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Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on immunity, health, and growth of dairy calves

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ABSTRACT

The objective of this study was to evaluate the effect of 2 subcutaneous injections of a multimineral preparation, each containing 60 mg of zinc, 10 mg of manganese, 5 mg of selenium, and 15 mg of copper at 3 and 30 d after birth on immunity, health, and growth of dairy calves during the preweaning period. The study was conducted in upstate New York in 2 commercial dairy farms. A total of 790 Holstein heifer calves were randomly allocated at birth into 1 of 2 treatments: trace mineral supplement (TMS) treated or control. Blood samples were collected at 3, 14, and 35 d after birth to evaluate glutathione peroxidase (GPx) activity, superoxide dismutase (SOD) activity, haptoglobin, and neutrophil and monocyte function. Incidence of diseases and average daily gain was evaluated in the first 50 d of life. At 14 d of life, TMS-treated calves had increased neutrophil activity compared with control calves. Moreover, TMS-treated calves had greater GPx activity on d 14 after birth than control calves. The TMS treatment reduced the incidence of diarrhea (TMS = 41.7% vs. control = 49.7%) and combined incidence of pneumonia or otitis or both (TMS = 41.7% vs. control = 49.1%). Additionally, GPx was greater for calves diagnosed with otitis at d 35 after birth. However, calves diagnosed with pneumonia had decreased GPx activity at d 35 after birth. Serum SOD and haptoglobin concentrations were not affected by treatment or disease. Moreover, no effects were observed on average daily gain and survivability between TMS-treated and control calves during the preweaning period. Supplementation with trace minerals at 3 and 30 d of life increased neutrophil function and GPx activity and reduced the incidence of health disorders.

Key words: trace mineral, dairy calf, neutrophil function, diarrhea

INTRODUCTION

Dairy replacement rearing success or failure is dependent on several complex and interrelated factors. Newborn calf health and growth can be impaired by poor maternal health (Lundborg et al., 2003), dystocia (Lombard et al., 2007), colostrum deprivation (Weaver et al., 2000), and poor calf nutrition (Ollivett et al., 2012). In the modern dairy industry, calves are reared artificially and early nutritional programs have been extensively studied to improve their performance during the preweaning period (Soberon et al., 2012). The physiological processes of a livestock animal, including the immune system, can be largely influenced by the availability of nutrient and trace minerals that are essential for multiple biochemical processes, including immune response, cell replication, and skeletal development, and are particularly relevant for the newborn (Carroll and Forsberg, 2007).

Studies that evaluate trace mineral depletion or supplementation focus on critical times in calves' lives and evaluate the effects of factors such as transportation (Crookshank et al., 1979), stress (Galyean et al., 1999), and diseases (Orr et al., 1990). For adult cattle, stress during the transition period can affect trace mineral status (zinc) and immune suppression can lead to greater susceptibility to diseases (Enjalbert et al., 2006). Likewise, stress can affect trace mineral status in dairy calves. Nockels et al. (1993) reported that calves under induced stress (intramuscular injections of ACTH) reduced their ability to retain trace minerals. An injectable trace mineral solution containing Zn, Cu, Mn, and Se was reported to increase liver concentrations of Cu and Se for at least a 15-d period, and increased plasma Zn and Mn for several hours in Angus and Simmental steers (Pogge et al., 2012).

Metabolic demands associated with stress and nutritional deficiency can lead to an increased production of reactive oxygen species (**ROS**; Sordillo and Aitken, 2009). When the production of ROS exceeds the antioxidant defense mechanisms present in the body, animals develop oxidative stress. Reactive oxygen species can initiate lipid peroxidation and cause cellular damage to tissues. Immune cells are particularly sensitive to oxidative stress because their membranes contain high

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concentrations of PUFA that are very susceptible to peroxidation, and produce large amounts of ROS when stimulated (Spears and Weiss, 2008). Several trace minerals are required for functioning of enzymes involved in the antioxidant defense system and may also affect immune cells via mechanisms distinct from antioxidant properties (Spears, 2000).

The role of early postnatal supplementation with injectable trace minerals on the immune response of newborn calves has not been investigated. Therefore, the objectives of this study were to evaluate the effect of supplementation of an injectable multimineral supplement containing Zn, Mn, Se, and Cu at 3 and 30 d after birth on peripheral blood neutrophil and lymphocyte function, oxidative stress markers, diseases (diarrhea, pneumonia, and otitis), and growth of Holstein heifer calves during the preweaning period.

MATERIALS AND METHODS

The study was conducted in 2 commercial dairy farms located near Ithaca, New York, from February to December, 2012. Farms were chosen because of their relationship with the Cornell University Ambulatory Clinic. Farm A was milking approximately 2,800 cows and farm B was milking approximately 1,600 cows.

Colostrum management was similar in both farms. Colostrum from primiparous and multiparous cows was pooled and refrigerated. Calves from farm A were fed approximately 4 L of raw colostrum within 4 h of birth at once by esophageal feeder (Oral Calf Feeder Bag with Probe; Jorvet; Jorgensen Laboratories, Loveland, CO). Calves from farm B were fed 2 L of raw colostrum within 2 h and another 2 L approximately 6 h later.

Both study farms had similar calf-rearing systems, which were individual pens in a greenhouse-type barn, positive ventilated, with individual pen dimensions of 1.5 m wide by 2 m long. The individual pens were isolated by plastic panels and bedded with a 0.5-m-deep gravel base that was covered with straw. Farm A and B had the same feeding system: calves were fed a total of 6 L of pasteurized (72°C for 15 s; T-600 and T-300; Goodnature Products Inc., Orchard Park, NY) nonsalable milk divided equally twice per day (0630 and 1700 h). Water and calf starter diets were offered ad libitum starting at d 3 of life to all calves in farms A and B. The starter composition for both farms is described in Table 1.

Study Design, Treatments, Blood Sampling, and Data Collection

A total of 790 calves were randomly allocated into the 2 treatments: trace mineral supplement (**TMS**)

Table 1. Nutrient composition of calf starter diets for farms A and B

	Starter diet			
Composition	Farm A	Farm B		
DM (%)	82.1	90.9		
CP (% of DM)	19.8	22.9		
NDF (% of DM)	29.4	31.8		
ADF (% of DM)	15.7	14.1		
TDN (% of DM)	72	77		
Ca (% of DM)	0.99	0.94		
P(% of DM)	0.64	0.78		
Mg (% of DM)	0.43	0.35		
K (% of DM)	1.40	1.01		
Na ($\%$ of DM)	0.35	0.39		
S (% of DM)	1.28	0.35		
Fe (mg/kg)	398	258		
Zn (mg/kg)	65	111		
Cu (mg/kg)	20	22		
Mo (mg/kg)	66	100		

treatment or control. Randomization was completed using the random number function in Excel software (Microsoft Corp., Redmond, WA). Calves allocated into the TMS treatment received two 1-mL subcutaneous injections (60 mg of Zn, 10 mg of Mn, 5 mg of Se, and 15 mg of Cu; Multimin North America Inc., Fort Collins, CO) at 3 and 30 d after birth. Calves allocated into the control group were left untreated. Body weight was measured weekly in both farms using a WayPig 15, 1.6-m (62-inch) digital scale (WayPig; Vittetoe Inc., Keota, IA) from birth until weaning, which occurred at 50 d of life.

Blood collection was performed via jugular venipuncture using an 18-gauge by 3.8-cm needle in 2 individual vacuum tubes: a 10-mL vacuum tube (Becton, Dickinson and Co., Franklin Lakes, NJ) without anticoagulant for serum and an 8-mL heparinized vacuum tube (Becton, Dickinson and Co.) for plasma. Serum was harvested following centrifugation at 2,000 $\times g$ for 15 min at 4°C and plasma was harvested after centrifugation at 1,000 $\times g$ for 10 min at 4°C. Serum and plasma samples were stored at -80° C. Blood was sampled on d 3 (immediately before treatment administration), 14, and 35 after birth to evaluate glutathione peroxidase (**GPx**), superoxide dismutase (**SOD**), and haptoglobin (**Hp**). Additionally, serum IgG concentration was measured only on d 3.

A random subset of serum samples from 10 calves (5 for treatment) were sent to Veterinary Diagnostic Laboratory, Iowa State University (Ames) for analysis of Ca, Cu, Fe, K, Mg, Mn, Mo P, Se, and Zn. Blood mineral levels were measured at 3, 14, and 35 d after birth. The concentrations of serum trace elements were analyzed by an inductively coupled plasma mass spectrometry (ICP-MS) system (Varian/Bruker 820 ICP-MS; Bruker Corp., Fremont, CA) through separation of analyte ions from spectral interferences. Serum

mineral concentrations were reported in milligrams per kilogram.

Serum IgG was measured using a radial immunodiffusion assay according to kit instructions (Bethyl Laboratories Inc., Montgomery, TX). Intraassay and interassay coefficients of variation for IgG were 3.2 and 3.6%, respectively. An SOD assay kit (Cayman Chemical Co., Ann Arbor, MI) was used, following the manufacturer's instructions for assaying SOD activity in plasma. The intraassay coefficient of variation for SOD was 3.7%. Likewise, a Cayman GPx assay kit was used, following the manufacturer's instructions to evaluate GPx activity. The intraassay coefficient of variation for GPx was 7.2%. Serum Hp was evaluated following the method described by Makimura and Suzuki (1982). The intraassay coefficient of variation for Hp was 9.8%.

Additionally, a random subset of 228 calves (112 control and 126 TMS-treated calves) from one of the farms was sampled to evaluate neutrophil and monocyte function at d 14 after birth. Neutrophil and monocyte function was measured by the neutrophil and monocyte phagocytic activity using a PHAGOTEST kit (Orpegen Pharma GmbH, Heidelberg, Germany) containing fluorescein-labeled opsonized Escherichia coli (E. coli-FITC), following the manufacturer's instructions. Cells were analyzed with a FACSCalibur flow cytometer (Becton, Dickinson and Co.) using a 488-nm argon-ion laser. Ten thousands events were collected for each cell population (neutrophils or monocytes). The results are reported as the percentage of cells performing phagocytosis from the total number of cells in the granulocyte gate and as the increase mean of the green fluorescence of the gated cells.

Case Definitions

All diseases were diagnosed by the farm personnel according to guidelines and training provided by the Ambulatory Production Medicine Service from Cornell University. Pneumonia was determined by the identification of 2 or more of the following clinical signs: cough, rectal temperature $>39.5^{\circ}$ C, respiratory rate >40 breaths/min, and increased cranioventral lung sounds or wheezes. Otitis was defined by observation of ear pain, evidenced by head shaking, scratching or rubbing the ears, epiphora, ear droop, and signs of facial nerve paralysis, combined with fever ($>39.5^{\circ}$ C). Calves were recorded as diarrheic when diarrhea (watery feces) was observed at least during 3 consecutive days.

Statistical Analyses

Descriptive statistics were performed using PROC FREQ, MEANS, and UNIVARIATE of SAS (SAS In-

stitute Inc., Cary, NC). The initial BW, IgG concentrations at d 3 after birth, percentage of calves born from primiparous cows, and assisted parturition percentage were evaluated to ensure similar distribution between TMS-treated and control calves within each farm and rearing systems.

For all health events [pneumonia; otitis; diarrhea; combined incidence of pneumonia, otitis, or both (CPO); and mortality] calves were right censored at the end of the study period (50 d of life). To evaluate the effect of treatment on pneumonia, otitis, diarrhea, CPO, and mortality, 5 multivariable mixed logistic regression models were fitted with a binary distribution and the logit link to the data using PROC GLIMMIX of SAS. An autoregressive covariance structure was used for the multivariable models. The variables treatment group, serum IgG concentration (3 d of life), and birth weight were included in all models. Additionally, the variable farm was included in all models as a random effect. Adjusted proportions of pneumonia, otitis, diarrhea, CPO, and mortality were obtained using the LSMEANS statement.

The effect of treatment on ADG and neutrophil and monocyte function was evaluated with linear mixed models using PROC MIXED of SAS. Average daily gain was calculated by subtracting the BW at weaning (50 d of life) by the birth weight, and subsequently dividing by 50. Farm effect was evaluated in the models and because no effects of farm were present, it was used as a random effect. Birth weight and serum IgG concentration at 3 d of life were used as independent variables. Least squares means and respective 95% confidence intervals were estimated for all categorical main effects.

Linear mixed models were fitted to the data using PROC MIXED of SAS to analyze the effect of treatment, pneumonia, otitis, and mortality from the repeated measures (3, 14, and 35 d of life) on blood minerals, GPx, SOD, and Hp. To control for repeated measures, the animal identification number (nested within farm) was included in all models as a random effect and serum IgG concentration (3 d of life) and birth weight were included as covariates. Separate models were used for treatment, pneumonia, otitis, and mortality to avoid multicollinearity. The interaction of each categorical variable and sampling time were offered to all models.

For all linear mixed models, the assumption that the residuals were normally distributed was assessed by visually evaluating the distribution plot of the studentized residuals. Statistical significance was declared at $P \leq 0.05$ and statistical tendencies were declared at $0.05 < P \leq 0.10$.

Table 2	2. Percentage of	calves born fr	rom primiparous	cows, assisted g	parturition per	centage, in	nitial BW (k	g), and calf	serum IgG o	concentrations
at d 3 a	fter birth (g/L)	, evaluated be	etween trace mir	ieral supplement	t (TMS)-treate	ed^1 and con	ntrol calves	within the fa	arm rearing	$systems^2$

	Fa	arm A treatment		Farm B treatment		
Item	Control	TMS	<i>P</i> -value	Control	TMS	<i>P</i> -value
Number of calves	330	310		70	80	
Calves born to primiparous cows (%)	34	33	0.92	43	45	0.79
Assisted parturition (%)	4	5	0.43	16	18	0.78
Initial $B\hat{W}^3$	38.1(0.3)	38.3(0.3)	0.65	41.3(0.6)	41.1(0.6)	0.79
Serum IgG^4	23.2(0.9)	23.8~(0.9)	0.59	26.7(1.8)	25.7(1.6)	0.69

¹The TMS-treated calves were treated at 3 and 30 d of age.

²The LSM and the respective SEM (in parentheses) are presented for initial BW and serum IgG concentration.

³Calf initial BW (kg) at 3 d of life.

⁴Calf serum IgG concentration (g/L) measured at 3 d of life, before the first treatment.

RESULTS

Descriptive Statistics

Average BW (kg) at enrollment, serum IgG concentration at 3 d of life, percentage of calves born to primiparous cows, percentage of assisted parturitions, and number of calves enrolled in each calf farm by rearing system were not different for calves on TMS and control treatments (Table 2).

Blood Minerals, Disease Incidence and ADG

Serum mineral concentrations analyzed revealed that Ca, Cu, Fe, K, Mg, Mn, Mo, P, Se, and Zn concentrations were not different for TMS-treated and control calves at d 3 after birth (baseline), right before the first TMS treatment was conducted (Figure 1). On d 14 after birth, TMS-treated calves had increased concentrations of Se (P < 0.01) and Zn (P < 0.01) compared with counterpart control calves (Figure 1). At d 14 after birth, serum concentrations of Ca, Cu, Fe, K, Mg, Mn, Mo, and P did not differ between TMS-treated and control calves (Figure 1). Moreover, no difference was detected between TMS-treated and control calves for any of the minerals evaluated at d 35 after birth (Figure 1).

The effect of treatment on adjusted incidences of mortality, diarrhea, otitis, pneumonia, and CPO is presented on Table 2. Briefly, TMS-treated calves had lower (41.7%) incidence of diarrhea compared with control group calves (49.1%; P = 0.034; Table 2). Additionally, control calves were at a 1.35 (range of 1.00–1.83) higher odds of CPO compared with calves in the TMS-treated group (P = 0.05). However, ADG was not significantly different between treatment groups (P = 0.49). The ADG for TMS-treated calves was 778 g/d (95% CI = 729–826) and 789 g/d (95% CI = 740–837) for calves in the control group. When pneumonia and otitis were analyzed separately, trace mineral treatment

numerically decreased the incidence of pneumonia and otitis but these results were not statistically significant (Table 3).

Blood Neutrophil and Monocyte Function

Peripheral blood neutrophil function was greater for TMS-treated calves than for control calves at 14 d after birth; however, no difference existed in monocyte function (Figure 2). Briefly, TMS-treated calves had increased neutrophils activity, showing an increased mean fluorescence [i.e., increased number of ingested bacteria by neutrophils (P = 0.025) and percentage of neutrophils that performed phagocytosis (P = 0.079) compared with control calves]. Additionally, blood neutrophil and monocyte function was not different for calves that were recorded with pneumonia, otitis, or diarrhea, and calves that died before the end of the study period compared with healthy calves that survived during the study period (Figure 3).

Oxidative Stress and Acute-Phase Protein Markers

Calves supplemented with trace minerals had increased (P = 0.03) GPx activity compared with control calves at d 14 after birth (Figure 4). Calves with pneumonia had increased GPx activity (P < 0.05) at d 3 after birth and reduced GPx activity (P < 0.05) on d 35 after birth compared with healthy counterparts (Figure 4). Moreover, calves with otitis had greater GPx activity (P < 0.05) on d 35 after birth than calves without otitis (Figure 4). Calves that died had increased GPx activity (P < 0.05) on d 3 after birth compared with calves that survived during the study (Figure 4). No effects of treatment on serum SOD activity and Hp optical density were detected at d 3, 14, and 35 after birth (Figures 5 and 6). Moreover, no effects of disease on calves' serum SOD activity and haptoglobin optic density were detected (Figures 5 and 6).

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- Control

- TMS



Figure 1. Effect of trace mineral supplement (TMS; 3 and 30 d of life) injections on serum concentrations at d 3, 14, and 35 after birth. The black lines represent calves in the control group and the light-gray lines represent calves in TMS group. Trace mineral-supplemented calves had greater serum concentrations of Se and Zn on d 14 after birth. Error bars represent SEM; * indicates P < 0.5.

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Health event	Adjusted i	ncidence (%)			
	TMS^2	Control	$\begin{array}{c} \text{Adjusted odds ratio} \\ (95\% \text{ CI}) \end{array}$	<i>P</i> -value	
Mortality	3.8	2.7	0.70(0.30 - 1.58)	0.40	
Diarrhea ³	41.7	49.7	1.38(1.02 - 1.86)	0.03	
Otitis	10.6	13.2	1.26(0.82 - 2.01)	0.31	
Pneumonia	35.2	40.0	1.23(0.91 - 1.65)	0.18	
CPO^4	41.6	49.1	1.35(1.00 - 1.83)	0.05	

Table 3. Effect of treatment on health events, evaluated using a mixed logistic regression model¹

¹Adjusted odds ratio and adjusted probabilities of pneumonia; otitis; diarrhea; combined incidence of pneumonia, otitis, or both (CPO); and mortality were obtained using the LSMEANS statement in SAS (SAS Institute Inc., Cary, NC). Adjusted odds ratios are presented with the respective 95% CI.

²Trace mineral supplement (TMS)-treated calves were treated at 3 and 30 d of age.

³Diarrhea = calves were affected with diarrhea for at least 3 d.

 ${}^{4}\text{CPO}$ = this health event is characterized by calves that were affected with otitis or pneumonia, or both.

DISCUSSION

Supplementation with injectable trace minerals at early postnatal life was beneficial to calf immunity and oxidative stress status. Calves supplemented with trace minerals had improved neutrophil function, increasing the ability to perform phagocytosis, and improved glutathione peroxidase activity. Ultimately, supplemented calves had lower incidence of diarrhea and a lower incidence of combined diseases (including calves diagnosed with pneumonia, otitis, or both).



Figure 2. Effect of trace mineral supplement (TMS; 3 and 30 d of life) injections on the mean fluorescence intensity (left y-axis; reflecting ingested bacteria) and percentage of cells [neutrophils (NEU) and monocyte (MON)] that performed phagocytosis (right y-axis). The dark-gray bars represent calves in the control group and the light-gray bars represent calves in the TMS group. Trace mineral-supplemented calves had greater overall neutrophil mean compared with control calves. Error bars represent SEM; * indicates $0.10 > P \ge 0.05$; ** indicates P < 0.5.

Trace minerals have an essential role in ensuring efficient immunocompetence in animals. Enjalbert et al., (2006) reported that marginal and deficient levels of trace minerals can increase risk of disease in adult cattle (metritis, mastitis, and lameness) and calves. A recent study reported a lower incidence of mastitis, decreased SCC, and lower incidence of endometritis for multiparous cows supplemented with an injectable trace mineral solution containing Zn, Cu, Mn, and Se (Machado et al., 2013). Additionally, supplementation of trace minerals for calves have been extensively study in calves and its response to transportation (Spears, 1996), vaccination (Mandal et al., 2007), and white muscle disease (Schubert et al., 1961).

Trace minerals such as Se, Zn, and Cu are required for functioning of enzymes involved in the antioxidant defense system and may also affect immune cells through mechanisms distinct from antioxidant properties (Rotruck et al., 1973). Selenium is an essential component of GPx enzymes destroying hydrogen peroxide and lipid hydroperoxides (Rotruck et al., 1973). Copper and Zn form the Cu-Zn superoxide SOD, which is responsible for dismutation of superoxide radicals to hydrogen peroxide in the cytosol (Halliwell, 1999). Zinc also induces synthesis of a metal-binding protein that may scavenge hydroxide radicals called metallothionein (Prasad, 2004). In addition to an antioxidant role, Zn may affect immunity via its important role in cell replication and proliferation (Spears and Weiss, 2008). The increased activity of the GPx enzyme suggests that the trace mineral supplementation can effectively reduce oxidative stress in calves in the first 2 wk of life. Considering the particular immune cell sensitivity to oxidative stress (Mittler, 2002), it is possible that the improved phagocytosis ability of neutrophils in the current study was a function of the enhanced antioxidant status provided by the TMS.

Phagocytosis is one of the most important functions of the neutrophils, playing a critical role in their ability 4222

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Figure 3. Mean fluorescence intensity (left y-axis; reflecting ingested bacteria) and percentage of performed phagocytosis (right y-axis) of neutrophils (NEU) and monocytes (MON) assessed on blood samples collected at 14 d of life. The dark-gray bars represent calves that were affected with otitis, pneumonia, diarrhea, and calves that died and light-gray bars represent calves that were not affected with disease and survived until the end of the study (50 d). Error bars represent SEM.

to recognize and eliminate foreign and infected cells. The effect of Se supplementation was already described to improve phagocytosis in white blood cell populations (Hogan et al., 1990). Moreover, Suwanpanya et al. (2007) reported an increase in the neutrophil phagocytic activity of *Staphylococcus aureus* in heifers dietary supplemented with 3 mg of Se and 4,000 IU of vitamin E per day. In the current study, 14 d-old-calf peripheral blood neutrophils were tested against opsonized *E. coli*

and the results indicated significant improvement in the phagocytic activity for calves supplemented with trace minerals including Se at 3 d of life compared with nonsupplemented calves. Although trace mineral supplementation significantly increased GPx activity at 14 d of age in this study, no effect of treatment was observed at 35 d of life. Interestingly, the current study reported decreased GPx activity for calves diagnosed with pneumonia and increased activity for calves diagnosed with EFFECT OF A TRACE MINERAL ON IMMUNITY, HEALTH, AND GROWTH OF CALVES



Figure 4. Plasma glutathione peroxidase (GPx) activity (nmol/min per milliliter) was assessed on d 3, 14, and 35 of the calves' lives. The light-gray lines represent the effects of trace mineral supplement (TMS)-treated calves (vs. control calves), calves with pneumonia, calves with otitis, and calves that died on the repeated measurements of GPx. The black lines represent calves that survived until the end of the study period (50 d). Error bars represent SEM; * indicates P < 0.05.

otitis. Cemek et al. (2006) reported similar results in an investigation of antioxidant status in children with acute pneumonia, where GPx activity was reduced in affected children compared with nonaffected children. Additionally, another study using human infants found a decreased level of plasma GPx activity in 57 patients with pneumonia compared with 87 controls (Cui et al., 1997).

Bonham et al. (2002) suggested that neutrophil function might be a valuable biomarker for Cu status. In the current study, supplemental trace minerals did not influence SOD, the antioxidant enzyme that uses Cu and Zn to be synthesized. Likewise, Hp, a marker of acute-phase proteins, was not affected by treatment. Acute-phase proteins are well described as quantifiable indicators of inflammation of infection in adult cattle. However, some factors exist that can significantly change acute-phase protein concentrations in neonatal calves, such as stress of birth, microbial challenge, and intake of colostral cytokines.

The beneficial effects of trace mineral supplementation on immune function were likely associated with the lower incidence of diarrhea and CPO. Oral Zn supplementation was reported to reduce the incidence of diarrhea among children in developing countries (Fuchs, 1998; Hambidge, 2000). Furthermore, the effectiveness of Zn to reduce diarrhea in children was related to improvement in the enzymatic functions of the brush borders, and enhancement of the intestinal mucosa repair (Sazawal et al., 1995: Strand et al., 2002). Moreover, Berger et al. (2006) reported a significant reduction of nosocomial pneumonia by intravenous multi-trace element supplements (Cu, Se, and Zn) in severely burned patients. Selenium is one of the trace minerals frequent-



Figure 5. Serum superoxide dismutase (SOD) activity (U/mL) was assessed on d 3, 14, and 35 of the calves' lives. The light-gray lines represent the effects of trace mineral supplement (TMS) treatment, pneumonia, otitis, and calves that died on the repeated measurements of SOD. The black lines represent calves that were healthy and survived until the end of the study period (50 d). No effect was observed on SOD activity for TMS-treated calves (vs. control calves), pneumonic calves (vs. healthy), calves diagnosed with otitis (vs. healthy), and calves that survived until the end of the study period (vs. calves that died). Error bars represent SEM.

ly associated with improved immunity and reduction of diseases (Rayman, 2000). Selenoenzymes are ubiquitous in mammalian cells, having 2 major roles. First, the selenoenzymes have antioxidant properties that protect cell components such as membranes, enzymes, proteins, and DNA. Second, selenoenzymes inhibit proinflammatory cell metabolisms by reducing the peroxide tone of intracellular water (Rayman, 2000).

The benefits of trace mineral supplementation herein reported did not translate into improved ADG. Clearly, within a calf-rearing program, a multitude of factors can play a role in the growth performance of calves. Although neutrophil function and oxidative stress status were improved in TMS-treated calves during the first 2 wk of life and the incidences of diarrhea and CPO were reduced, the absence of effects on ADG and survivability for TMS-treated calves warrants further investigation.

CONCLUSIONS

Trace minerals supplementation improved neutrophil function and GPx activity of dairy calves in the first 2 wk after birth. Moreover, TMS-treated calves had reduced incidence of diarrhea and CPO. However, the benefits for immunity-related parameters and health did not translate into improved growth performance and reduced mortality in the first 50 d of life. Future research should focus on the investigation of the length and magnitude of trace mineral supplementation needed to sustain an appropriate immunological status in the preweaning period to maximize health benefits and growth performance of dairy calves.



Figure 6. Serum haptoglobin (Hp) optical density (OD) was evaluated on d 3, 14, and 35 of calves' lives. The light-gray lines represent the effect of trace mineral supplement (TMS) treatment, pneumonia, otitis, and calves that died on the repeated measurements of superoxide dismutase. The black lines represent calves that were healthy and survived until the end of the study period (50 d). No effect was observed on Hp OD for TMS-treated calves (vs. control calves), pneumonic calves (vs. healthy), calves diagnosed with otitis (vs. healthy), and calves that survived until the end of the study period (vs. calves that died). Error bars represent SEM.

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