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Evaluation of the stress-reducing effect of trace mineral injection in beef calves

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Abstract

Background: Little is known about the effects of trace mineral supplementation on the stress response in beef calves.

Objectives: To investigate the effect of injectable trace mineral supplementation (ITM) on the stress response in beef calves exposed to different types of stress.

Animals: Thirty weaned Angus and Angus crossbred calves.

Methods: The enrolled calves were randomly assigned to 2 groups: ITM, 15 calves received modified-live virus vaccine (MLV) and ITM SC and 15 calves received MLV and saline SC (CONT). The calves were exposed to 3 types of stress: the stress of MLV vaccination (d0), nasal aerosol with bovine viral diarrhea virus-2 (BVDV-2) challenge (d5), and liver biopsy (d26). The calves' body weights and health status were monitored. Leukocyte counts, serum cortisol concentration ([cort]), BVDV-2 serum neutralizing antibodies (SNA), and percentages of $CD4^+$, $CD8^+$, $WC1^+$, and $CD25^+$ T-lymphocytes were measured.

Results: Serum cortisol concentration ([cort]) showed strong associations with the percentage of $CD8^+$ ($r_s = .50$), BVDV2-SNA ($r_s = -.43$), and $WC1CD25^+$ ($r_s = .41$) cells, and rectal temperature ($r_s = .40$). The highest [cort] was reported 3 days after aerosol BVDV-2 challenge. Serum [cort] was decreased in ITM-treated calves 3 days post-BVDV-2 challenge, compared with CONT calves, with an average decrease of 18.5 ng/μL (95% confidence interval [CI], −6.07 to −31.3). The ITM-treated calves were heavier and healthier ($P < .01$) than the CONT calves.

Conclusions and Clinical Importance: Trace mineral supplementation appears to have stress mitigation effects in beef cattle that may reflect positively on growth and health performance. Viral exposure is associated with a high degree of stress, which is considered a major welfare concern.

KEYWORDS

beef calves, bovine viral diarrhea, cortisol, stress, trace minerals

Abbreviations: [cort], cortisol concentration; ACTH, adrenocorticotropin-releasing hormone; BVDV, bovine viral diarrhea virus; BW, body weight; Cu, copper; Fe, iron, Temp, body temperature; GRA, granulocytes; HPA, hypothalamic-pituitary-adrenal; HS, health score; ITM, injectable trace-minerals supplementation; LYM, lymphocytes; Mb, molybdenum; MLV, modified-live virus; Mn, manganese; MON, monocytes; ncp, noncytopathic; PLT, blood platelets count; SC, subcutaneously; Se, selenium; SNA, serum-neutralizing antibodies; WBC, white blood cell count; Zn, zinc.

1 | INTRODUCTION

Stress was defined in the nineteenth century as a perturbation of the body's homeostasis caused by real or perceived internal or external adverse effects, known as stressors. Stressors and their impact on the body vary markedly in their intensity.[1] In the current United States beef production systems, beef calves, from weaning to slaughter, are subjected to various levels of stress that impact their health and production.[2] Beef calves exposed to more stress have lower dry matter intake, lower average daily gains, and lower carcass quality.[3]

The primary response to stressful stimuli is activation of the hypothalamic-pituitary-adrenal (HPA) axis culminating in the secretion of glucocorticoids, particularly cortisol.[1] Cortisol serves numerous functions that orchestrate aspects of the physiological stress response, immunity, and metabolism in humans and animals.[4,5] Therefore, cortisol is the most commonly used biomarker for assessing the level of stress in the beef industry.[6] It is noteworthy that slight variation in cortisol exposure, particularly during the early stages of animal development, can have long-term effects on immunity and growth performance.[7,8] Additionally, long-term increases in cortisol concentrations may inhibit nonadaptive processes such as reproduction, rendering the animal infertile.[9]

Selenium supplementation has been shown to lower serum cortisol concentration ([cort]) by inhibiting the adrenal gland's capacity to generate corticosterone concentration in response to the anterior pituitary gland's release of adrenocorticotropic hormone (ACTH). Additionally, it can decrease the oxidative damage caused by the body's stress response.[10] In humans, zinc supplementation was found to have an inhibitory effect on cortisol secretion.[11] In cattle, chromium supplementation mitigates the stress response by decreasing [cort].[12,13] We therefore hypothesized that injectable trace minerals (ITM) could decrease the stress response in beef calves subjected to a variety of stressors. Our main objective was to investigate the stress mitigation effects of ITM in beef calves exposed to various types of stress.

2 | MATERIALS AND METHODS

All procedures were approved by the University of Georgia Institutional Animal Care and Use Committee (UGA-AUP# A2014 02-005-Y3 A8). The study reported here was part of a larger study investigating the effect of ITM on the immune response to modified live virus vaccine (MLV) in beef calves.[14]

2.1 | Calf husbandry

Thirty weaned Angus and Angus crossbred calves (7 months old) were enrolled in the study. Sample size was determined from the effect size and variation observed in another study.[14] A sample size of 12 calves in each group was sufficient to identify a serum [cort] difference of 15 ng/μL as significantly different with 95% confidence and 80% power. Considering attrition of 20% during the experiment, a final sample size of 15 calves per group was enrolled in the proposed study. The calves were purchased from a commercial ranch in Calhoun, Georgia, and transferred to the University of Georgia (UGA) Oconee Farm (Watkinsville, Georgia) 2 days before starting the study treatments. All calves were deemed healthy based on physical examinations. During the first 2 months of life, bull calves were dehorned and castrated. At the time of weaning, all calves were dewormed using a combination of injectable (doramectin; DECTOMAX; 1 mL/50 kg SC; Zoetis, Parsipanny, New Jersey) and white paste (fenbendazole; Safe-guard; Merck Animal Health, Rahway, New Jersey) dewormers. All calves were seronegative against bovine viral diarrhea virus (BVDV) 1 and 2 based on standard virus neutralization testing and immunohistochemistry.[14] The calves and their dams were not vaccinated with bovine respiratory disease (BRD) vaccines on the farm of origin and kept in an isolated pasture away from the main herd to maintain the calves' BVDV-naive status. Before being transferred to UGA Oconee farm, calves grazed on a pasture of ryegrass (*Lolium hybridum*) with free access to Bermuda grass hay (*Cynodon dactylon*) and water ad libitum. Five days after treatments were administered (d0) to the calves at the UGA Oconee farm, all calves were grouped together (d5) in a pasture with fescue grass (*Festuca arundinacea*) and free access to bermuda grass hay (*Cynodon dactylon*), and water ad libitum. Additionally, the enrolled calves were supplemented (2.5 kg per calf/day) with a commercial ration (Cattleman's Special Beef; Godfreys Warehouse; Madison, Georgia) offered in 2 meals per day. The commercial ration consisted of soybean hulls (45.03%), corn gluten (50.04%), molasses (2.25%), calcium (1.5%), salt (1.0%), trace minerals (0.1%), and vitamins A, D, and E (0.05%). Oral mineral supplementation was not provided during the study. The nutrient and mineral contents of pasture forage, hay, and feed concentrate were determined at the Diagnostic Center for Population and Animal Health at Michigan State University, Lansing, MI, on sampling days (Table 1).

2.2 | Calf vaccination and treatments (first type of stress)

Calves were randomly assigned to one of 2 treatments (d0): (a) calves were vaccinated with 2 mL of a 5-way modified live vaccine (MLV) containing bovine herpesvirus 1 (BHV1), bovine viral diarrhea virus 1 and 2 (BVDV1 and 2), bovine respiratory syncytial virus (BRSV), and parainfluenza 3 virus (PI3V; Express 5, Boehringer Ingelheim, Vetmedica, St. Joseph, Missouri) SC and received ITM (Multimin 90, Multimin USA Inc, Fort Collins, Colorado) at a dosage of 1 mL/45 Kg of body weight SC (ITM; n = 15, 8 steers and 7 heifers) and (b) calves were vaccinated and received an injection of sterile saline (Vetone Sterile Saline; Nova-Tech Inc., Grand Island, Nebraska) in the same manner as the first group (CONT; n = 15, 7 steers and 8 heifers).

2.3 | Bovine viral diarrhea virus intranasal challenge (second type of stress)

All enrolled calves were intranasally challenged with a noncytopathic (ncp) BVDV2 isolate (strain 890) 5 days (d5) after vaccination and

TABLE 1 Mineral composition and bromatological analysis of feedstuffs offered to calves during the study period.

Item	Grass	Hay	Concentrate
(% DM)			
CP	14.53	13.8	17.08
ADF	38.0	41.5	19.41
NDF adj	64.47	71.7	37.45
Ca	0.48	0.49	0.06
P	0.5	0.29	0.81
Mg	0.35	0.29	0.3
K	2.8	2.2	1.28
Na	0.01	0.03	0.18
mg/kg (DM basis)			
Fe	257	354.7	121.1
Zn	25.43	38.71	49.85
Cu	7.14	10.42	5.14
Mn	94.86	48.71	18.85
Se	0.05	0.06	0.16
Mb	3.6	0.97	1.68
Co	0.47	0.38	0.36
S	0.28	0.36	0.4

Note: Values are averages based on the dry matter basis (DM).
Abbreviations: ADF, acid detergent fiber; Ca, calcium; Co, cobalt; CP, crude protein; Cu, copper; DM, dry matter; Fe, iron; K, potassium; Mb, molybdenum; Mg, magnesium; Mn, manganese; Na, sodium; NDF adj, adjusted neutral detergent fiber; P, phosphorus; S, sulfur; Se, selenium; Zn, zinc.

treatment administration (d0). The details of the intranasal challenge of BVD were described previously.[14]

2.4 | Liver biopsy (third type of stress)

Liver biopsies were performed on day 26 (d26) relative to d0. Briefly, liver biopsy samples were collected using sterile surgical TRU-CUT semi-automatic biopsy needles (14 g and 4 inches long; Merit Medical Systems Inc., South Jordan, Utah) with the assistance of ultrasonography (Ibex, E.I. Imaging, Loveland, Colorado) for liver visualization after local anesthesia (lidocaine injectable; Aspen Veterinary Resources Ltd, Liberty, Missouri). Serum trace mineral concentrations and trace mineral content in liver samples were determined at the Diagnostic Center for Population and Animal Health at Michigan State University, Lansing, Michigan.

2.5 | Sampling collection and analysis

Blood samples were collected from the jugular vein using 18 gauge × 2.5-cm single sample needles (Vacuette; Nipro Medical Industries Ltd, Gunma, Japan) into vacuum tubes (Vacutainer, BD Diagnosis, Franklin Lakes, New Jersey) without anticoagulant and with EDTA anticoagulant on study days 0, 1, 5, 8, 10, 12, 14, 16, 19, 23, 26, and 33 relative to d0. Complete blood cell counts were performed using EDTA-containing tubes using an automatic cell counter (HESKA CBC-Diff, Vet Hematology System, Des Moines, Iowa) at the University of Georgia (UGA) Department of Veterinary Pathology in Athens, Georgia. The expression of T-cell phenotyping ($CD4^+$, $CD8^+$, $CD25^+$, and $WC1^+$) was measured by flow cytometry as described previously.[14] Calves were monitored for hydration, attitude, ocular health and nasal discharge, and fecal consistency using the University of Wisconsin's scoring system (http://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf) and rectal temperature was monitored by 3 experienced veterinarians on the same days of blood collection as described previously.[14] The daily sum health score was calculated.[14] Additionally, body weight (BW) was measured on days 0, 5, 12, 19, 26, and 33.

Serum samples were separated and stored at −80°C for further analysis. Serum [cort] was measured using a solid-phase competitive enzyme-amplified chemiluminescent immunoassay (IMMULITE 2000; Siemens Healthcare Diagnostics, Deerfield, Illinois) in the Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, Virginia. Serum neutralizing antibody (SNA) titers to BVDV2 and 1 were measured at the University of Georgia Athens Veterinary Diagnostic Laboratory (Athens, Georgia) using a standard virus neutralization protocol as previously described.[14]

2.6 | Statistical analysis

All continuous data were evaluated for normality and homogeneous variances. Logarithmic transformation was used for non-normally distributed data. Data are expressed as mean ± SD or mean and 95% confidence intervals (CI) and $P \leq .05$ was considered significant. The associations between serum [cort] and various measures were analyzed using Spearman's Rho correlation coefficient (r_s). To test the effects of treatments (3 levels) and time and the interaction between treatment and time on serum [cort], BW, and health score (HS), repeated-measures analysis of variance (ANOVA) was used using the MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, North Carolina). Calves were included in the model as a random effect whenever the between-subjects variation was determined to be significant. An autoregressive covariance structure was used based on the lowest value for Akaike's information criterion. Whenever the *F*-test was significant, Bonferroni-adjusted *P*-values were used to assess differences between 2 treatment groups at different times and between sampling times within the treatment group.

3 | RESULTS

Associations between serum [cort] and variables of interest are summarized in Table 2. Serum cortisol concentrations showed marked negative associations with BVDV-SNA ($r_s = -.43$; $P < .01$), and positive associations with body temperature ($r_s = 0.40$; $P < .001$), and percentage of $CD8^+$ ($r_s = .50$); $P < .001$), and $WC1^+$ and $CD25^+$ ($r_s = .41$; $P < .001$) T cells.

TABLE 2 Spearman correlation coefficients between serum cortisol concentration and variables of interest for 30 beef calves.

	SNA	Se	Cu	Zn	Mn	Mb	Fe	Temp	HS	BW	WBC	LYM	MON	GRA	PLT	CD4	CD8	WC1CD25
Cort	−0.43**	0.07	0.12*	−0.06	0.07	−0.19**	−0.09	0.40**	0.03	0.03	−0.19**	−0.23**	−0.20**	−0.04	−0.11*	−0.22*	0.50**	0.41**
SNA		−0.02	−0.03	0.20*	−0.01	0.32**	0.15	−0.35**	0.13	0.23	−0.07	0.13	−0.04	−0.16	0.19	0.12	−0.35**	0.10
Se			0.55**	0.11*	0.13*	−0.10	−0.17**	0.16**	−0.05	−0.31**	0.01	−0.15*	−0.04	0.10	0.30**	−0.04	0.03	−0.06
Cu				0.05	−0.10	−0.20**	−0.27**	0.18**	0.02	−0.27**	0.02	−0.20**	0.01	0.14	0.17**	0.33**	0.01	0.21*
Zn					0.14*	0.30**	0.37**	−0.16*	−0.03	0.17	−0.09	0.13*	−0.11	−0.17**	0.08	−0.02	0.11	0.03
Mn						−0.01	−0.06	0.05	−0.22**	0.30**	0.17**	0.01	0.00	0.18**	0.06	0.25**	−0.37**	−0.24**
Mb							0.21**	−0.14	0.04	−0.09	−0.03	0.24**	−0.07	−0.18**	−0.14*	0.26**	−0.30**	−0.10
Fe								−0.38**	0.06	0.30**	−0.10	0.15*	−0.12	−0.20**	0.13*	0.03	0.10	−0.13
Temp									−0.02	−0.31**	0.08	−0.18**	0.02	0.22**	−0.05	−0.20**	0.30**	0.07
HS										−0.13	−0.09	0.13	0.10	−0.20**	−0.02	0.17*	0.20**	0.10
BW											0.12	0.02	0.06	0.12	−0.09	−0.02	−0.28**	0.33**
WBC												0.41**	0.68**	0.76**	0.04	0.14*	0.11	0.09
LYM													0.41**	−0.27**	0.08	0.04	0.08	0.00
MON														0.35**	0.07	−0.02	0.15*	0.08
GRA															−0.01	0.17*	0.11	−0.07
PLT																0.14	0.08	−0.05
CD4																	0.36**	−0.12
CD8																		0.12

Abbreviations: Bw, body weight; $CD4^+$, percentage of $CD4^+$; CD8, percentage of $CD8^+$; cort, serum cortisol concentration; Cu, serum copper concentration; Fe, serum iron concentration; GRA, granulocytes count; HS, sum of health score; LYM, lymphocytes count; Mb, serum molybdenum concentration; Mn, serum manganese concentration; MON, monocytes count; PLT, blood platelets count; Se, serum selenium concentration; SNA, serum neutralizing antibodies to BVDV-1 and BVDV-2; Temp, body temperature; WBC, white blood cell count; WC1CD25, percentage of $WC1^+$ T-cell activated $CD25^+$; Zn, serum zinc concentration.

*$P \leq .05$; **$P \leq .01$.

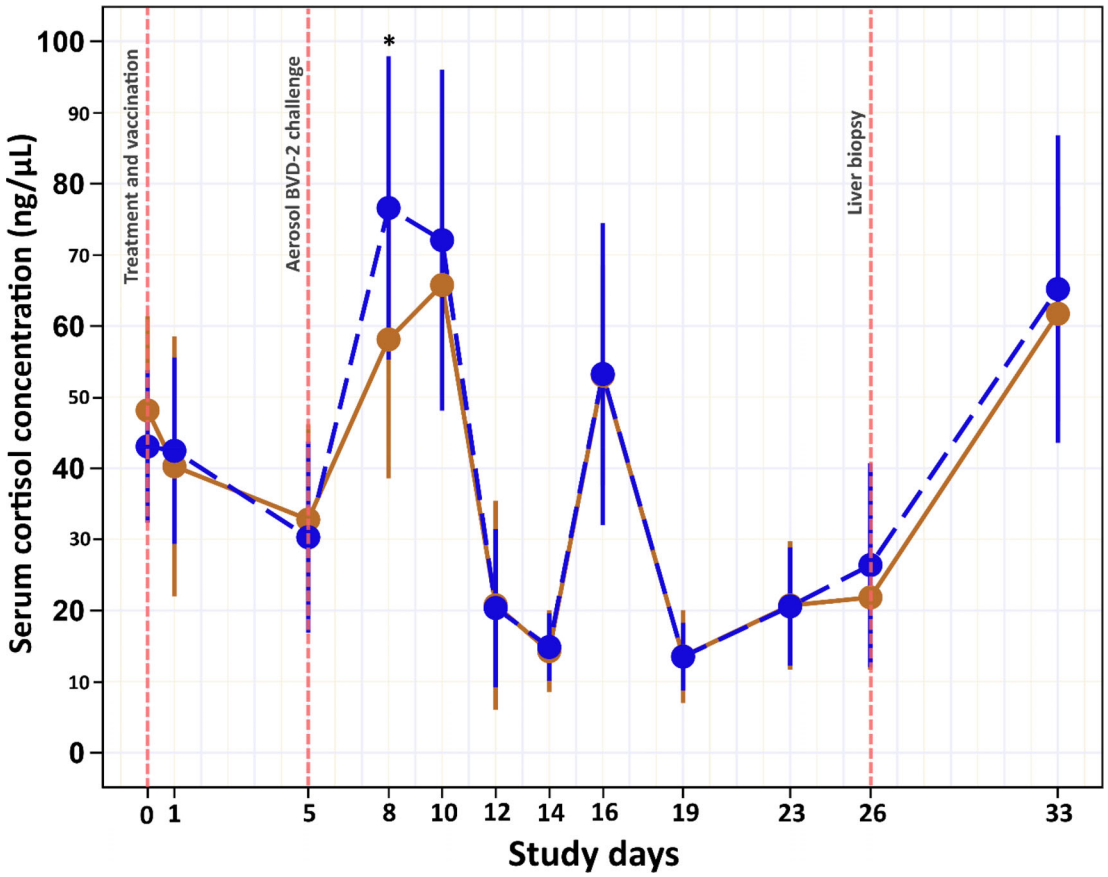

FIGURE 1 Mean ± SD of serum cortisol concentrations in vaccinated and injectable trace minerals-treated calves (ITM; solid-orange) and vaccinated and saline-treated calves (CONT; dashed-blue): treatment, days relative to the day of vaccination and treatment (d0), interaction treatment by day. Black asterisks indicate significantly different between treatment groups ($P \leq .05$).

Compared with CONT calves, serum [cort] was markedly ($P < .001$) decreased in ITM-treated calves with an average reduction of 18.5 ng/µL (95% CI, −6.07 to −31.3) 3 days post-BVDV-2 challenge (Figure 1). Our results showed that serum [cort] was markedly increased in a biphasic mode, 5 days (mean ± SD, 68.7 ± 17.5 ng/µL; $P < .001$) and 11 days (51.7 ± 20.0 ng/µL; $P < .001$) post-BVDV-2 challenge, compared with 33.7 ± 12.5 ng/µL on the day of the BVDV-2 challenge. Post-liver biopsy stress, serum [cort] was markedly increased, reaching a concentration of 61.5 ± 16.9 ng/µL 7 days post-challenge, compared with 25.7 ± 11.7 ng/µL on the day of liver biopsy challenge (Figure 1).

The overall average of BW was significantly higher in the ITM-treated calves (278.8 ± 20.9 kg) than in CONT calves (272.3 ± 16.8 kg; $P < .001$). The ITM-treated calves showed higher BW than CONT calves between d19 and d33 ($P < .01$; Figure 2). The distribution of health scores in both groups at different time points is shown in Figure 3. The ITM-treated calves had lower health scores than CONT calves from d10 to d19.

4 | DISCUSSION

Over the course of the past 2 decades, considerable attention has been provided to animal welfare and stress reduction during the production cycle of livestock. The main goal of our study, therefore, was to evaluate the stress-minimizing effect of ITM in post-weaning beef calves exposed to various types of stress. To the best of our knowledge, our is the first study to have investigated the effects of ITM on stress-related hypothalamic-pituitary-adrenal (HPA) axis responses in beef calves subjected to various types of stress. The first main finding of our study was that ITM seems to have stress mitigation effects in post-weaning beef calves, particularly with high levels of stress such as viral infections, and a positive impact on growth and health performance. The second finding was that peak serum [cort] was observed after exposure to the BVDV-2 challenge, indicating that viral infection may be a major cause of stress in beef calves. The third finding was that serum [cort] was associated with BVDV2 immunological response outcomes, suggesting that stress may modulate the immune response to vaccination or infection.

Activation of the HPA axis is the main physiological response to any external or internal stimuli that disturb the body's homeostatic balance.[1] Viral infection, in general, is associated with concomitant activation of the sympathetic nervous system and HPA axis culminating in the secretion of catecholamines and glucocorticoids, respectively. Both catecholamines and glucocorticoids are essential in orchestrating the so-called fight or flight response to assist the body

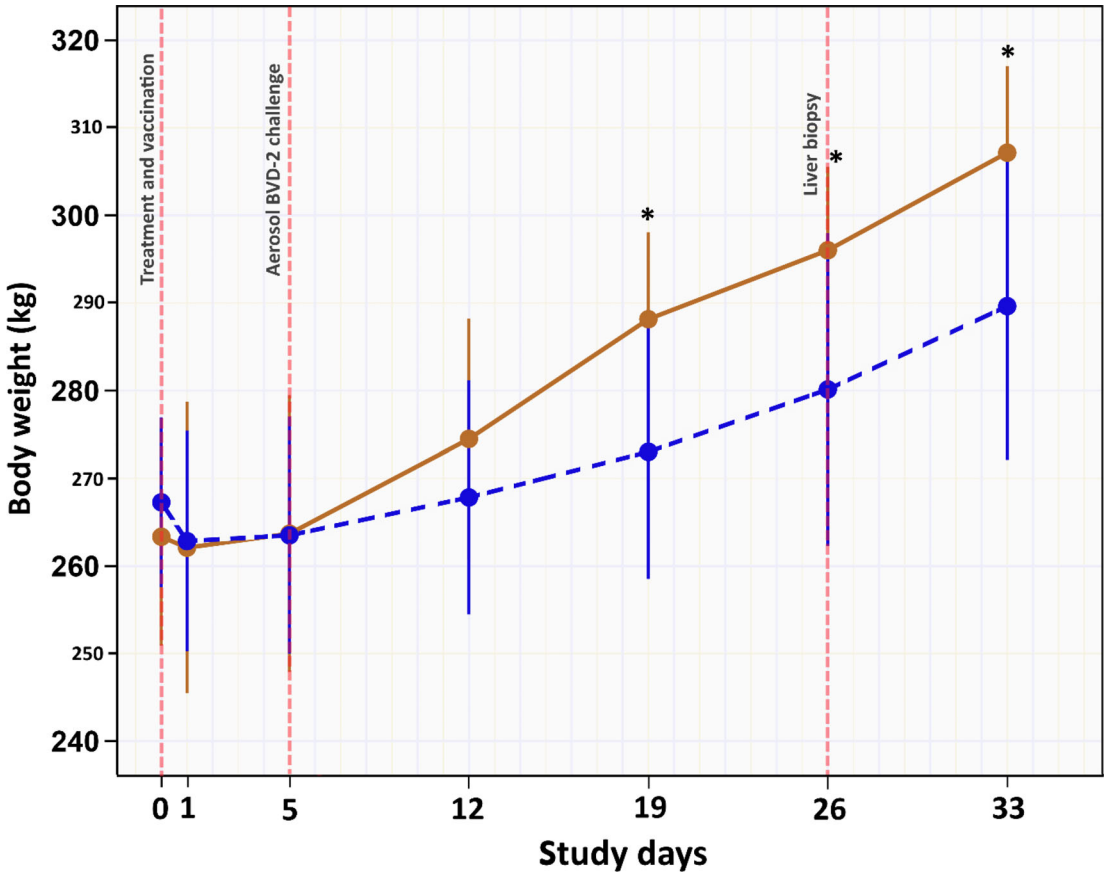

FIGURE 2 Mean ± SD of body weight of vaccinated and injectable trace minerals-treated calves (ITM; solid-orange) and vaccinated and saline-treated calves (CONT; dashed-blue): treatment, days relative to the day of vaccination and treatment (d0), interaction treatment by day. Black asterisks indicate significantly different between treatment groups ($P \leq .05$).

in coping with this challenge.[15,16] This mechanism is supported by the first wave of hypercortisolemia observed in our study post-BVDV-2 challenge. Additionally, viral infection is associated with changes in immune-endocrine cross-talk modulated by the HPA axis resulting in hypercortisolism and cortisol resistance.[17] Immune activation caused by viral infection results in the release of protein hormones called cytokines from various immune cell types. Cytokines have marked effects on the neuroendocrine system, particularly the HPA axis, resulting in the release of glucocorticoids. In turn, glucocorticoids, particularly cortisol, suppress the immune system from the further release of cytokines.[18] Therefore, HPA activation by cytokines plays an essential role in mediating the antiviral defense and shaping the immune response in order to protect the host from the detrimental effects of the aggressive immune response, such as tissue damage, autoimmune disease, septic shock, and others.[19] Also, this mechanism is supported by the second wave of hypercortisolemia reported here after the BVDV-2 challenge.

The post-liver biopsy hypercortisolemia reported in our study is consistent with earlier studies that also reported long-term cortisol secretion after invasive surgical procedures.[20-23] The mechanism of hypercortisolemia in response to surgical stress starts with signals that arise from afferent nerves at the surgical site, which stimulate the HPA axis and consequently stimulate cortisol secretion.[20] Recently, no marked difference in cortisol response was observed between moderately and highly invasive surgeries. Serum [cort] after moderately invasive surgeries, such as liver biopsy, should reach its peak 18 hours postsurgery and persist up to 1 week after surgery, which is consistent with our findings.[23] Interestingly, surgical stress can cause hypercortisolemia without a corresponding increase in plasma ACTH concentration. This might be because the marked increase in circulating cytokines postsurgery directly stimulates the release of glucocorticoids from the adrenal glands, providing an alternative pathway.[23]

The decrease in serum [cort] in calves that received ITM, particularly after BVDV-2 challenge is an important new finding. This unexpected finding may improve our understanding of the beneficial effects of ITM at various stages in the beef cattle production cycle. Because of a lack of research on the stress-reducing effect of trace mineral supplementation, it is difficult to determine the exact mechanisms for this effect. However, dietary zinc deficiency is associated with hypercortisolemia through direct activation of the HPA axis.[24] Zinc supplementation has an acute inhibitory effect on cortisol secretion.[11] Zinc directly suppresses the adrenal cortex, where adrenal hyperplasia and reduction in plasma 11-hydroxysteroids were observed in rats fed zinc-rich diets.[11] Not only zinc, but also selenium,

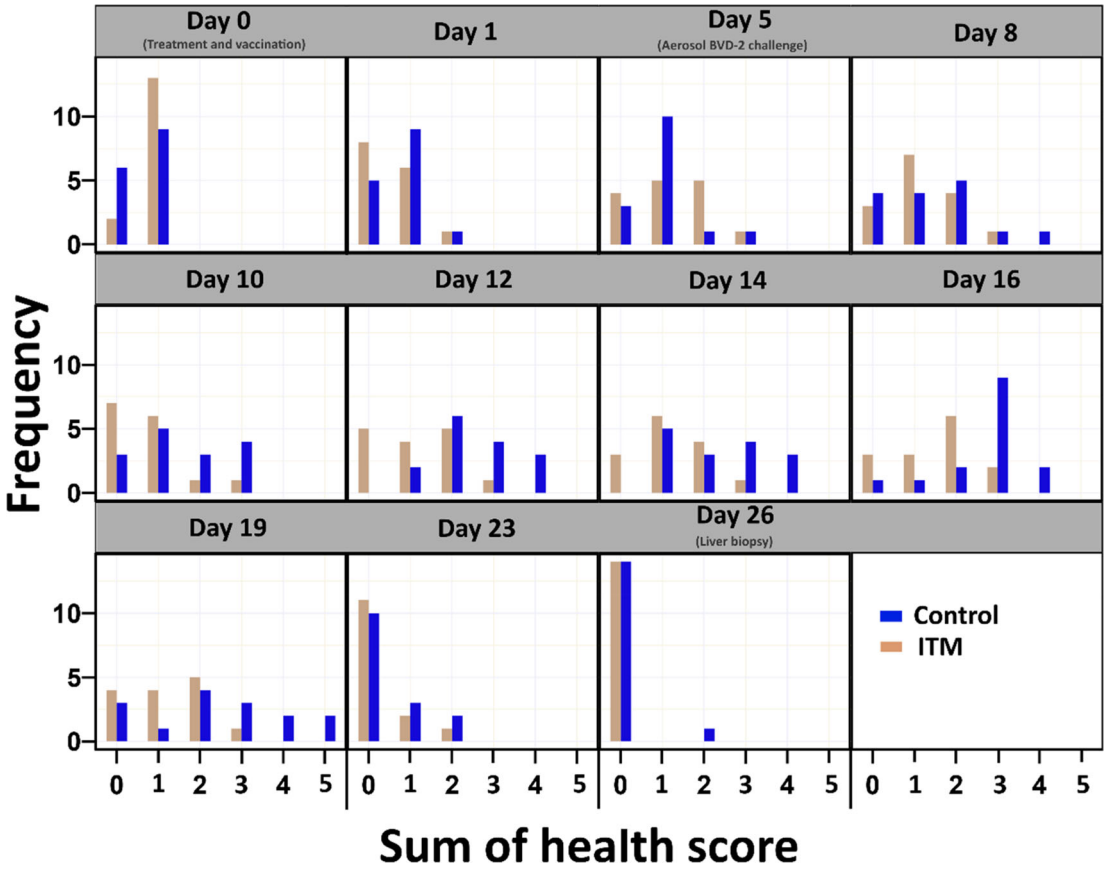

FIGURE 3 Histogram plots for health scores of vaccinated and injectable trace minerals-treated calves (ITM; orange) and vaccinated and saline-treated calves (CONT; blue).

has protective effects against stress. Recently, it has been found that dietary selenium supplementation normalizes the function of the HPA axis with potential for therapeutic benefit.[10] Thus, ITM thus may facilitate autonomic recovery from stress. Surprisingly, ITM-treated calves were heavier and healthier than CONT calves. This finding could be associated with the fact that these calves appear to have lower responses than CONT calves to stress, which normally has an adverse effect on growth and health performance. Additionally, trace mineral supplementation also helps to optimize the metabolism and immune system function of animals.[24,25]

Our study had some limitations. Data were only collected at a single follow-up time point after the liver biopsy because of funding limitations. Future research with additional follow-up time points is required to validate our findings. Because of the inherent variability in cortisol response, the sample size used in our study may have been inadequate. Additional studies with a large sample size are required to validate our findings. Additionally, our calves had a short acclimation period (2 days), and the calves may have been stressed from shipping and transitioning to new diets. Thus, studies with longer acclimation periods are required. Furthermore, the health scoring system used in our study is applicable to nursing dairy calf populations and not optimal for weaned beef cattle. Therefore, additional research would be useful to validate our findings using more appropriate scoring systems and standards by which respiratory health can be assessed in beef cattle, such as depression, appetite, respiration, and temperature (DART) or clinical illness scores (CIS) systems.

5 | CONCLUSIONS

In summary, we showed that ITM has promising stress-mitigation effects in beef cattle, which positively impact growth and health performance. However, more research is needed to validate our findings and better understand the mechanism of ITM's stress-reducing effects and their implications for beef cattle health and production. Viral infection challenges are associated with a high level of stress, which is a major welfare concern.

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CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

The authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the University of Georgia IACUC, number UGA-AUP# A2014 02-005-Y3 A8.

HUMAN ETHICS APPROVAL DECLARATION

The authors declare human ethics approval was not needed for this study.

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