OXFORD

AMERICAN SOCIETY OF **ANIMAL** SCIENCE

Effects of supplemental Zn concentration and trace mineral source on immune function and associated biomarkers of immune status in weaned beef calves received into a feedlot

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Abstract

Low-risk, weaned Angus-crossbred steers ($n = 72$; 284 ± 25 kg) were used in a 42-d receiving study. Steers were housed in pens ($n = 6$ steers per pen) equipped with GrowSafe bunks for determination of individual animal feed disappearance. Dietary treatments ($n = 24$ steers per treatment) included: 1) trace minerals (**TM**) from an organic source (Availa4; Zinpro Corp., Eden Prairie, MN) at 7 g·steer^{-1}·d^{-1}; for 42 d (ORG); 2) ORG for entire 42-d plus AvailaZn (Zn amino acid complex, Zinpro Corp., Eden Prairie, MN) to provide 1,000 mg Zn·steer^{-1}·d^{-1} for first 14 d (ORG+Z); 3) inorganic TM sources to supplemented at equivalent concentration as in ORG for 42-d (ING). Cattle were weighed on day -1, 0, 14, 41, and 42. Whole blood was collected ($n = 72$ steers) on day 0, 14, and 42. Liver biopsies were conducted ($n = 36$ steers; 3 steers per pen) on day 0, 14, and 42. Flow cytometry measures were conducted using whole blood on day 1, 14, and 42 for determination of circulating frequencies of immune cell populations. There was a tendency for improved overall average daily gain ($P = 0.07$) where both ORG and ORG+Z were greater than ING. Final body weight did not differ ($P = 0.21$) and overall dry matter intake was unaffected by dietary treatment ($P \geq 0.18$). However, overall gain-to-feed ratio was improved ($P = 0.01$) in steers supplemented organic TM (ORG and ORG+Z) compared to ING. Plasma Zn concentration did not differ at any time point during the study ($P \geq 0.20$). Liver Zn concentration did not differ between treatments on day 0 or 42; however, on day 14 ING tended ($P = 0.09$) to be greater than ORG+Z with ORG being intermediate. Plasma Cu was unaffected by dietary treatment ($P \geq 0.34$) on day 0, 14, and 42. Plasma Fe did not differ on day 0 or 42 but tended to be greater in ORG and ORG+Z compared to ING ($P = 0.08$) on day 14. Dietary treatment did not alter ($P \geq 0.22$) liver Fe or Mn concentration at any time point. Frequency of total circulating natural killer (**NK**) and CD8 T cells measured on day 0, 14, and 42 did not differ ($P \geq 0.07$). However, cell surface markers of activation (CD16, CD44, and CD8) on NK cells measured on day 14 did differ because of treatment ($P \leq 0.05$). Results presented herein indicate TM from an organic source supplemented to steers during receiving can positively influence growth rate and feed efficiency. Regardless of source, TM supplementation affected markers of immune function but did not influence the prevalence of circulating NK and CD8 T-cell populations.

Lay Summary

The receiving phase of the beef cattle production cycle occurs when calves are initially placed into the feedlot. During this time cattle are often exposed to stressors such as new environments, unfamiliar feedstuffs, and new pathogens. Together these stressors can result in lesser feed consumption. Along with lower total feed consumption, it is during this time that cattle likely require greater amounts of specific trace minerals (**TM**) to mount an effective immune response and maintain adequate growth. Therefore, this study aimed to evaluate the effects of supplemental Zn concentration and TM source on the immune function and associated biomarkers of immune status in weaned beef calves received into a feedlot. In this study, the more bioavailable, organic TM source supplemented to steers during receiving positively influenced growth rate and feed efficiency. Plasma TM concentration of steers in this study was adequate and was minimally influenced by TM source or concentration. These results also show TM supplementation, regardless of source, can alter markers of activation within immune cell populations.

Introduction

Within the feedlot production cycle, the receiving phase is particularly crucial. Newly received calves placed in a feedlot are exposed to a wide array of stressors that can vary based on time of year, geographical location, and from feedlot to feed-

lot. Common stressors encountered by newly received calves include transportation, lack of feed and water, introduction to unfamiliar feed resources, different environmental conditions, and foreign pathogens (Loerch and Fluharty, 1999). These factors can ultimately result in decreased dry matter intake

(**DMI**) (Blom, 2019). Dry matter intake of healthy calves during the first-week post-arrival is approximately 1.6% of body weight (**BW**); however, morbid calves reportedly consume less than 1% of BW (Hutcheson, 1987). Decreased DMI, along with the amalgamation of stressors, elicits physiological changes resulting in compromised immunity. Stress-induced decreases in DMI intake in newly received beef animals manifest themselves readily as inadequate metabolizable energy intake and impaired immune function. However, one must consider trace mineral (**TM**) intake during this period equally critical to the immunocompetency of the newly received feedlot animal. Trace minerals, a relatively small component of the diet on a percentage of dry matter (**DM**) basis, play a profound and diverse role related to immune function (Erickson et al., 2000; Spears, 2000; Suttle, 2010). Therefore, during the earlier portion of the receiving phase, additional fortification of the diet with TM such as Zn may prove useful.

Beyond TM intake, another vital consideration to be made when assessing TM concentration in a diet includes source of TM. Either inorganic (sulfate-bound TM) or an organic source (those TM bound to an amino acid or other organic compound) offer different relative bioavailability to the animal. Thus making this consideration relevant not only to animal performance but to economic analysis as well (Wedekind et al., 1992; Kegley and Spears, 1994). Previous work investigating supplementation of Zn, Cu, Mn, and Co from organic sources compared to inorganic sources showed improved growth performance of shipping-stressed cattle supplemented with TM from an organic source vs. those supplemented equivalent concentrations of Zn, Cu, Mn, and Co from inorganic sources (Kegley et al., 2012). Zinc in particular appears to be critical in receiving cattle diets. Dietary Zn, regardless of source, has been shown to improve growth in cattle during the growing period (Spears and Kegley, 2002). Along with a host of other growth-related physiological roles, Zn plays numerous immunological roles (Wessels et al., 2021). Chirase et al. (1991) reported positive effects of supplementing an organic Zn source to cattle challenged with infectious bovine rhinotracheitis virus (**IBRV**). Cattle supplemented with Zn methionine complex (ZnMet; Zinpro Corporation, Eden Prairie, MN) regained pre-challenge DMI more quickly (control diet Zn = 31 mg/kg; ZnMet Zn = 90 mg/kg). Cattle-fed ZnMet had lower mean rectal temperatures on day 7 and 12 post-IBRV challenge. More work is needed investigating the specific influence of Zn on immune function and associated biomarkers of immune status. Thus, the objective of the present study was to evaluate the effects of supplemental Zn concentration and TM source on immune function, associated biomarkers of immune status, TM status, and growth performance in weaned beef calves being received into a feedlot.

Materials and Methods

All procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC-21-206).

Experimental design

Newly received, low-risk weaned Angus-crossbred steers ($n = 72$; initial BW = 284 ± 25 kg) were enrolled in a 42-d feedlot receiving study to determine the effects of dietary supplemental TM concentration and source on biomarkers of immune status, TM status, and growth performance. Steers

in this study were considered low-risk as they originated from a known source, were weaned, vaccinated, and a complete health history was known. This study was conducted at the Beef Nutrition Farm (BNF) located in Ames, IA, from early November to mid-December 2021. Steers were housed in pens measuring 45.2 m², for an average of 11.3 m² per head. Twelve pens equipped with GrowSafe feed bunks ($n = 1$ GrowSafe feed bunk/pen of six steers; $n = 4$ pen replicates per dietary treatment; GrowSafe Systems Ltd, Airdire, AB, Canada) were used for measurement of individual animal feed intake. Steers were adapted to GrowSafe feed bunks for 13 d prior to study initiation. All steers were implanted with a Component E-S with Tylan (donated by Elanco Animal Health, Greenfield, IN) on day 0. Bodyweight measurements were recorded for individual steers on consecutive dates at study initiation (day 0) prior to placement in final study pen and at study conclusion (day 42). A single interim BW was also recorded for all steers on day 14.

Weaned steers ($n = 72$) were assigned to one of three dietary treatments containing supplemental TM of differing source and concentration that were included as premixes in a common receiving diet: 1) steers were supplemented TM from an organic source (Availa4; Zn amino acid complex, Mn amino acid complex, Cu amino acid complex, and Co glucoheptonate; Availa4, Zinpro Corp., Eden Prairie, MN) at 7 g·steer⁻¹·d⁻¹; for the entirety of the 42-d receiving trial (ORG); 2) steers were supplemented inorganic TM sources to provide equivalent supplemental TM concentrations relative to the commercially available product Availa4 for the entirety of the 42-d receiving trial (ING); 3) or steers were fed the ORG supplement with additional AvailaZn to provide 1,000 mg Zn·steer⁻¹·d⁻¹ for first 14 d of study (ORG+Z).

Dietary management

Cattle were fed treatment diets (Table 1) once daily at approximately 0800 h according to standard procedure at the BNF. Ad libitum feed was provided for steers during the entirety of the study and managed to ensure residual feed in GrowSafe bunks prior to daily feeding. Dietary treatments were included into the total mixed ration (**TMR**) in the form of a premix that utilized dried distiller's grains (**DDG**) as a carrier; treatment premixes replaced DDG in the diet. Premix inclusion as a percentage of diet DM was adjusted every 14 d to target desired TM intake. Diets presented in Table 1 represent actual DM diet composition from weekly ingredient DM analysis. Water was provided ad libitum throughout the study via automatic waterers available in each pen. Water tanks were checked daily and cleaned by feedlot personnel as needed to ensure a constant and clean water supply to the test animals.

Sample collection and analytical procedures

Samples of TMR were collected weekly during the 42-d receiving study. Samples were subsequently dried in a forced-air oven at 70 °C for 48 h for DM determination. Individual steer DMI was calculated using as-fed intakes (i.e., feed disappearance tracked by the unique electronic identification tag given to each steer prior to study initiation) corrected for the DM of weekly TMR samples. Proximate analysis on weekly TMR samples of the CON diet was dried, ground, and composited for analysis of nitrogen,

Table 1. Diet composition and nutrient analysis[4]

	ING	ORG	ORG+Z
Ingredient, % DM basis			
Corn silage	40	40	40
Sweetbran	40	40	40
DDGS	10	10	5
Farm basal[1]	5	5	5
ING premix	5.5	—	—
ORG premix	—	5.5	5.5
Zn premix[2]	—	—	5
Analyzed composition[3], %			
DM		55.4	
Crude protein[3]		18.9	
NDF[3]		34.5	
Ether extract[3]		5.02	
NEm, Mcal/kg		1.85	
NEg, Mcal/kg		1.26	

[1]Provided vitamins at 2016 NASEM recommendations.
[2]DDGS were carrier for Zn treatment from day 1 to day 14 to supply AvailaZn at a rate of 1,000 mg Zn·steer^{-1}·d^{-1} for first 14 d.
[3]Values determined by Dairyland Laboratories (Arcadia, WI).
[4]DDGS, dried distiller's grains with solubles; DM, dry matter; NDF, neutral detergent fiber; NEg, net energy for gain; NEm, net energy for maintenance.

Table 2. Trace mineral (TM) TMR analysis[1, 2]

Period	TM, mg/kg DM	Treatment		
		ING	ORG	ORG+Z
Day 1–14	Cu	22.6	23.6	23.1
	Mn	43.7	46.1	49.0
	Zn	135.5	103.1	229.4
Day 14–28	Cu	18.9	24.6	25.0
	Mn	47.6	53.4	54.5
	Zn	120.7	108.9	116.3
Day 28–42	Cu	22.7	22.8	20.4
	Mn	49.8	49.8	46.4
	Zn	116.7	106.8	101.0

[1]Values obtained via inductively coupled plasma optical emission spectroscopy (PerkinElmer) analysis.
[2]DM, dry matter; TMR, total mixed ration.

neutral detergent fiber, and ether extract by a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI). Feed efficiency (gain-to-feed ratio [**G:F**]) was calculated from the total gain and total DMI during weighing intervals (i.e., day 1 to 14 and day 14 to 42). Composited feeds were acid-digested using TM grade nitric acid as previously described (Genther-Schroeder et al., 2016) before analysis for Cu, Fe, Mn, and Zn concentrations (Table 2) using inductively coupled plasma optical emission spectroscopy (PerkinElmer, Waltham, MA).

Whole-blood samples were collected from all steers (n = 72) on day 0, 14, and 42 relative to study initiation. Whole-blood samples (collected in tubes containing either no additive for serum, K_2EDTA for TM analysis, or sodium heparin for additional analyses) were centrifuged at 1,000 × g for 20 min at

4 °C. Plasma and serum were harvested and aliquoted for storage at –20 °C and –80 °C, respectively, prior to subsequent analyses. Flow cytometry measures were taken on day 0, 14, and 42 using whole blood that was processed immediately following sample collection for determination of circulating frequencies of immune cell populations as described (Mahmoud et al., 2020). Plasma samples were analyzed for Cu, Fe, and Zn concentration using methods previously described (Pogge and Hansen, 2013). A human serum reference sample from UTAK Laboratories Inc. (Valencia, CA) was included in all analyses to verify instrument accuracy.

Liver biopsies were taken from steers selected at random within pen prior to study initiation (n = 3 steers per pen). Liver biopsies were done on day 0, 14, and 42 relative to study initiation using the method outlined by Engle and Spears (2000). A bovine liver reference sample from National Institutes of Standards and Technology (Gaithersburg, MD) was included in all analyses to verify instrument accuracy. Liver samples were dried and acid-digested in preparation for subsequent analysis of Cu, Fe, Mn, and Zn concentration using methods previously described (Pogge and Hansen, 2013).

Packed red blood cells from blood samples (collected into K_2EDTA for following removal of plasma for TM analysis) on day 0, 14, and 42 from sentinel steers (n = 12 steers per treatment) were lysed with four volumes of ice-cold molecular-grade water (Cayman Chemical), vortexed, and centrifuged at 10,000 × g for 15 min at 4 °C. The supernatant (red blood cell lysate [**RBCL**]) was removed, aliquoted, and stored at –80 °C until subsequent analysis of superoxide dismutase (**SOD**) activity (item number 706002, Cayman Chemical); intra- and inter-assay coefficient of variation (CV) for SOD were 5.1% and 7.9%, respectively. A unit of SOD activity was defined as the amount of the enzyme required to dismutase 50% of the superoxide radical and the activity is expressed per gram of hemoglobin (1,000 units of SOD activity/g hemoglobin). Hemoglobin was determined using methods described by Hansen et al. (2010).

Serum collected from each animal (n = 72) on day 0, 14, and 42 and was stored in a –20 °C freezer prior to analysis using D2Dx technology. The D2Dx immunity test will be referred to herein as a nanoparticle-enabled immunity test. Before testing, all frozen serum samples were thawed in a refrigerator at 4 °C overnight. Prior to testing, serum samples were allowed to equilibrate to room temperature for approximately 3 h before testing. The nanoparticle-enabled immunity test uses gold nanoparticles (**AuNP**) as a pseudo-virus pathogen to probe the humoral immunity in blood plasma or serum samples. The AuNP are designed to react with type 1 and type 2 immunity-associated Immunoglobulin G (IgG) subclasses in a blood plasma or serum sample (Nano Discovery Inc.). The nanoparticle-enabled immunity test was conducted by adding 50 µL of nanoparticle reagent to the cuvette. To the reagent solution, 10 µL of undiluted serum sample was added. After which it was briefly vortexed for 5 s. The cuvette containing the mixed solution is then inserted into the CT-100 reader device (Nano Discovery Inc.). The resulting color change was read automatically by the device in 30 s. The test score was expressed as 10 times the absorbance change.

Statistical analysis

Feedlot performance and blood measure data were analyzed as a completely randomized design using the MIXED

procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Individual steer served as the experimental unit. The model included the fixed effect of dietary treatment. Initial BW served as a covariate in performance data analysis. Initial values for blood and liver tissue parameters served as a covariate for analysis of subsequent data. Initial values for cell population frequency and markers of activation within flow cytometry data were used as covariates for statistical analysis. Initial values were not used as a covariate for mean fluorescence intensity (**MFI**) measures as MFI measures cannot be properly compared across different time points. Measures of Mn SOD, Cu/Zn SOD, or total SOD activity as well as nanoparticle-enabled immunity test measurements for assessing immune response and health in cattle on day 0, 14, and 42 were analyzed as repeated measures. Dry matter intake and G:F data from a single pen ($n = 6$ steers) were removed from analysis due to chronic malfunctions in that particular GrowSafe feed bunk; however, no abnormalities in growth performance data for steers in that pen were noted and were included in the final analysis. Statistical outliers were determined to be data that were beyond 3 SDs from the mean for a particular parameter. Significance was determined as $P \leq 0.05$ and tendencies were declared when $0.05 < P \leq 0.10$.

Results

Feedlot growth performance

Growth performance data are presented in Table 3. Body weight did not differ at time of trial initiation ($P = 0.25$). During the day 0 to 14 interim period, day 14 BW, average daily gain (**ADG**), DMI, and G:F were not affected by dietary treatment ($P \geq 0.18$). From day 14 to 42 G:F was increased for ORG+Z steers ($P = 0.02$) compared to ORG and ING. However, no significant response ($P \geq 0.14$) was noted for day 42 BW, or day 14 to 42 ADG, or day 14 to 42 DMI.

Overall (day 0 to 42), there was a tendency for improved cumulative ADG ($P = 0.07$) for steers supplemented TM from an organic source where ORG and ORG+Z were increased 9.0% and 12.0%, respectively, compared to ING. Cumulative growth performance data (day 1 to 42) indicate DMI was unaffected ($P \geq 0.18$) by dietary treatment. Organic TM supplementation improved G:F ($P = 0.01$). When compared to ING, overall G:F for steers from ORG and ORG+Z was increased 13.2% and 20.3%, respectively.

Total Zn intake

Total Zn intake data are presented in Table 4. By design, total Zn intake (mg/d) differed ($P = 0.01$) from day 1 to 14 when the ORG+Z treatment received an additional 1,000 mg Zn from AvailaZn and thus ORG+Z was greater than both ING and ORG (ING = 1,174 mg·steer^{-1}·d^{-1}, ORG = 914 mg·steer^{-1}·d^{-1}, ORG+Z = 1,900 mg·steer^{-1}·d^{-1}). Total Zn intake from day 14 to 42 was significantly greater ($P = 0.01$) for ING compared to both organic treatments (ING = 1,054 mg·steer^{-1}·d^{-1}, ORG = 969 mg·steer^{-1}·d^{-1}, ORG+Z = 931 mg·steer^{-1}·d^{-1}). ORG+Z had the greatest ($P = 0.01$) cumulative total Zn intake (day 1 to 42) with ORG being the lowest and ING intermediate (ING = 1,095 mg·steer^{-1}·d^{-1}, ORG = 951 mg·steer^{-1}·d^{-1}, ORG+Z = 1,258 mg·steer^{-1}·d^{-1}).

Table 3. Effects of dietary supplemental Zn concentration and trace mineral source on growing steer growth performance[1, 5]

	Treatments			SEM[2]	*P*-value
	ING	ORG	ORG+Z		
Steers	$n = 24$	$n = 24$	$n = 24$	—	—
Initial BW, kg[3]	284	284	284	5.0	0.99
Period: day 1 to 14					
Day 14 BW, kg[3]	312	315	315	1.4	0.25
ADG, kg/d	2.01	2.23	2.21	0.100	0.23
DMI, kg/d[4]	8.67	8.87	8.29	0.250	0.18
G:F[4]	0.240	0.254	0.262	0.0132	0.45
Period: day 14 to 42					
Day 42 BW, kg[3]	359	366	366	3.1	0.21
ADG, kg/d	1.67	1.80	1.87	0.070	0.14
DMI, kg/d[4]	8.88	8.97	8.55	0.260	0.43
G:F[4]	0.183[b]	0.199[b]	0.219[a]	0.0090	0.02
Overall					
ADG, kg/d	1.78[y]	1.94[x]	1.99[x]	0.069	0.07
DMI, kg/d[4]	8.81	8.94	8.42	0.110	0.21
G:F[4]	0.193[b]	0.217[a]	0.231[a]	0.0091	0.01

[1]Initial BW was used as a covariate for all growth performance calculations.
[2]Greatest SEM reported.
[3]BW presented as BW * 0.96.
[4]$n = 18$ for ING.
[5]ADG, average daily gain; BW, body weight; DMI, dry matter intake; G:F, gain-to-feed ratio.
[a,b]Within a row, means with unlike superscripts differ $P \leq 0.05$.
[x,y]Within a row, means with unlike superscripts differ $0.1 \geq P > 0.05$.

Liver TM concentration

Liver TM concentration data are presented in Table 5. There was no difference ($P \geq 0.22$) for day 0 liver TM concentration for any treatment group. On day 14, cattle-supplemented TM from an inorganic source tended to have greater ($P = 0.09$) liver Zn concentration compared to ORG+Z with ORG being intermediate. No difference was noted ($P = 0.28$) for day 42 liver Zn concentration. Dietary treatment elicited a similar response for liver Cu concentration on both day 14 and 42 where cattle-supplemented TM from an inorganic source had greater ($P \leq 0.01$) concentrations of liver Cu than cattle fed an organic source of TM. Neither liver Mn nor Fe was affected ($P \geq 0.22$) by dietary treatment at any point of measurement during the study.

Plasma TM concentration

Plasma TM concentration data are presented in Table 6. No differences ($P \geq 0.19$) in plasma TM status existed between treatment groups at study initiation. Dietary treatment did not elicit an effect ($P \geq 0.20$) on plasma Zn or Cu at any remaining sampling points. Plasma Fe tended ($P = 0.08$) to be lesser in ING compared to ORG and ORG+Z on day 14 but was not different between treatments on day 42.

RBCL SOD activity

Red blood cell lysate SOD data are shown in Figure 1. No treatment by day interactions were noted ($P \geq 0.15$) for Mn SOD, Cu/Zn SOD, or total SOD. No day effect was noted ($P = 0.34$) for Cu/Zn SOD activity. However, there was a day effect ($P = 0.02$) for Mn SOD where Mn SOD activity was greatest at day 1, least on day 14, and intermediate by day 42. Likely driven by the statistical trend noted for Mn SOD activity, a similar tendency for a day effect was noted ($P = 0.07$) in total SOD activity. Total SOD activity tended to be greatest on day 1, least on day 14, and intermediate on day 42 ($P = 0.82$).

Immune cell phenotypic markers of activation and nanoparticle-enabled immunity test value

Percentage of immune cell population and phenotypic markers of activation as well as MFI data are presented in Tables 7–12, respectively. Frequency of total circulating natural killer (**NK**) cells activated CD8+ NK cells, and CD8 T cells measured on day 0, 14, and 42 did not differ ($P \geq 0.07$). However, markers of activation (CD16, CD44) on CD8+ NK cells measured on day 14 did differ due to treatment ($P \leq 0.05$). On day 14 the percentage of CD16+ of CD8+ NK cells was greatest in the ORG+Z treatment with ORG being the lowest and ING intermediate. On day 14 percentage of CD44+ of CD8+ NK cells was greatest in ORG compared to both ORG+Z and ING.

Nanoparticle-enabled immunity test data are presented in Figure 2. No treatment by day effects or treatment effects were noted ($P \geq 0.30$) for nanoparticle-enabled immunity test measures. However, a day effect was noted ($P = 0.01$) where the measured nanoparticle-enabled immunity test value was intermediate on day 0, greatest at day 14, and least by day 42.

Table 4. Total Zn intake by growing steers[1, 2]

Period	Treatments			SEM	*P*-value
	ING	**ORG**	**ORG+Z**		
Steers	*n* = 18	*n* = 24	*n* = 24	—	—
Day 1 to 14 Zn intake mg/d	1,174[b]	914[b]	1,900[a]	41	<0.001
Day 14 to 42 Zn intake mg/d	1,054[a]	969[b]	929[b]	29	<0.001
Day 1 to 42 Zn intake mg/d	1,095[b]	951[c]	1,258[a]	29	<0.001

[1]Calculated using DMI for a given period and TMR Zn concentration obtained via analysis using inductively coupled plasma optical emission spectroscopy.
[2]DMI, dry matter intake; TMR, total mixed ration.
[a–c]Within a row, means with unlike superscripts differ $P \leq 0.05$.

Table 5. Liver trace mineral (TM) concentration in growing beef steers with different supplement dietary Zn concentration and TM source[2]

| TM, mg/kg DM	Day	Treatment			SEM	*P*-value
		ING	**ORG**	**ORG+Z**		
Steers		*n* = 12	*n* = 12	*n* = 11		
Zn	0	138	135	139	12	0.97
	14	130[a]	123[a,b]	105[b]	8	0.09
	42	118	112	102	8	0.28
Cu	0	265	294	252	26	0.51
	14	310[a]	281[b]	258[b]	10	0.01
	42	366[a]	288[b]	248[b]	16	0.01
Mn	0	7.0	7.8	7.7	0.38	0.22
	14	7.8	8.1	7.7	0.31	0.65
	42	7.9	7.6	8.1	0.30	0.39
Fe	0	130	136	124	8	0.53
	14	124	121	127	5	0.64
	42	129	129	124	5	0.70

[a,b]Within a row, means with unlike superscripts differ $P \leq 0.05$.
[2]DM, dry matter.

Table 6. Plasma trace mineral (TM) concentration in growing beef steers with different supplement dietary Zn concentration and TM source

TM, mg/L	Day	Treatment			SEM	*P*-value
		ING	ORG	ORG+Z		
Cu	0	1.10	1.04	1.03	0.038	0.35
	14	1.04	1.02	0.98	0.039	0.34
	42	1.02	0.94	0.93	0.512	0.35
Zn	0	1.05	1.11	1.02	0.042	0.32
	14	1.07	1.14	1.15	0.359	0.20
	42	1.13	1.15	1.13	0.035	0.86
Fe	0	1.58	1.86	1.61	0.122	0.19
	14	1.40^y	1.70^x	1.69^x	0.110	0.08
	42	1.63	1.55	1.61	0.101	0.82

^{x,y}Within a row, means with unlike superscripts differ $0.1 \geq P > 0.05$.
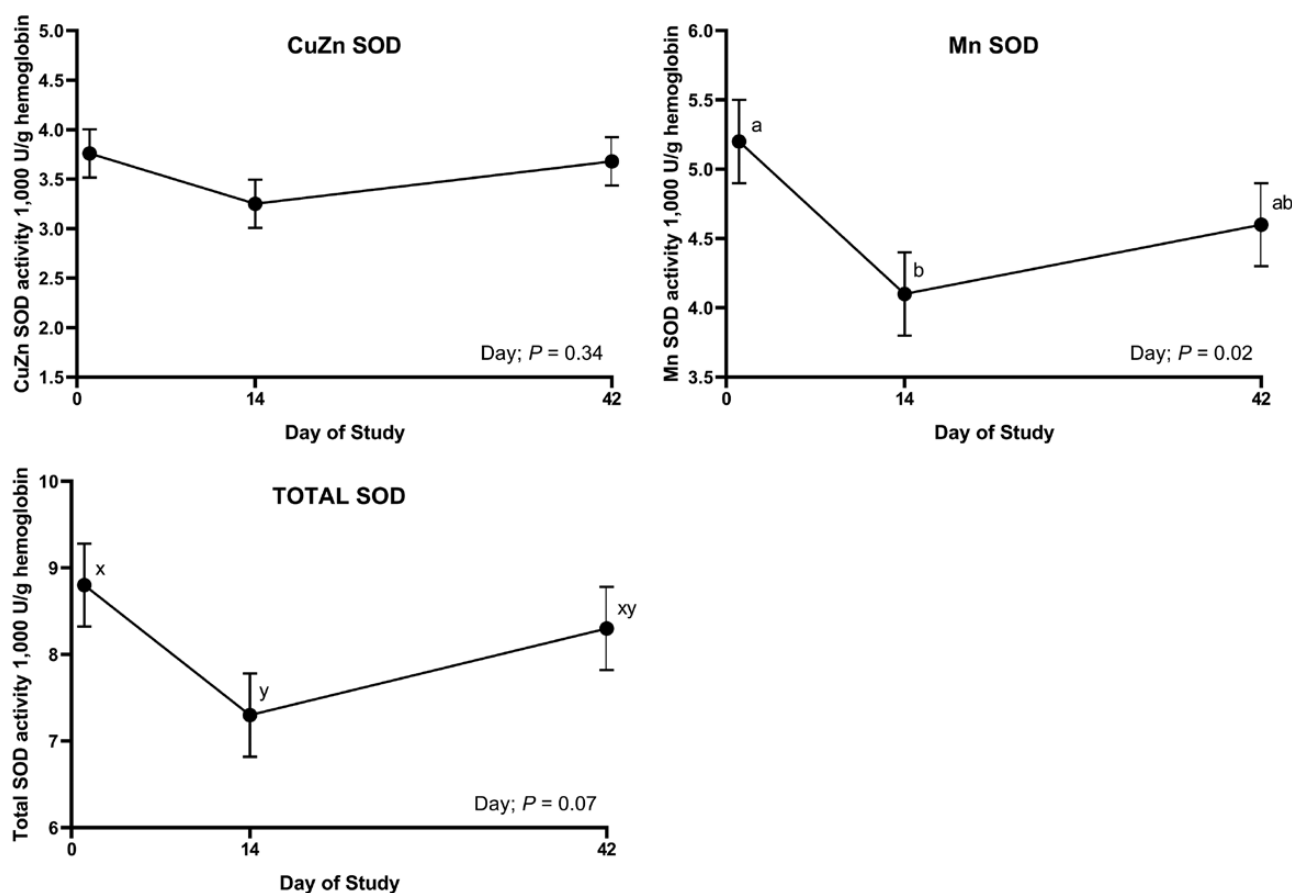
Figure 1. Effect of day on Mn SOD, Cu/Zn SOD, or total SOD activity (1,000 U/g hemoglobin) based on repeated measures analysis of blood samples collected on day 0, 14, and 42. Values within a panel with unlike superscripts (a, b, and c) differ ($P \leq 0.05$) across sampling days and superscripts that differ (x, y) denote a tendency ($0.05 < P \leq 0.10$). SOD, superoxide dismutase.

Discussion

Our objective was to evaluate the effect of TM source (organic vs. inorganic) on biomarkers of immune status and growth performance in weaned beef calves as well as the effect of additional organic Zn offered in the form of AvailaZn for the first 14 d of the study, thus targeting the period of the receiving phase when disease challenge is most likely and dietary TM fortification may be most effective. Organic TM supplementation appears beneficial when cattle are stressed such as in cases of shipping (Kegley et al., 2012; Heiderscheit and Hansen, 2022); or disease (Chirase et al., 1991), whereas in other work (Gunter et al., 2001) organic TM supplementation did not improve cattle performance. In the present study, the value of supplementing TM from an organic source (ORG and ORG+Z) to beef steers received into the feedlot was evidenced by improvements in overall efficiency and performance. Steers from ORG and ORG+Z tended to

Table 7. Effects of dietary supplemental Zn concentration and trace mineral source on percentage of immune cell phenotypic markers of activation and population measures on day 0[2]

	Treatments			SEM[1]	P-value
	ING	ORG	ORG+Z		
Day 0					
Steers	n = 23	n = 24	n = 23	—	—
CD8+ T cells					
Freq. of live, %	7.80[x,y]	8.20[x]	6.55[y]	0.563	0.10
% CD2+	94.22	94.61	96.11	0.741	0.17
% CD16+	2.08	1.40	1.75	0.281	0.23
% CD25+	2.89	2.97	2.46	0.252	0.31
% CD44+	80.46	81.04	83.12	1.750	0.53
% CD45RO+	17.09[b]	15.26[b]	22.22[a]	1.425	0.01
CD8+ NK cells					
Freq. of live, %	0.26	0.29	0.28	0.040	0.82
% CD2+	79.24	74.84	75.64	2.137	0.30
% CD16+	28.02[b]	26.34[b]	34.75[a]	2.323	0.03
% CD25+	26.46[x]	22.01[x,y]	20.39[y]	2.061	0.10
% CD44+	54.56	51.26	47.06	3.942	0.41
% CD45RO+	37.84	38.04	42.42	2.346	0.30
NK cells					
Freq. of live, %	6.04	6.36	5.31	0.725	0.58
% CD2+	82.18	84.12	83.63	1.246	0.51
% CD16+	44.22	41.82	45.96	1.686	0.22
% CD25+	3.02	2.82	2.62	0.309	0.66
% CD44+	17.61	14.84	17.47	1.399	0.29
% CD45RO+	24.77	22.46	27.02	1.959	0.26

[1]Highest SEM of any treatment.
[2]NK, natural killer.
[a,b]Within a row, means with unlike superscripts differ $P \leq 0.05$.
[x,y]Within a row, means with unlike superscripts differ $0.1 \geq P > 0.05$.

have greater overall ADG, where ORG and ORG+Z relative to ING were 9.0% and 12.0% better, respectively. Driven by improvements in ADG and no differences in DMI, ORG and ORG+Z displayed 13.2% and 20.3% improvements in G:F, respectively, compared to ING. Similarly, increased AvailaZn supplementation to dairy cows before calving and during lactation improved feed efficiency (Nayeri et al., 2014). In received beef cattle, Dorton et al. (2006) reported supplementation with organic TM resulted in greater ADG than steers supplemented equivalent amounts of TM from inorganic sources.

While results from the present study align with previous work, showing improvements in performance in receiving cattle, it should be noted steers in this study underwent adaptation to GrowSafe bunks prior to initiation of the trial. This is important to consider as one interprets results herein as cattle intakes had risen to adequate levels prior to study initiation. Overall DMI was unaffected by dietary treatment and, by design, ORG+Z had the greatest total Zn intake from day 1 to 14. Total Zn intake from day 14 to 42 was greater for ING compared to either organic treatment because of differences in analyzed TM content of the diets as well as numerically different intakes. Overall, ORG+Z had the greatest total Zn intake from day 1 to 42. Data from this study provide evidence that even in low-risk cattle that

are eating well, and TM adequate, organic TM can improve growth performance.

Reliable Zn biomarkers when cattle are not Zn-deficient are lacking. At study initiation, cattle were well within the adequate range of liver and plasma Zn when compared to standard reference ranges (Kincaid, 2000). With cattle eating well already at trial initiation and all treatments being fed diets adequate in Zn, the continued adequacy in liver and plasma Zn concentrations observed during the trial was unsurprising.

Following the initial 14-d supplementation with AvailaZn at rate of 1,000 mg Zn·steer^{-1}·d^{-1}, ORG+Z steers tended to have the lowest liver Zn concentration, albeit still within the accepted range of adequacy. This decrease in liver Zn may indicate increased use of the Zn for productive functions, resulting in lesser hepatic storage. This supposition aligns with the previously discussed improvements in overall growth performance. In this study, no difference between dietary treatment existed for plasma Zn at any point. Spears and Kegley (2002) reported no effect of Zn concentration or source on plasma Zn concentration during the growing phase although these workers supplemented lower concentrations of Zn compared to the present study. Perhaps the most valuable TM biomarker reported herein is the observed improvement in growth performance for cattle-supplemented organic TM.

Table 8. Effects of dietary supplemental Zn concentration and trace mineral source on percentage of immune cell phenotypic markers of activation and population measures on day 14[2, 3]

	Treatments			SEM[1]	*P*-value
	ING	ORG	ORG+Z		
Day 14					
Steers	*n* = 24	*n* = 24	*n* = 24	—	—
CD8+ T cells					
Freq. of live, %	4.43	4.55	4.56	0.572	0.98
% CD2+	97.58	97.84	97.43	0.275	0.52
% CD16+	0.10	0.10	0.09	0.770	0.52
% CD25+	0.38	0.35	0.31	0.088	0.81
% CD44+	75.81	79.99	79.19	2.080	0.31
% CD45RO+	26.91	24.53	23.17	2.303	0.50
CD8+ NK cells					
Freq. of live, %	0.31	0.18	0.27	0.051	0.19
% CD2+	89.12	90.21	88.67	1.409	0.71
% CD16+	18.08[a,b]	14.32[b]	21.70[a]	2.185	0.05
% CD25+	5.57	5.23	4.36	1.434	0.81
% CD44+	41.62[b]	55.06[a]	38.78[b]	4.819	0.04
% CD45RO+	40.53	37.97	44.24	3.633	0.45
NK cells					
Freq. of live, %	2.49	2.62	3.27	0.423	0.36
% CD2+	89.12	87.33	89.83	1.863	0.61
% CD16+	22.23	18.45	21.53	2.136	0.39
% CD25+	0.47	0.92	0.83	0.200	0.23
% CD44+	8.71	9.51	8.13	1.076	0.64
% CD45RO+	35.79	31.58	34.46	3.346	0.65

[1]Highest SEM of any treatment.
[2]Day 14 data are covariate-adjusted using day 0 values.
[3]NK, natural killer.
[a,b]Within a row, means with unlike superscripts differ *P* ≤ 0.05.

Heiderscheit and Hansen (2022) supplemented increasing concentrations of Zn bisglycinate (0, 70, and 120 mg supplemental Zn/kg DM) prior to and after a transit event. Prior to transit, supplemental Zn minimally affected plasma Zn concentration; however, following transit, plasma Zn reflected dietary Zn more closely, suggesting stress (transit) increases Zn requirements. This may also indicate plasma Zn is a useful transient Zn status index when cattle experience marginal intakes or stress.

In cattle, hepatic storage of Cu is substantially more malleable than hepatic stores of Zn and Mn. In this study equivalent concentrations of Cu were supplemented to treatment diets from either inorganic or organic sources. In this study, inorganic supplementation of Zn resulted in greater liver Cu concentration for ING on both day 14 and 42. Cattle were in the adequate range of liver Cu concentration at all study time points, and plasma Cu did not differ between dietary treatments throughout. In studies utilizing beef cows with much lower initial liver Cu concentrations inorganic Cu increased liver Cu (Muehlenbein et al., 2001) or had no effect on liver Cu (Olson et al., 1999) when compared to feeding organic Cu.

Taken together, liver Zn and Cu concentration results suggest the organic source of supplemental Zn was more available for productive functions and may have interacted with and inhibited Cu more strongly. If cattle are Cu adequate no additional Cu fortification would be recommended to overcome this Zn antagonism, especially if it is an interim fortification with Zn lasting only the first several weeks of receiving.

Measures of SOD activity can be indicative of ability to respond to oxidative stress. Both Cu and Mn play catalytic roles in their respective enzymatic SOD systems while Zn serves an important structural component of the Cu/Zn SOD enzyme. Although no treatment effects were noted, greater Mn SOD activity and a tendency for increased total SOD activity at the beginning of the feeding period (day 0) may indicate cattle were undergoing a greater degree of oxidative stress at this time. These results in combination with those of Deters and Hansen (2019) where newly received cattle had greater Mn SOD activity in RBCL at arrival (post-transit) suggest Mn SOD upregulation may be a marker of stress early in the receiving phase.

An interesting and recognizable pattern exists in the liver and plasma TM data. Over the course of the study, liver Zn decreased with a concurrent increase in plasma Zn, while plasma Cu decreased. Taken together, these data suggest steers may have been exhibiting signs of nutritional immunity during the early portion of this study. Classically, nutritional immunity can be described as the physiological response to an infection where vertebrates sequester Fe, Zn, and Mn both intracellularly and extracellularly to protect

Table 9. Effects of dietary supplemental Zn concentration and trace mineral source on percentage of immune cell phenotypic markers of activation and population measures on day 42[2, 3]

	Treatments			SEM[1]	*P*-value
	ING	ORG	ORG+Z		
Day 42					
Steers	*n* = 24	*n* = 24	*n* = 23	—	—
CD8+ T cells					
Freq. of live, %	4.70	4.34	4.56	0.337	0.73
% CD2+	92.89	93.91	92.37	0.663	0.25
% CD16+	1.94	2.36	2.19	0.528	0.85
% CD25+	6.72	5.42	5.52	1.935	0.87
% CD44+	83.71	85.97	84.51	1.825	0.66
% CD45RO+	14.11	12.72	11.98	1.433	0.55
CD8+ NK cells					
Freq. of live, %	0.40[x]	0.22[y]	0.29[x,y]	0.058	0.08
% CD2+	63.27	66.26	59.70	3.598	0.43
% CD16+	43.20	42.01	47.55	4.357	0.64
% CD25+	38.20	43.75	34.79	3.777	0.24
% CD44+	55.47	60.25	51.97	4.678	0.45
% CD45RO+	37.83	39.77	35.76	2.572	0.55
NK cells					
Freq. of live, %	2.76	2.70	2.53	0.263	0.81
% CD2+	76.20	79.14	77.06	2.404	0.66
% CD16+	70.13[x]	68.73[x,y]	64.48[y]	1.744	0.06
% CD25+	5.74	4.58	5.55	1.844	0.89
% CD44+	24.78	24.23	25.97	1.648	0.74
% CD45RO+	17.96	15.32	15.06	1.492	0.32

[1]Highest SEM of any treatment.
[2]Day 42 data are covariate-adjusted using day 0 values.
[3]NK, natural killer.
[x,y]Within a row, means with unlike superscripts differ $0.1 \geq P > 0.05$

against infection (Kehl-Fie and Skaar, 2010) while plasma Cu will rise as in response to infection with increased concentration of Cu-dependent acute phase proteins such as ceruloplasmin.

Deficiencies and excess concentrations of TM can influence parameters of the immune system, such as antibody responses, cell-mediated immunity, NK cell activation, and T-cell activity and development (Mocchegiani et al., 1995; Mingari et al., 1998; Beck, 1999). In the present study, dietary treatment did not affect frequency of total circulating NK cells, activated CD8+ NK cells, and CD8 T cells measured on day 0, 14, and 42. However, within the NK cell population, phenotypic markers of activation (CD16, CD44, and CD8) on NK cells measured on day 14 differed due to TM treatment. Markers of activation assessed via flow cytometry can be indicative of different innate properties of these cell types. On day 14, percent CD16+ CD8+ NK cells were greatest in ORG+Z steers, least in ORG, and intermediate in ING. However, on day 14 ORG steers had greater percentage of CD44+ CD8+ NK cells compared to ING and ORG+Z. By day 42, no treatment effects for any phenotypic markers of activation for CD8+ NK cells were noted; however, ING tended to have the greatest percentage of circulating live CD8+ NK cells, where ORG was least and ORG+Z was intermediate. Beyond that, among NK cells, ING tended to have the

greatest percentage of CD16+ NK cells, ORG+Z the lowest percentage, with ORG being intermediate. In humans, Zn supplementation increased the activity and percentage of NK cells within cultured leukocytes in vitro (Metz et al., 2007). Previous work in humans also indicates that serum Zn concentration may be related to NK cell function (Ravaglia et al., 2000) and that Zn deficiency has also been shown to adversely influence NK cell number and activity (Prasad, 2000). However, in the present study, cattle were never deficient in liver nor plasma Zn and plasma Zn did not differ between treatment.

As a crucial component of the adaptive immune system, T cells function to provide cellular immunity (Gammoh and Rink, 2019). Within the T-cell population, dietary treatment did not influence any phenotypic markers of activation. However, previous work in human and rodent models indicates Zn can influence the activity of T cells (Mingari et al., 1998; Kahmann et al., 2008) as well as T-cell development (Mocchegiani et al., 1995).

Flow cytometry results from the present study indicate TM source and concentration can alter phenotypic markers of immune activation. However, underlying mechanisms are unclear. Very limited research has examined these types of measures in beef cattle. Rients et al. (2021) evaluated the influence of *Saccharomyces cerevisiae* fermentation product (**SCFP**) on immune cell populations and markers of activation

Table 10. Effects of dietary supplemental Zn concentration and trace mineral source on MFI (mean fluorescence intensity) for immune cell phenotypic markers of activation MFI on day 0[2]

	Treatments			SEM[1]	P-value
	ING	ORG	ORG+Z		
Day 0					
Steers	*n* = 23	*n* = 24	*n* = 23	—	—
CD8+ T cells					
CD16+	166.0[x,y]	148.7[y]	181.8[x]	10.05	0.07
CD25+	129.4	119.6	146.7	11.79	0.24
CD44+	1,480.7	1,408.8	1,486.0	44.95	0.39
CD45RO+	2,221.9	1,845.5	2,348.7	142.27	0.04
CD8+ NK cells					
CD16+	652.5[y]	875.4[y]	1,064.8[x]	130.71	0.09
CD25+	722.7[x]	517.5[x,y]	461.6[y]	80.06	0.06
CD44+	1,282.4	1,125.0	1,067.6	95.77	0.27
CD45RO+	12,931.0	14,100.0	10,836.0	1,356.40	0.22
NK cells					
CD16+	468.2	443.4	482.7	13.51	0.12
CD25+	228.4	211.4	233.7	25.60	0.80
CD44+	219.5[x]	186.1[y]	214.5[x]	11.53	0.09
CD45RO+	6,158.2[a]	3,831.8[b]	4,964.8[a,b]	523.89	0.01

[1]Highest SEM of any treatment.
[2]NK, natural killer.
[a,b]Within a row, means with unlike superscripts differ $P \leq 0.05$.
[x,y]Within a row, means with unlike superscripts differ $0.1 \geq P > 0.05$.

Table 11. Effects of dietary supplemental Zn concentration and trace mineral source on MFI (mean fluorescence intensity) for immune cell phenotypic markers of activation MFI on day 14[2]

	Treatments			SEM[1]	
	ING	ORG	ORG+Z		P-value
Day 14					
Steers	*n* = 24	*n* = 24	*n* = 24	—	—
CD8+ T cells					
CD16+	143.7	148.9	164.2	11.92	0.44
CD25+	50.3	53.8	50.7	2.88	0.64
CD44+	744.4	764.5	759.8	44.13	0.95
CD45RO+	2,793.3	2,850.8	2,476.5	229.29	0.47
CD8+ NK cells					
CD16+	900.4[a]	895.1[a]	522.0[b]	124.99	0.05
CD25+	178.0	190.5	144.0	27.43	0.45
CD44+	517.3[y]	748.3[x]	529.5[y]	77.27	0.07
CD45RO+	18,602.0[x]	23,582.0[x]	16,708.0[y]	2,331.75	0.10
NK cells					
CD16+	297.3	280.5	297.1	12.19	0.53
CD25+	84.7	97.3	94.2	4.71	0.15
CD44+	100.3	106.8	96.5	10.03	0.76
CD45RO+	8,320.3	8,228.8	7,952.7	1,127.18	0.97

[1]Highest SEM of any treatment.
[2]NK, natural killer.
[a,b]Within a row, means with unlike superscripts differ $P \leq 0.05$.
[x,y]Within a row, means with unlike superscripts differ $0.1 \geq P > 0.05$.

in beef steers fed ractopamine hydrochloride. These workers observed increased percentage of activated gamma delta T cells and NK cells in circulation, suggesting SCFP can influence the innate immune system. In the present study, due to the variable relationship in the response to dietary treatment for immune cell phenotypic markers of activation, the

Table 12. Effects of dietary supplemental Zn concentration and trace mineral source on MFI (mean fluorescence intensity) for immune cell phenotypic markers of activation MFI on day 42[2]

	Treatments			SEM[1]	P-value
	ING	ORG	ORG+Z		
Day 42					
Steers	n = 24	n = 24	n = 23	—	—
CD8+ T cells					
CD16+	176.2	168.7	155.1	9.66	0.29
CD25+	241.9[a]	184.6[b]	176.5[b]	18.28	0.02
CD44+	2,149.6	2,148.3	2,177.3	74.41	0.95
CD45RO+	1,556.6	1,538.5	1,340.7	153.21	0.54
CD8+ NK cells					
CD16+	877.0	1,075.6	832.9	216.96	0.70
CD25+	938.7	1,050.5	695.9	163.60	0.29
CD44+	1,622.4	1,819.6	1,525.9	159.59	0.41
CD45RO+	5,715.4	5,261.8	5,028.6	908.62	0.86
NK cells					
CD16+	646.5[x]	637.3[x,y]	594.0[y]	18.28	0.10
CD25+	299.9	354.1	388.6	47.09	0.41
CD44+	290.7	301.0	310.9	18.70	0.74
CD45RO+	2,049.0	1,750.3	2,394.1	282.15	0.28

[1]Highest SEM of any treatment.
[2]NK, natural killer.
[a,b]Within a row, means with unlike superscripts differ $P \leq 0.05$.
[x,y]Within a row, means with unlike superscripts differ $0.1 \geq P > 0.05$.
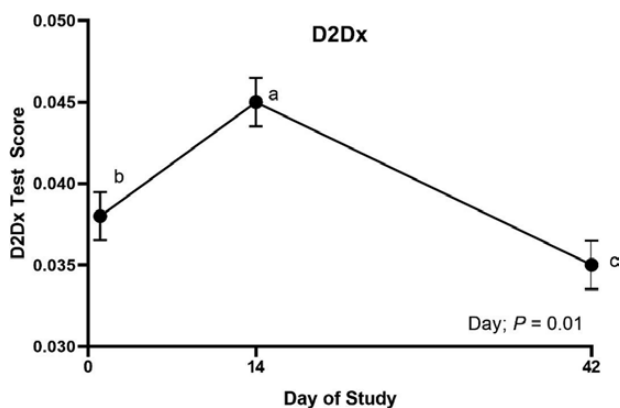
Figure 2. Effect of day on nanoparticle-enabled immunity test value based on repeated measures analysis of blood samples collected on day 0, 14, and 42. Values within a panel with unlike superscripts (a, b, and c) differ ($P \leq 0.05$) across sampling days.

relationship between TM and immune cell phenotypic markers of activation requires further work.

The nanoparticle-enabled immunity test was conducted as another measure to assess immune status in these steers throughout the study. This test uses AuNP as a pseudo-virus pathogen to probe the humoral immunity in blood plasma or serum samples. The AuNP are designed to react with type 1 and type 2 immunity-associated IgG subclasses. This rapid blood-based immune test has been suggested to detect antibody-mediated immune responses in cattle (Zheng et al., 2020). In the present study, we did not observe any treatment by day interactions or treatment effects; however, the nanoparticle-enabled immunity test

score did change over time and was intermediate on day 0, greatest at day 14, and lowest by day 42. This may indicate a shift in antibody-mediated immune responses or changes in components of the complement system over time during this study, but more work is needed to understand the biological relevance of the nanoparticle-enabled immunity test measures in receiving cattle.

Overall, in low stress cattle with adequate liver TM and plasma TM concentration, supplementation with organic TM tended to improve overall ADG and did improve overall G:F during the 42-d receiving period. Additionally, TM supplementation, regardless of source, influences immune cell phenotypic markers of activation that can be indicative of changes in immune cell function. This study provides justification to further investigate the relationship between TM supplementation and immune function in beef cattle. Further work examining phenotypic markers of activation paired with functional immune assays may be a logical next step to improve our understanding of the relationship between immune cell function and TM supplementation and status in freshly received cattle.

Acknowledgments

Conflict of Interest Statement

M.E.B. is an employee of Zinpro Corporation. The authors D.T.S., J.L.M., and S.L.H. declare no conflicts of interest.

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