A randomized trial to evaluate the effect of a trace mineral premix on the incidence of active digital dermatitis lesions in cattle

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ABSTRACT

A balanced, parallel-group, single-blinded randomized efficacy study divided into 2 periods was conducted to evaluate the effect of a premix containing higher than typically recommended levels of organic trace minerals and iodine (HOTMI) in reducing the incidence of active digital dermatitis (DD) lesions acquired naturally and induced by an experimental infection challenge model. For the natural exposure phase of the study, 120 healthy Holstein steers 5 to 7 mo of age without signs of hoof disease were randomized into 2 groups of 60 animals. The control group was fed a standard trace mineral supplement and the treatment group was fed the HOTMI premix, both for a period of 60 d. On d 60, 15 steers free of macroscopic DD lesions were randomly selected from each group for the challenge phase and transported to an experimental facility, where they were acclimated and then challenged within a DD infection model. The same diet group allocation was maintained during the 60 d of the challenge phase. The primary outcome measured was the development of an active DD lesion greater than 20 mm in diameter across its largest dimension. No lesions were identified during the natural exposure phase. During the challenge phase, 55% (11/20) and 30% (6/20) of feet were diagnosed with an active DD lesion in the control and treatment groups, respectively. Diagnosis of DD was confirmed by histopathologic demonstration of invasive *Treponema* spp. within eroded and hyperplastic epidermis and ulcerated papillary dermis. All DD confirmed lesions had dark-field microscopic features compatible with DD and were positive for *Treponema* spp. by PCR. As a secondary outcome, the average DD lesion size observed in all feet was also evaluated. Overall mean (standard deviation) lesion size was 17.1 (2.36) mm and 11.1 (3.33) mm for the control and treatment groups,

respectively, with this difference being driven by acute DD lesions >20 mm. A trend existed for the HOTMI premix to reduce the total DD infection rate and the average size of the experimentally induced lesions. Further research is needed to validate the effect of this intervention strategy in the field and to generate prevention and control measures aimed at optimizing claw health based on nutritional programs.

Key words: digital dermatitis, experimental challenge, organic trace minerals

INTRODUCTION

Bovine digital dermatitis (DD), also called papillomatous digital dermatitis (PDD) or foot warts, is a contagious disease of the feet of cattle that responds to antibiotics, found worldwide (Blowey and Sharp, 1988; Read and Walker, 1998a). The precise etiology of DD is undefined. However, compelling evidence exists that several phylotypes of *Treponema* spp. play an important role in its pathogenesis (Read et al., 1998; Gomez et al., 2012). Prolonged exposure to slurry in the environment and poor foot hygiene predispose cattle to development of DD (Rodriguez-Lainz et al., 1999; Somers et al., 2005). The disease has a high incidence in countries with intensive cattle husbandry (Holzhauer et al., 2006; USDA, 2009) and DD-associated lameness results in significant economic and welfare problems, particularly in the dairy industry (Bruijnjs et al., 2010; Cha et al., 2010; Ettema et al., 2010). Numerous efforts have been made to prevent and control DD, currently including strategies based on frequent use of disinfecting footbaths at the herd level (Jorritsma et al., 2007; Holzhauer et al., 2008) and topical treatments of acute DD lesions for individual animals (Dreher et al., 1999; Berry et al., 2010; Loureiro et al., 2010). However, these strategies have obtained variable success (Relun et al., 2013). In a cross-sectional study including 22,454 adult cows in 383 herds, single-herd prevalence was reported as high as 83%, with an average of 21.2% of the cows

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affected (Holzhauer et al., 2006). Furthermore, the traditional approach to help prevent DD infection has overlooked animals receiving less attention with regard to lameness, such as beef or replacement dairy heifers. However, evidence exists to suggest that replacement dairy cattle should be a primary focus for DD management in dairy herds. Laven and Logue (2007) showed that heifers infected with DD before calving were more susceptible to DD infection during lactation. Similarly, Holzhauer et al. (2012) reported an increased average risk (odds ratio) of 5.97 of recurrent or persistent lesions during the first lactation in animals previously diagnosed with DD during the rearing period and Capion et al. (2009) reported a 15% DD prevalence in heifers before parturition. One of the limitations to control DD in young animals is the lack of adequate facilities to implement footbaths and treat individual cases in addition to a significant increase in labor. Therefore, the development of management strategies aimed at optimizing the immune response to DD and increasing skin quality of the foot are attractive components of an integrated approach to DD control.

Thus, one approach to enhance disease resistance of cattle in general is to provide an adequate supply of available trace minerals, such as Zn, Mn, Cu, or I. Zinc is of importance in maintaining the health and integrity of skin due to its role in cellular repair and replacement, and has a critical role in wound healing (Moynahan, 1981; Miller et al., 1988). Zinc and Mn also help regulate a wide variety of immune system components, including those of T lymphocytes, natural killer cells, IL-2, IL-6, and tumor necrosis factor α (Wirth et al., 1984; Pinna et al., 2002; Ibs and Rink, 2003). Both cell-mediated and humoral immunity are greatly depressed by Cu deficiency (Prohaska and Failla, 1993). In addition, low Cu status in steers was associated with a reduced response of peripheral-blood lymphocytes to stimulation with T-cell mitogens following weaning and IBRV challenge (Gengelbach et al., 1997; Wright et al., 2000), and I affects the local inflammatory response and the ability of macrophages to form the cytotoxic compounds that kill phagocytized pathogens (Klebanoff and Clark, 1978; Siddiqui et al., 1993). Furthermore, the trace mineral uptake depends on the form trace minerals are offered in the diet and, therefore, conditioning the level of trace mineral requirements achieved (Wedekind et al., 1992).

An important gap exists in the research of alternative strategies to manage young stock in relation to DD. Aiming at preventing DD in heifers, the use of elevated levels of organic trace minerals (OTM) and iodine might be an easily implemented management strategy that might increase the success of systematic prevention and control of DD at the herd level.

In the current study, the objective was to evaluate the efficacy of a trace mineral premix containing higher than typically recommended levels of OTM and iodine (**HOTMI**) in reducing the incidence of naturally and experimentally induced DD lesions in a randomized controlled trial in Holstein steers.

MATERIALS AND METHODS

The study was approved before commencement by the School of Veterinary Medicine Animal Care and Use Committee under Protocol No. V-1429 and issued by the Research Animal Resources Center of the University of Wisconsin-Madison.

Trial Design

We conducted a balanced, parallel-group, single-blinded randomized efficacy study divided into 2 periods (natural exposure phase and challenge phase). The study was conducted between January and April 2010. The natural exposure phase corresponded to the first 60 d after enrollment and aimed to evaluate the efficacy of the HOTMI premix in preventing natural DD infection. The challenge phase corresponded to 60 to 120 d after enrollment, and aimed to evaluate the efficacy of the HOTMI premix in preventing the occurrence of experimentally induced active DD lesions (Figure 1).

Study Groups (Participants)

Holstein steers purchased from a local custom steer-raising operation were selected to be part of the study and were eligible if they appeared healthy by clinical inspection and had a BW between 135 and 270 kg. The steers were excluded if a DD lesion was diagnosed on any of the 4 feet after inspection in a restraint chute. During the natural exposure phase, the steers were housed at a commercial rearing operation in 4 group pens in a naturally ventilated building with a bedded pack system and free water and feed access. During the challenge phase, the steers were housed in a closed building under positive pressure ventilation in 2 slatted floor pens, with free water and feed access at the Livestock Laboratory of the University of Wisconsin-Madison. Feed was offered once per day in both locations.

Interventions

Natural Exposure Phase. One hundred twenty steers were randomly allocated into 2 groups of 60 and fed for a period of 60 d HOTMI premix containing OTM (Availa 4; Availa Zn; Zinpro Corp., Eden Prairie, MN)

Figure 1. Flow diagram of the trial, including natural exposure and challenge phases and number of animals allocated to the supplement with higher than typically recommended levels of organic trace minerals and iodine (HOTMI) and the standard mineral supplement. OTM = organic trace minerals.

and ethylenediamine dihydroiodide (**EDDI**; treatment group) or a standard trace mineral supplement (Cargill Inc., Minneapolis, MN; control group). The HOTMI was formulated to provide daily (per 45 kg of BW) 103 mg of Zn, 68 mg of Mn, 14 mg of Cu, 1.1 mg of Co, 4 mg of I, and 0.32 mg of Se. Metal amino-acid complexes (Zinpro Corp.) supplied 59.2, 24.6, and 76.9% of the Zn, Mn, and Cu, respectively, with the remainder supplied by sulfate forms of these metals. Cobalt glucoheptonate (COPRO; Zinpro Corp.) and EDDI supplied 100% of the Co and I, respectively. All of the Se was supplied by sodium selenite. The standard trace mineral premix was formulated to provide daily (per 45 kg of BW) 35 mg of Zn, 37 mg of Mn, 12 mg of Cu, 0.53 mg of Co, 0.42 mg of I, and 0.32 mg of Se. Sulfates supplied all of the Mn and Cu, and EDDI, cobalt carbonate, and sodium selenite supplied all the I, Co, and Se, respectively. Zinc was supplied by a combination of Zn sulfate and Zinpro Zn methionine complex (Zinpro Corp.).

Allocation to the interventions was performed by randomly assigned ear tags numbered between 1 and 120. Animals with numbers from 1 to 60 were allocated to the HOTMI premix-fed group and numbers 61 to 120 were allocated to the standard trace mineral mix group. Baseline characteristics after randomization are shown in Table 1. The same base ration was used for both groups and was composed of alfalfa haylage, corn silage, and a mixed concentrate of cereals and macrominerals (Table 2). Diets were formulated to meet

or exceed nutrient requirements to support an average daily gain of 0.8 kg, including trace mineral requirements (NRC, 2001). Two evaluations (at d 30 and 60) were made in a stand-up restraint chute to detect possible DD infections naturally acquired on the farm on any of the 4 feet. The timeline of the study is shown in Figure 2a.

Challenge Phase. Fifteen steers free of macroscopic DD lesions were randomly selected from each of the treatment and control groups on d 60 after enrollment and transported to the Livestock Laboratory. The steers were selected from each group by using a random list of numbers generated using Excel software (Microsoft Corp., Redmond, WA). The same diet group allocation was maintained for the 60 d of the challenge phase. The HOTMI premix was formulated as for the natural exposure phase. The standard trace mineral premix was formulated to provide daily (per 45 kg of BW) 74 mg of Zn, 65 mg of Mn, 15 mg of Cu, 1.0 mg of Co, 1.4 mg of I, and 0.42 mg of Se. In contrast to the natural exposure phase of the study, all Zn was supplied by Zn sulfate.

Attempts to induce experimental DD lesions were made according to the Gomez et al. (2012) modifications of the Read and Walker (1998b) infection model. Digital skin of 20 feet in each group was inoculated with a homogenate of DD lesion biopsy material obtained from commercial dairy operations; 10 animals were inoculated in the right rear foot and 5 animals

Table 1. Baseline characteristics (means, with SD in parentheses) of steers for the natural exposure and challenge phases

Item	Natural exposure phase (0 to 60 d after enrollment)		Challenge phase (60 to 120 d after enrollment)	
	Control group (n = 60)	Treatment group (n = 60)	Control group (n = 15)	Treatment group (n = 15)
Age (mo)	5.9 (0.25)	5.8 (0.27)	7.9 (0.22)	7.9 (0.21)
Weight (kg)	194.9 (18.2)	194.4 (16.6)	256.8 (17.3)	252.5 (21.4)
DD lesions ¹ (%)	0	0	0	0

¹DD = digital dermatitis.

were inoculated in both rear feet. The remaining 10 left rear feet were used as sham inoculation. Animals were selected randomly to receive sham inoculations and equivalent inoculation distribution was performed across treatments. Using a stand-up restraint chute, gross visual examination of DD lesions was performed twice per week on all inoculated feet, including sham inoculations, until completion of the study (Figure 2b).

Outcomes

According to the DD classification described by Döpfer et al. (1997), the primary outcome of the study was defined as an active ulcerative DD lesion ≥ 20 mm across the largest dimension (an **M2** lesion). Digital dermatitis lesions < 20 mm were classified as **M1** lesions. Given that in a single foot several DD lesions might be present, a secondary outcome was defined as the diameter of M1 and M2 lesions present in the foot upon diagnosis of an M2 lesion or the diameter of M1

lesions at d 60 of the challenge phase when only M1 lesions were present. The outcomes were determined by clinical inspection and by measuring the largest dimension of the lesions with a caliper [General Pocket Caliper (132me); General Tools LLC, New York, NY].

Additional Analysis

To evaluate potential differences in blood and liver trace mineral contents between the groups, blood samples were taken on d 1, 14, and 29 of the challenge phase and upon detection of an acute DD lesion, using a Vacutainer tube (BD reference number 369737; royal blue trace element serum tube; BD, Franklin Lakes, NJ). Within 12 h of collection, blood was centrifuged at $1,500 \times g$ for 10 min at room temperature ($\sim 23^\circ\text{C}$), serum was harvested into microcentrifuge tubes (1.7 mL; VWR International LLC, Radnor, PA) and frozen at -80°C until serum was analyzed for Co, Cu, Fe, Mn, Mo, Zn, Se, and I concentrations (Diagnostic Center for

Table 2. Nutritional composition analysis of the diets fed during the natural exposure and challenge phases

Nutrient content	Natural exposure phase		Challenge phase	
	Control	Treatment	Control	Treatment
NE _M (Mcal/kg)	1.66	1.60	1.41	1.43
CP (%)	16.09	14.28	16.72	17.34
Fat (%)	3.06	2.76	3.45	3.45
Ash (%)	6.81	6.74	8.70	8.63
ADF (%)	22.67	24.41	26.42	27.48
NDF (%)	37.78	41.96	40.15	39.41
NFC (%)	36.27	34.26	31.81	32.03
TDN (%)	71.14	69.08	64.5	62.81
Ca (%)	0.99	0.83	1.15	1.16
P (%)	0.34	0.34	0.43	0.42
Mg (%)	0.33	0.32	0.4	0.41
K (%)	1.66	1.61	2.08	2.07
Na (%)	0.18	0.13	0.28	0.27
Cl ⁻ (%)	0.44	0.62	0.87	0.89
S (%)	0.24	0.26	0.19	0.19
Mn (mg/kg)	42.5	115.5	119	109
Zn (mg/kg)	64	161.5	103	121
Cu (mg/kg)	30	37.5	23	20
Fe (mg/kg)	174.5	239	246	237
Mo (mg/kg)	0.57	0.54	0.87	0.85

Figure 2. Timeline of the natural exposure (a) and challenge (b) phases, showing maceration wrap and inoculation periods, evaluation number (E), interval days (d) between events, and digital dermatitis (DD) diagnosis (arrows). M2 = ulcerative DD lesion >20 mm across the largest dimension.

Population and Animal Health, Michigan State University, Lansing). Liver biopsies were taken upon detection of an acute DD lesion or on d 60 of the challenge phase for animals without M2 diagnosis. Liver biopsies were performed as described in Corah and Arthington (1995) using a True-Cut biopsy instrument (Baxter Healthcare, Valencia, CA), stored in a trace elements serum tube (BD reference number 369737; royal blue trace elements serum tube; BD) and analyzed on a DM basis for Co, Cu, Fe, Mn, Mo, Zn, and Se concentrations (Diagnostic Center for Population and Animal Health, Michigan State University).

After clinical identification of a lesion compatible with M2 DD, a full-skin thickness punch biopsy was taken, following the procedure reported in Gomez et al. (2012), and inspected for motile spirochetes using a wet mount of a drop of ground biopsy in culture medium under a dark-field microscope at 200 $\times$ (80i; Nikon, Milwaukee, WI). Polymerase chain reaction was used to test for the presence of *Treponema*-like microorganisms in all samples, including sham inoculations (Evans et al., 2008). A second biopsy of the same lesion was placed in 10% neutral buffered formalin and submitted for histopathologic evaluation by one of the authors (D. H. Read). Tissues were processed routinely to wax; sectioned at 5 μ m; stained by hematoxylin-eosin, May-Grünwald-Giemsa, and modified Steiner silver staining; and subjected to an immunohistochemical (IHC) procedure using a polyclonal antiserum to a PDD/DD-associated *Treponema* spp. [Walker isolate 1-9185MED,

phenotype group 1, phylotype group 1, most closely resembling (mcr) *Treponema denticola*; Walker et al., 1995]. Twelve additional control biopsies were taken from equivalent dewclaw areas in sham feet, without biopsy homogenate inoculation, to evaluate possible cross-contamination. Histopathologic diagnosis of DD and classification was blind to clinical classification and made according to the guidelines prescribed by Berry et al. (2010). A diagnosis of DD was made if the following was observed: loss of stratum corneum, invasion of eroded and hyperplastic epidermis and ulcerated papillary dermis by spirochete-dominant bacterial flora, and reactive inflammation. Steiner silver stain was used to detect spirochetes and the IHC procedure was used to determine if the spirochetes were antigenically related to PDD/DD-associated *Treponema* sp. (mcr) *T. denticola*. After M2 diagnosis or biopsy, and cleaning using a disposable towel, the lesions were treated topically in the stand-up restraint chute using liquid oxytetracycline (Oxy-Mycin 100 mg/mL; Agripharm Products, West Lake, TX) under a light wrap (Coban; 3M, Saint Paul, MN), which was removed in 24 h.

Blinding

The staff responsible for the feeding of the animals, and the evaluator of the histopathology samples were blinded to treatment allocation. However, the investigator and 2 technicians were aware of the group allocation.

Sample Size and Statistical Analysis

To detect a reduction in the total incidence of M2 infections of 50% during the challenge phase with a type I error of 5%, a type II error of 20%, and a predicted incidence of experimentally induced lesions of 95%, 15 steers were randomized to each group. The sample size estimate accounted for 10% possible losses to follow-up due to health issues or unexpected adverse events.

Logistic regression was used to analyze the presence of an M2 DD lesion, with the foot being the unit of observation (primary outcome). The nesting characteristic of both rear feet on the same steer was considered as a random term in the regression model. A linear mixed regression model was used to evaluate the lesion size as a secondary outcome as indicated in Equation 1:

$$\begin{aligned} \text{Lesion size}_{ij} \sim & \beta_0 + \beta_1 \text{DD lesion type}_{ij} \\ & + \beta_2 \text{Treatment group}_{ij} + \beta_3 \text{DD lesion type}_{ij} \\ & \times \text{Treatment group} + \nu_i + \varepsilon_j, \end{aligned} \tag{1}$$

where j = j th lesion in a foot, i = i th steer unique identifier, β_0 = intercept, β_1 = estimate of the mean lesion size difference between treatment and control groups, β_2 = estimate of the mean M1 DD lesion size, β_3 = estimation of the difference in M2 lesion size between the control and treatment groups, ν_i = random steer term, and ε_j = residual error. Lesion size = millimeters across the largest dimension of the lesion, DD lesion type = M1 or M2 DD, Treatment group = treatment or control group (reference), DD lesion type $_{ij}$ $\times$ Treatment group = interaction term between lesion type and treatment group. Similarly, a random term was included in the model to account for the nesting of both rear feet in the same animal and diagnostic plots were checked to assess the assumption about constant variance of the residuals and potential linear trends. The R software (R Core Team, 2013) was used to perform the statistical analysis.

RESULTS

Primary and Secondary Outcomes

During the natural exposure phase, no DD infections were diagnosed. Therefore, the evaluation of the nutritional supplement effect under natural conditions was inconclusive.

A summary of the challenge phase results are shown in Tables 3 and 4 for the primary and secondary outcomes, respectively. Fifty-five and 30% of feet with an M2-stage DD lesion were diagnosed in the control and treatment groups, respectively. The median (95% CI) time to diagnosis was 28 (22 to 34) and 32 (30 to 40) d in the control and treatment groups, respectively ($P = 0.11$; Figure 3). The level of M1 DD observed was found equivalent between the groups up to d 10 postinoculation. An M1 DD infection was identified in 70% (14/20) and 60% (12/20) of feet in the treatment and control groups, respectively. During the study and without topical antibiotic intervention, M1 lesions evolved either to an M2 lesion, maintained the M1 status until the termination of the study, or healed with total restoration of the skin.

When M1 and M2 lesions were considered, similar M1 lesion size was found in the treatment group (7.23 mm; SE = 0.75 mm) versus the control group (7.35 mm; SE = 0.75 mm; Figure 4). However, larger M2 lesions were observed at diagnosis in the control group (mean = 27.58 mm; SE = 2.13 mm) compared with the treatment group (mean = 23.85 mm; SE = 1.65 mm). Although the adjusted difference of -4.15 mm observed in the treatment group was not found to be statistically significantly different from the control group ($P = 0.11$), it demonstrated a beneficial trend resulting from feeding the HOTMI premix (Table 4).

Serum and Liver Mineral Content

During the challenge phase, significant differences in liver Co and Se, and serum Co and I concentrations

Table 3. Absolute and relative risk of developing an infection with an active ulcerative digital dermatitis (DD) lesion ≥ 20 mm across the largest dimension (M2 DD) during the challenge phase

	Percentage (no. of feet)		Risk ratio ¹ (95% CI)	Risk difference % (95% CI)
	Control supplement (n = 20)	Treatment supplement (n = 20)		
Primary outcome				
Proportion of feet developing an M2 DD infection	55 (11)	30 (6)	0.54* (0.18 to 1.09)	−25 (−49.6 to 5)

¹Corrected odds ratio (Zhang and Yu, 1998).
* P -value logistic regression = 0.11.

Table 4. Summary of the estimation of the digital dermatitis (DD) lesion size differences observed between the treatment and control groups considering DD stages with lesions <20 mm (M1) and active ulcerative DD lesions >20 mm across the largest dimension (M2)

Item	Secondary outcome: DD lesion size			
	Mean	SE	95% CI	P-value
Intercept (control)	7.57	1.23	5.24 to 9.70	0.98
Treatment	-0.03	1.56	-3.03 to 2.81	
M1 stage	Reference	—	—	<0.01
M2 stage	19.96	1.7	16.6 to 23.4	
Treatment × M2 stage	-4.15	2.6	-8.89 to 1.54	0.11

were observed between the treatment groups (Table 5). Furthermore, liver concentrations of Co, Mn, and Se in the control group and liver concentrations of Co and Cu in the treatment group were significantly lower in animals diagnosed with an M2 lesion (Table 6).

Histopathology and Direct Observation

All lesions diagnosed clinically as M2 stage of DD were confirmed histopathologically as DD. Spirochetes were consistently detected by dark-field microscopy in all biopsies collected from M2 lesions. Histopathologically, Steiner silver stain consistently detected invasive spirochetes in stratum spinosum and papillary dermis, and the IHC procedure consistently demonstrated that the spirochetes were antigenically related to PDD/DD-associated *Treponema* spp. in all M2 lesions. The presence of *Treponema* spp. organisms were confirmed

by PCR. Spirochetes were not identified in any biopsies from comparable locations in sham inoculation feet ($n = 12$) by any of the staining techniques.

DISCUSSION

This study compared the effect of feeding HOTMI premix versus a standard trace mineral premix on incidence and size of DD lesions in Holstein steers under natural and experimental infection settings. No DD lesions were observed during the natural exposure phase and, therefore, comparison between HOTMI and standard trace minerals could not be made. However, for experimentally induced lesions, the results showed a decreased number of M2 lesions in the HOTMI group (30 vs. 55% of feet diagnosed in the control group), as well as decreased size of M2 lesions [23.85 mm (SE = 1.65 mm) vs. 27.58 mm (SE = 2.13 mm) in the control group], suggesting a beneficial effect of the HOTMI premix in preventing DD lesions. However, none of these

Figure 3. Survival curve of digital dermatitis (DD) diagnosis involving an active ulcerative DD lesion >20 mm across the largest dimension (M2 DD) during the challenge phase of the study. + = censored observations.

Figure 4. Final mean (with SEM) digital dermatitis (DD) lesion sizes for lesions <20 mm (M1) and active ulcerative DD lesions ≥20 mm across the largest dimension (M2) observed during the challenge phase of the study in treatment and control groups, and statistical significance of the difference evaluation.

Table 5. Mean (SEM) serum trace mineral content from samples taken on d 1, 14, and 29 of the challenge phase and liver trace mineral content upon diagnosis of an active ulcerative digital dermatitis (DD) lesion >20 mm across the largest dimension (M2) or termination of the study

Micromineral	Serum analysis				Liver analysis			
	Units	Control	Treatment	<i>P</i> -value	Units	Control	Treatment	<i>P</i> -value
Co	ng/mL	0.67 (0.06)	2.03 (0.09)	<0.001**	µg/g	0.35 (0.02)	0.51 (0.02)	<0.001**
Cu	µg/mL	0.76 (0.02)	0.72 (0.03)	0.59	µg/g	514.4 (44.45)	519.7 (62.87)	0.81
Fe	µg/dL	125.4 (5.99)	131.6 (8.52)	0.56	µg/g	226.8 (15.13)	253.1 (21.40)	0.23
Mn	ng/mL	1.31 (0.17)	1.53 (0.24)	0.11	µg/g	9.86 (0.48)	10.0 (0.68)	0.83
Mo	ng/mL	43.0 (5.85)	51.1 (8.3)	0.32	µg/g	3.84 (0.23)	3.86 (0.32)	0.95
Zn	µg/mL	0.90 (0.02)	0.90 (0.03)	0.42	µg/g	164.4 (10.36)	154.1 (14.66)	0.46
Se	ng/mL	60.5 (0.86)	58.0 (1.20)	0.40	µg/g	1.84 (0.08)	1.52 (0.12)	0.02**
I	ng/mL	107.5 (7.62)	248.4 (10.8)	<0.001**				

***P* < 0.05.

differences were statistically significant ($P = 0.11$; Table 3). The original sample size calculation was conditional on an infection rate of 95% in the control group and a proposed relative reduction in active DD in the HOTMI premix group of 50% and none of these thresholds were achieved. The final number of steers included in the study corresponded to a power of 60% and this was not sufficient to demonstrate the 45% difference achieved at a significance level of 5%. Future trials using the current DD experimental infection model should take into account the incidence of infection achieved in the present study to calculate appropriate sample sizes and achieve adequate power.

It is difficult to determine whether the response was the result of providing an increased supply of one mineral or a combination of minerals. Significant differences in the levels of some blood and liver trace minerals were found between treatment and control groups. Iodine may have an important role in the beneficial effects of the HOTMI premix on incidence and severity of DD lesions, as the group being fed HOTMI premix had higher serum I levels ($P < 0.01$) than the control group. Iodine in the form of EDDI, included in the HOTMI premix, has been used previously in bovine skin-related disease prevention (Çam et al., 2007).

Ethylenediamine dihydroiodide has been shown to be efficacious in preventing experimentally induced bovine foot rot using mouse and cow models (Berg et al., 1984). Currently, Food and Drug Administration regulations limit the use of EDDI to 49.9 mg/animal per day (Food and Drug Administration Office of Regulatory Affairs, Compliance Policy Guide for EDDI; CPG Sec. 651.100, 2000). Furthermore, if milk iodine concentrations are excessive, the milk is considered adulterated and the dairy producer is liable. Therefore, the HOTMI premix fed in this study should be restricted to nonlactating animals. Steers in the HOTMI group consumed greater amounts of some trace minerals (i.e., 29 mg of Zn and 2.6 mg of I per 45 kg of BW more were consumed by the HOTMI group, respectively, than by the control group) and, therefore, environmental accumulation of heavy metals by the elevated mineral ingestion could be a concern (López-Alonso, 2012); diets were specifically formulated below the dietary maximum allowance of mineral levels in livestock production.

The HOTMI premix group had lower concentrations of Se in the liver and higher Co concentrations in both serum and liver compared with the control group (Table 5). The higher liver Se concentrations for the control group may be due, in part, to the control heifers being

Table 6. Liver trace mineral content (means, with SD in parentheses) observed based on diagnosis of an active ulcerative digital dermatitis (DD) lesion ≥20 mm across the largest dimension (M2 DD) and intervention group

Micromineral	Control group			Treatment group		
	M2	No M2	<i>P</i> -value	M2	No M2	<i>P</i> -value
Co (µg/g)	0.31 (0.04)	0.39 (0.02)	<0.01**	0.45 (0.09)	0.56 (0.06)	0.01**
Cu (µg/g)	429 (219)	589 (62.8)	0.07*	423 (161)	600 (244)	0.04**
Fe (µg/g)	211 (30.0)	240 (47.1)	0.19	213 (40.3)	279 (77.4)	0.07*
Mn (ng/g)	8.75 (2.08)	10.8 (1.58)	0.04**	9.23 (1.44)	10.5 (1.44)	0.14
Mo (µg/g)	3.52 (0.44)	4.11 (0.66)	0.07*	3.55 (0.60)	4.06 (1.32)	0.39
Zn (µg/g)	180 (48.7)	150 (43.0)	0.21	162 (46.3)	147 (18.3)	0.37
Se (µg/g)	1.54 (0.29)	2.11 (0.25)	<0.01**	1.36 (0.18)	1.63 (0.30)	0.07*

* $P < 0.1$; ** $P < 0.05$.

fed more Se per unit of BW during the challenge phase of the study. The higher serum and liver Co concentrations may be due, in part, to steers fed the HOTMI premix consuming approximately twice as much Co during the natural exposure phase. However, during the challenge phase of the study, both groups of steers received approximately the same amount of Co per unit of BW (1.1 and 1.0 mg of Co per 45 kg of BW for HOTMI and control premixes, respectively). Another possible explanation for steers fed the HOTMI premix having higher serum and liver Co concentrations than control steers was that they were fed Co in the form of Co glucoheptonate, whereas control steers were fed Co in the form of Co carbonate. Akins et al. (2013) found no difference in liver Co concentrations when dairy cattle received 25 mg of Co from either feed-grade Co carbonate or feed-grade Co glucoheptonate from 60 d prepartum through 150 d postpartum. For both the treatment and HOTMI steers, liver Co concentrations were lower when steers were diagnosed with an M2 lesion (Table 6). It is not certain whether having lower liver Co concentrations predisposed steers to M2 lesions or whether the development of an M2 lesion resulted in lower Co concentrations in the liver. Cobalt is primarily utilized by rumen microbes for the formation vitamin B₁₂ and may also aid in fiber digestion (NRC, 2001). No known function exists for Co once absorbed and it does not appear to be recycled back to the rumen after absorption (NRC, 2001). Thus, we know of no potential mode of action for increased Co status increasing the ability of the animal to resist M2 lesions.

When compared with the control premix, the HOTMI premix provided animals with approximately 1.6 times more Zn and 2.9 times more I. In addition, all the Zn, Mn, Cu, and Co in the control premix was supplied by inorganic minerals, whereas in the HOTMI premix, a portion of the Zn, Mn, and Cu were supplied by amino acid complexes.

Association of organic compounds, mainly amino acids, with trace minerals has previously been shown to increase their bioavailability (Wedekind et al., 1992; Spears, 1996). This could explain differences in the performance of diets including OTM against diets based on inorganic sources of trace minerals (Nocek et al., 2006; Siciliano-Jones et al., 2008; DeFrain et al., 2009).

However, no differences were found between the treatment and control groups in Zn serum and liver concentrations. Given that several tissues, such as bones, are responsible for the retention and release of Zn, influencing plasma turnover of Zn, blood and liver Zn levels are considered poor indicators of Zn bioavailability (Kincaid, 1999). However, the role of increased Zn supply in reducing the severity and size of DD lesions cannot

be dismissed, given its role in immune function, specifically modulating IL-8 (Zhao et al., 2011). Interleukin 8 has been correlated with keratinocyte activity in DD pathogenesis (Refaai et al., 2013). Manganese may also have a role in the responses observed when the HOTMI premix was fed. First, liver Mn concentrations were lower ($P < 0.05$) in animals diagnosed with M2 lesions. Second, a trend ($P < 0.11$) existed for serum Mn to be higher in HOTMI steers compared with control steers. The role of Mn in the positive response to the HOTMI premix may be related to its role as an antioxidant, with implications for wound healing and immune cell function (Keen and Zidenberg-Cherr, 1996; Muszyńska et al., 2000; Son et al., 2007).

As previously noted, in steers with M2 lesions, several trace minerals were significantly decreased relative to steers without M2 lesions (Table 6). This suggests that during a DD infection, some trace minerals are more rapidly consumed, have elevated metabolism, or protect or solve DD infection. However, the exact role of trace minerals during DD infection remains speculative, as no previous research is available to support it. Alternatively, the potential decrease in feed intake due to pain and discomfort as a consequence of DD lesions might have modified the trace mineral levels observed in DD-affected animals (Bareille et al. 2003), although this seems less plausible, given that no signs of lameness or changes in behavior were observed during the trial.

Current nutritional recommendations by NRC (2001) are calculated for animals without special health-related needs, such as stress or presence of antagonists. Therefore, trace mineral dietary recommendations are formulated for normal situations. But there is a lack of knowledge regarding trace mineral needs of livestock during periods of disease and how blood and liver trace mineral concentrations are modified within the normal range during situations of increased need. In critically ill human patients, however, increased trace mineral requirements are well documented to be related to disease processes (Berger et al., 2007; Heyland et al., 2008; Doley, 2010). Discussions regarding trace mineral profiles need to take into consideration the dependency of the trace mineral content on dietary availability of minerals and the variation of the reference values used by different laboratories or literature sources (Puls, 1994; Herdt and Hoff, 2011).

Specifically for animals, strong evidence exists to support an association between cellular immune response, skin health, and wound healing with adequate nutritional plane and bioavailability of trace minerals (Tenaud et al., 2000; Richelle et al., 2006; Kharaeva et al., 2009). This can be particularly important in situations where the skin health is compromised by external

stressors (Tomlinson et al., 2004). During the challenge phase of the current study, the hind feet were subjected to continuous moisture (maceration) and reduced access to air (closure) by the wrapping procedure. Changes in the skin were evident throughout the study, manifesting themselves as marked skin maceration in all wrapped feet. Other skin infections, such as ulcerative mammary dermatitis (Evans et al., 2010) or ringworm (Çam et al., 2007), may also benefit from this type of nutritional supplementation.

The final level of DD incidence induced using the infection model in this study (55%) is encouraging to recommend this approach as a gold standard for investigation into the efficacy of DD intervention strategies. This is particularly true if future studies take this incidence into account and adjust power calculations accordingly. Of particular importance is the ability of this experimental infection model to eliminate many of the confounding factors prevalent in field studies (Wells et al., 1999; Berry et al., 2002; Teixeira et al., 2010) that may contribute to inconclusive results. In addition, *Treponema*-like organisms were not identified in any of the samples collected from equivalent dewclaw areas in sham inoculations, confirming successful isolation of the experimentally induced lesions from the surrounding environment and from possible cross-contamination during the numerous feet manipulations.

In addition to the previously discussed lack of power, another limitation of the study was the possible introduction of observer bias in the evaluation of the DD lesions. The principal author and 2 technicians, all of whom were aware of the treatment allocation, were responsible for diagnosis of the DD lesions. However, clinical DD diagnosis is simple in the experimental setting, and photographs of the lesions and measurements were recorded for later reassessment, if needed.

CONCLUSIONS

Although more research is needed to confirm the results observed in the present study, the trend of decreased DD incidence and decreased lesion size is suggestive that the HOTMI premix could be a novel strategy for controlling DD in the field. This control strategy could focus on high-risk groups such as beef cattle, dairy heifers, and transition cows before parturition. Additionally, the bovine DD infection model appears to be adequate to evaluate the efficacy of intervention strategies aimed at controlling DD, with the advantage of decreasing the number of confounding factors often encountered in field trials and minimizing the number of animals needed to demonstrate efficacy.

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