported 1,600 km. These two factors likely explain much of the difference in haptoglobin responses between the two studies.

There was a tendency ($P = 0.06$) for a treatment × day, but not treatment × day × year, treatment × year, and year effects ($P \geq 0.13$), for plasma ceruloplasmin concentrations. Plasma ceruloplasmin concentrations increased ($P < 0.001$) for all treatments, peaking on d 2 (Fig. 1B). On d 2, plasma ceruloplasmin concentrations were greatest for ADJ ($P \leq 0.04$) and did not differ between SAL and ITM heifers ($P =$ 0.39; Fig. 1B). On d 7, plasma ceruloplasmin concentrations of heifers receiving ADJ were greater ($P = 0.005$) than SAL, but not ITM ($P =$ 0.37), while ITM tended ($P = 0.064$) to have greater plasma ceruloplasmin concentrations than SAL. Plasma ceruloplasmin concentrations did not differ ($P \geq 0.19$) among treatments on d 15 and 21. The increase in plasma ceruloplasmin concentrations of heifers assigned to the ADJ treatment was expected and considered a normal physiological process in the activation of the acute phase reaction. Previous research (Arthington et al., 2014) showed similar results to the current study, where plasma ceruloplasmin concentrations 6 d after injection were

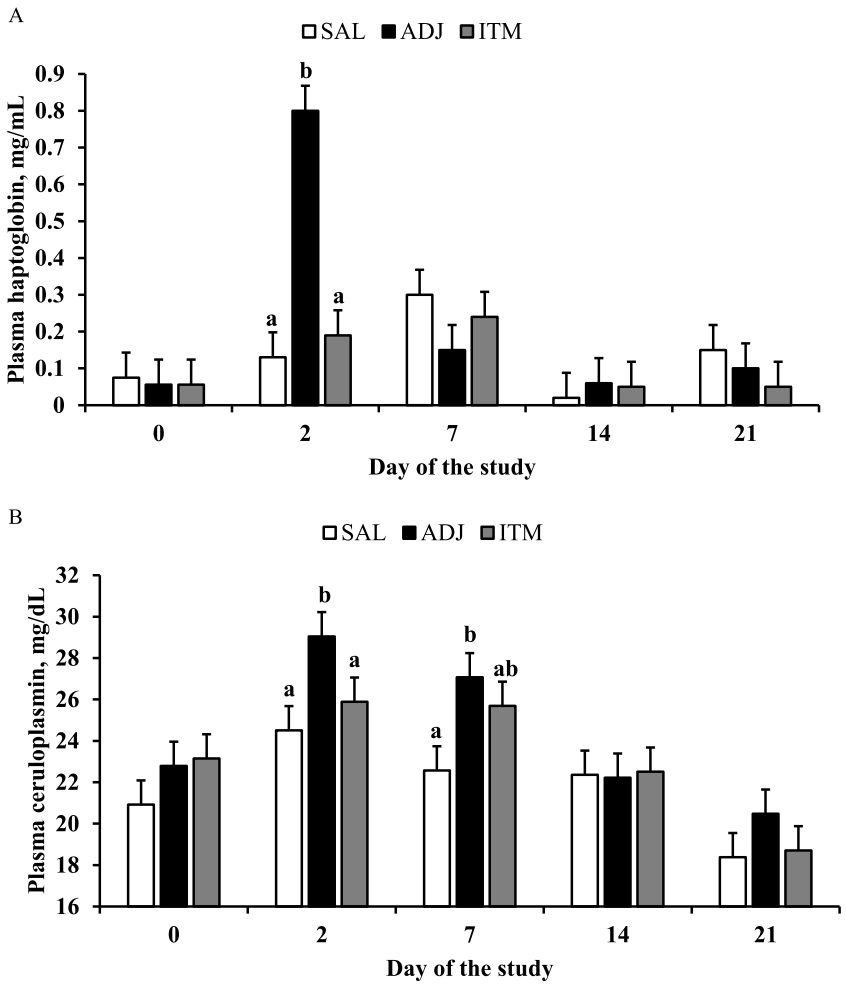

Fig. 1. Plasma concentrations of haptoglobin (A, yr 2 only) and ceruloplasmin (B, yr 1 and 2) of beef heifers receiving a 2.5-mL s.c. injection of sterile saline (SAL: 0.9% NaCl), a squalene-based oil-in-water adjuvant (ADJ; AddaVax), or MultiMin 90 (ITM; 60, 10, 15 and 5 mg/mL of Zn, Mn, Cu and Se) on d 0. Plasma haptoglobin concentrations of ITM and SAL heifers in yr 1 were below the detection limits (<0.003 mg/mL) and not included in the statistical analyses. Effects of treatment × day were detected ($P < 0.0001$) for plasma concentrations of haptoglobin ($P < 0.0001$) and ceruloplasmin ($P = 0.06$). [a-b]Within day, means without a common superscript differ ($P \leq 0.05$).

greater for heifers receiving ITM than saline.

Although ADJ and ITM heifers had similar plasma ceruloplasmin concentrations on d 7, it is important to note that the mechanisms for the increase may differ for both treatments. The response observed for ADJ heifers is likely related to the activation of the pro-inflammatory reaction caused by the adjuvant (Coffman et al., 2010), while the response seen for the ITM heifers may be assisted by a greater availability of Cu to be incorporated into apoceruloplasmim in the liver and therefore resulting in elevated plasma ceruloplasmin concentrations (Hellman and Gitlin, 2002).

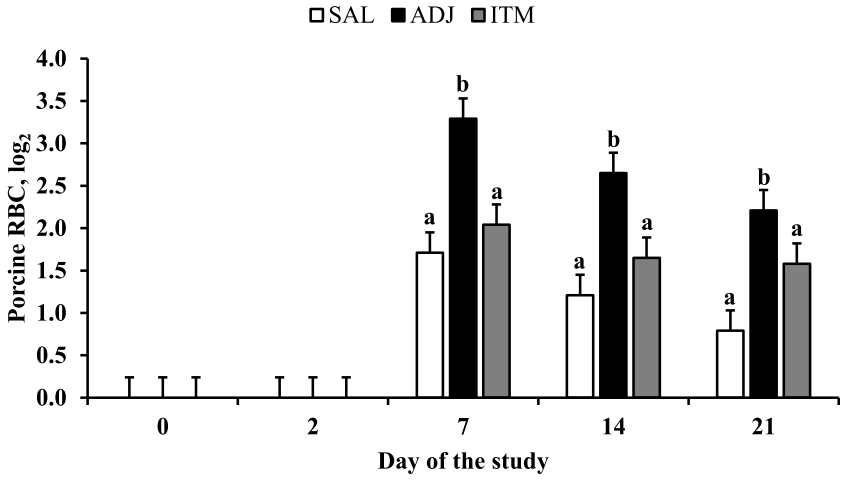

Fig. 2. Average serum porcine red blood cells (PRBC) titers (yr 1 and 2) of beef heifers receiving a 2.5-mL s.c. injection of sterile saline (SAL: 0.9% NaCl), a squalene-based oil-in-water adjuvant (ADJ; AddaVax), or MultiMin 90 (ITM; 60, 10, 15 and 5 mg/mL of Zn, Mn, Cu and Se) on d 0. Effects of treatment × day were detected ($P < 0.0001$) for serum PRBC titers. [a-c]Within day, means without a common superscript differ ($P \leq 0.05$).

3.4. Serum Neutralizing Antibody Titers

Effects of treatment × day ($P < 0.0001$), but not treatment × day × year, treatment × year, and year effects ($P \geq 0.85$), were detected for serum PRBC titers. Serum PRBC titers were not detected on d 0 and 2, but increased sharply for all treatments on d 7 (Fig. 2). Serum PRBC titers did not differ ($P \geq 0.23$) between ITM and SAL heifers, but both were less ($P \leq 0.004$) than ADJ heifers on d 7 and 15 (Fig. 2). On d 21, serum PRBC titers only tended to differ ($P = 0.07$) between ADJ and ITM heifers, but both were greater ($P \leq 0.03$) than SAL (Fig. 2). These results suggest a lengthened humoral immune response by heifers receiving ITM. Similar to the current study, Arthington et al (2014) reported greater PRBC titers for heifers receiving ITM vs. saline. In another study, Arthington and Havenga (2012) reported greater BHV-1 titers for heifers administrated ITM compared to saline injections (7 mL). Conversely, Palomares et al (2016) reported no increase in antibody titers to BHV-1 after priming or booster vaccination of calves receiving ITM or saline injections (1 mL/45 kg). In their study, calves were younger (3.5 mo) than the current study and the authors attribute the lack of improvement in antibody production to the presence of maternally-derived antibodies that may have inhibited the development of a humoral immune response to BHV-1. It is important to highlight that ADJ was included in this study as a method to stimulate the humoral immune response, and more importantly, ADJ was included for comparison purposes rather than a practice to be incorporated by commercial operations. Collectively, these data suggest that the use of ITM may enhance the humoral immune of calves by mechanisms additional to, or complimentary to, increased trace mineral status. This improvement appears to be dependent upon ITM dose. Additional studies are warranted, particularly using differing ITM dosages, to better elucidate this repeatable enhancement of humoral immunity when ITM is administered concurrent to vaccine.

Declaration of Competing Interest

Partial financial support for this research was provided by Micronutrients USA, LLC, Fort Collins, CO, USA.

References

Arthington, J.D., Cooke, R.F., Maddock, T.D., Araujo, D.B., Moriel, P., DiLorenzo, N., Lamb, G.C., 2013. Effects of vaccination on the acute-phase protein response and measures of performance in growing beef calves. J. Anim. Sci. 91, 1831–1837. https://doi.org/10.2527/jas.2012-5724.

Arthington, J.D., Eicher, S.D., Kunkle, W.E., Martin, F.G., 2003. Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake of newly weaned beef calves. J. Anim. Sci. 81, 1120–1125. https://doi.org/10.2527/2003.8151120x.

Arthington, J.D., Swenson, C.K., 2004. Effects of trace mineral source and feeding method on the productivity of grazing Braford cows. Prof. Anim. Sci. 20, 155–161. https://doi.org/10.15232/S1080-7446(15)31290-0.

Arthington, J.D., Corah, L.R., 1995. Liver biopsy procedures for determining the trace mineral status in beef cows. Part II. Video, AI 9134. Extension TV. Dep. Comm. Coop. Ext. Serv. Kansas State Univ., Manhattan.

Arthington, J.D., Havenga, L.J., 2012. Effect of injectable trace minerals on the humoral immune response to multivalent vaccine administration in beef calves. J. Anim. Sci. 90, 1966–1971. https://doi.org/10.2527/jas2011-4024.

Arthington, J.D., Moriel, P., Martins, P.G.M.A., Lamb, G.C., Havenga, L.J., 2014. Effects of trace mineral injections on measures of performance and trace mineral status of pre- and post-weaned beef calves. J. Anim. Sci. 92, 2630–2640. https://doi.org/10.2527/jas.2013-7164.

AOAC, 2006. Official methods of analysis, 18th ed. AOAC Int., Arlington, VA.

Baumann, H., Gauldie, J., 1994. The acute phase response. Immunol. Today. 15, 74–80. https://doi.org/10.1016/0167-5699(94)90137-6.

Bittar, J.H.J., Paolomares, R.A., Hurley, D.J., Hoyos-Jaramillo, A., Rodriguez, A., Stoskute, A., Hamrick, B., Norton, N., Adkins, M., Saliki, J.T., Sanchez, S., Lauber, K., 2020. Immune response and onset of protection from Bovine viral diarrhea virus 2 infection induced by modified-live virus vaccination concurrent with injectable trace minerals administration in newly received beef calves. Vet. Immunol. Immunopath. 225, 1–11. https://doi.org/10.1016/j.vetimm.2020.110055.

Braselton, W.E., Stuart, K.J., Mullaney, T.P., Herdt, T.H., 1997. Biopsy mineral analysis by inductively coupled plasma-atomic emission spectroscopy with ultrasonic nebulization. J. Vet. Diagn. Invest. 9, 395–400. https://doi.org/10.1177/104063879700900409.

Coffman, R.L., Sher, A., Seder, R.A., 2010. Vaccine adjuvants: Putting innate immunity to work. Immunity 33, 492–503. https://doi.org/10.1016/j.immuni.2010.10.002.

Cooke, R.F., Arthington, J.D., 2013. Concentrations of haptoglobin in bovine plasma determined by ELISA or a colorimetric method based on peroxidase activity. J. Anim. Physiol. Anim. Nutr. (Berl). 97, 531–536. https://doi.org/10.1111/j.1439-0396.2012.01298.x.

Demetriou, J.A., Drewes, P.A., Gin, J.B., 1974. Pages 857-864 in Clinical Chemistry. In: Cannon, D.C., Winkelman, J.W. (Eds.), Ceruloplasmin. Harper and Row, Hagerstown, MD.

Engle, T.E., Spears, J.W., Brown Jr., T.T., Lloyd, K.E, 1999. Effect of breed (Angus vs. Simmental) on immune function and response to a disease challenge in stressed steers and preweaned calves. J. Anim. Sci. 77, 516–521. https://doi.org/10.2527/1999.773516x.

Ferket, P.R., Qureshi, M.A., 1992. Performance and immunity of heat-stressed broilers fed vitamin- and electrolyte-supplemented drinking water. Poult. Sci. 71, 88–97. https://doi.org/10.3382/ps.0710088.

Genther-Schroeder, O.N., Hansen, S.L. 2015. Effect of a multielement trace mineral injection before transit stress on inflammatory response, growth performance, and carcass characteristics of beef steers. 93, 1767–1779. doi:10.2527/jas2014-8709.

Gozho, G.N., Plaizier, J.C., Krause, D.O., Kennedy, A.D., Wittenberg, K.W., 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. J. Dairy Sci. 88, 1399–1403. https://doi.org/10.3168/jds.S0022-0302(05)72807-1.

Heegaard, P.M.H., Godson, D.L., Toussaint, M.J.M., Tjornehoj, K., Larsen, L.E., Viuff, B., Ronsholt, L., 2000. The acute phase response of haptoglobin and serum amyloid A (SAA) in cattle undergoing experimental infection with bovine respiratory syncytial virus. Vet. Immunol. Immunopathol. 77, 151–159. https://doi.org/10.1016/s0165-2427(00)00226-9.

Hellman, N.E., Gitlin, J.D., 2002. Ceruloplasmim metabolism and function. Annu. Rev. Nutr. 22, 439–458. https://doi.org/10.1146/annurev.nutr.22.012502.114457.

Herdt, T.H., Hoff, B., 2011. The Use of Blood Analysis to Evaluate Trace Mineral Status in Ruminant Livestock. Vet. Clin. North Am. - Food Anim. Pract. 27, 255–283. https://doi.org/10.1016/j.cvfa.2011.02.004.

King, J., 1965. Ceruloplasmin. Pages 108-110 in Practical Clinical Enzymology. Van Nostrand, London.

Makimura, S., Suzuki, N., 1982. Quantitative determination of bovine serum haptoglobin and its elevation in some inflammatory disease. Japanese J. Vet. Sci. 44, 15–21.

NRC, 2000. Nutrient Requirements of Beef Cattle. revised, 7th ed. Natl. Acad. Press, Washington, DC.

Palomares, R.A., Hurley, D.J., Bittar, J.H.J., Saliki, J.T., Woolums, A.R., Moliere, F., Havenga, L.J., Norton, N.A., Clifton, S.J., Sigmund, A.B., Barber, C.E., Berger, M.L., Clark, M.J., Fratto, M.A., 2016. Effects of injectable trace minerals on humoral and cell-mediated immune responses to Bovine viral diarrhea virus, Bovine herpes virus 1 and Bovine respiratory syncytial virus following administration of a modified-live virus vaccine in dairy calves. Vet. Immunol. Immunopathol. 178, 88–98. https://doi.org/10.1016/j.vetimm.2016.07.003.

Petersen, H.H., Nielsen, J.P., Heegaard, P.M.H., 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res. 35, 163–187. https://doi.org/10.1051/vetres:2004002.

Pogge, D.J., Richter, E.L., Drewnoski, M.E., Hansen, S.L., 2012. Mineral concentrations of plasma and liver after injection with a trace mineral complex differ among angus and simmental cattle. J. Anim. Sci. 90, 2692–2698. https://doi.org/10.2527/jas.2012-4482.

Puls, R., 1988. Mineral levels in animal health. In: Puls, R. (Ed.), Sherpa International. Clearbrook, BC, Canada.

Richeson, J.T., Kegley, E.B., 2011. Effect of supplemental trace minerals from injection on health and performance of highly stressed, newly received beef heifers. Prof. Anim. Sci. 27, 461–466. https://doi.org/10.15232/S1080-7446(15)30519-2.

Roberts, S.L., May, N.D., Brauer, C.L., Gentry, W.W., Weiss, C.P., Jennings, J.S., Richeson, J.T., 2016. Effect of injectable trace mineral administration on health, performance, and vaccine response of newly received feedlot cattle. Prof. Anim. Sci. 32, 842–848. https://doi.org/10.15232/pas.2016-01543.

Spears, J.W., 2000. Micronutrients and immune function in cattle. Proc. Nutr. Soc. 59, 587–594. https://doi.org/10.1017/S0029665100000835.

Stokka, G.L., Edwards, A.J., Spire, M.F., Brandt, R.T., Smith, J.E., 1994. Inflammatory response to clostridial vaccines in feedlot cattle. JAVMA 204, 415–419.

Suffredini, A.F., Fantuzzi, G., Badolato, R., Oppenheim, J.J., O'Grady, N, 1999. New insights into the biology of the acute phase response. J. Clin. Immunol. 19, 203–214. https://doi.org/10.1023/a:1020563913045.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to nutrition animal. J. Dairy Sci. 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Weiss, W.P., Conrad, H.R., St. Pierre, N.R, 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim. Feed Sci. Technol. 39, 95–110. https://doi.org/10.1016/0377-8401(92)90034-4.