Source of supplemental dietary copper, zinc, and manganese affects fecal microbial relative abundance in lactating dairy cows

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ABSTRACT

Appropriate trace mineral supplementation can improve immune response and hoof health in cattle and at much higher rates of supplementation to swine and poultry can alter microbial colonization of the gut, resulting in improved gut health. Diet can influence fecal microbial excretion in cattle, and the fecal microbiome may serve as a means for assessing gastrointestinal microbial changes. We hypothesized that feeding diets that differed in source of supplemental Cu, Zn, or Mn would alter the relative abundance of fecal microbes in lactating dairy cattle and that organic Zn would have the greatest effect. Twenty-four cows were fed diets devoid of supplemental Cu, Zn, and Mn for a 16-d preliminary phase (basal diet provided 9, 29, and 32 mg/kg of Cu, Zn, and Mn, respectively), and then were randomly assigned to 1 of 3 treatment diets ($n = 8$ cows/treatment): one group of cows was fed supplemental Cu, Zn, and Mn from sulfate minerals; the second group was fed glycinate minerals; and the third group was fed Cu and Mn sulfate with glycinate Zn. Assayed total dietary concentrations were approximately 21, 73, and 72 mg/kg for Cu, Zn, and Mn, respectively. Milk production (averaged 38.8 kg/d), DMI (averaged 25.8 kg/d), and milk components were not affected by treatment. Fecal DNA was extracted, amplified using a universal primer targeting the V4-V5 hypervariable region of the 16S rRNA gene, and sequenced to compare microbial community composition between treatments. Relative abundances of *Treponema* species-level operational taxonomic units (OTU) were less for animals fed Cu and Mn sulfate with glycinate Zn compared with sulfates alone, but were similar to animals fed glycinate mineral sources. Relative abundances for exclusive glycinate mineral and sulfate mineral treatments were similar. *Treponema* OTU and cultured representatives are of-

ten associated with bovine digital dermatitis. These data may provide an additional link between organic Zn supplementation and improved hoof health. To our knowledge this is the first report of a dietary treatment decreasing the relative abundance of *Treponema* OTU in cattle feces; however, the potential benefits of this response on overall animal health and the mechanism for the observed responses are unknown and warrant further investigation.

Key words: fecal microbiome, trace minerals, digital dermatitis

INTRODUCTION

Pregastric microbiomes in ruminant animals have been studied in great detail and are highly documented (Kim et al., 2011). Although there is still much to learn regarding pregastric microbiomes, even less is known regarding other segments of the bovine gastrointestinal tract. Feeding supplemental trace minerals at dietary concentrations in the 100 to 1,000s mg/kg range can influence the enteric microbiome populations of poultry (Shao et al., 2014) and swine (Broom et al., 2006). Specifically, Zn supplementation improves gut health and performance in swine (Broom et al., 2006).

Bovine colonic microbes are derived from an accumulation of microbe populations and actions, and fecal samples provide a convenient sample for analyses of these populations (Wells et al., 2014). Fecal bacterial populations can be influenced by diet (Shanks et al., 2011). Supplementation of glycinate minerals has been shown to alter milk fatty acid profiles in lactating dairy cattle (Faulkner, 2016), which may reflect changes in rumen microbial populations. Organic Zn supplementation reduces SCC in lactating dairy cows (Kellogg et al., 2004) and may improve hoof health (Siciliano-Jones et al., 2008). We hypothesized that feeding diets that differed in source of supplemental Cu, Zn, or Mn would alter the relative abundance of fecal microbes in lactating dairy cattle and that organic Zn would have the greatest effect. Therefore, the objectives of the current study were to (1) determine whether source of supplemental trace minerals fed to lactating dairy

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Table 1. Ingredient composition of the diet¹ (% of DM)

Ingredient	Sulfate	Glycinate	Sulfate + glycinate Zn
Corn silage	27.0	27.0	27.0
Alfalfa silage	27.5	27.5	27.5
Alfalfa hay	5.0	5.0	5.0
Ground corn	20.5	20.5	20.5
Soybean meal, 48% CP	4.5	4.5	4.5
Treated soybean meal ²	8.0	8.0	8.0
Soy hulls	4.5	4.5	4.5
Animal/vegetable fat	0.70	0.70	0.70
Limestone	0.81	0.81	0.81
Dicalcium phosphate	0.30	0.30	0.30
Magnesium oxide	0.07	0.07	0.07
Iodized salt	0.52	0.52	0.52
Selenium premix ³	0.16	0.16	0.16
Vitamin mix ⁴	0.43	0.43	0.43
Cu sulfate	0.00004	—	0.00004
Zn sulfate	0.0001	—	—
Mn sulfate	0.0001	—	0.0001
Glycinate Cu ⁵	—	0.00004	—
Glycinate Zn ⁵	—	0.0002	0.0002
Glycinate Mn ⁵	—	0.0002	—

¹The sulfate and glycinate treatments provided all supplemental Cu, Zn, and Mn from sulfate or glycinate sources, respectively. The sulfate + glycinate Zn treatment provided supplemental Cu and Mn from sulfate sources and Zn from glycinate.

²Aminoplus, Ag Processing Inc., Omaha, NE.

³Sodium selenate, 200 mg/kg.

⁴Contained 767 kIU of vitamin A/kg, 279 kIU of vitamin D/kg, 4,900 IU of vitamin E/kg, and 209 mg of biotin (Rovimix Biotin, DSM Nutritional Products, Belvidere, NJ)/kg.

⁵B-TRAXIM 2C (Pancosma S.A., Geneva, Switzerland). The products contained 270,000, 260,000, and 220,000 mg of Cu, Zn, or Mn/kg, respectively.

cattle would alter fecal microbial relative abundance and (2) specifically investigate whether feeding glycinate Zn combined with Cu and Mn sulfate minerals affects fecal microbial relative abundance assessed by 16S rRNA gene sequencing differently than all sulfate or all glycinate mineral treatments alone.

MATERIALS AND METHODS

Cows and Treatments

All procedures using animals were approved by The Ohio State University Institutional Animal Care and Use Committee.

Twenty-four multiparous Holstein cows [166 ± 54 DIM ($\pm$ SD) at the start of mineral supplementation] were randomly assigned to 1 of 3 dietary treatments (Tables 1 and 2) in a completely randomized design to evaluate the effects of trace mineral source on fecal microbial populations. The experiment lasted 16 d. Prior to the experiment, cows were housed in a common freestall pen and fed the experimental diet, but devoid of supplemental Cu, Zn, and Mn (basal diet provided 9, 29, and 32 mg/kg of Cu, Zn, and Mn, respectively) for 30 d. This was done to put cows on a similar trace nutrient nutritional plane and to allow

previously supplemented trace mineral sources to pass from the gastrointestinal tract. No experimental data were collected during the preliminary phase.

Following the preliminary phase, cows were moved into the tie stall barn and fed a common diet except for changes in trace minerals. Treatments were (1) supplemental Cu, Zn, and Mn from sulfates; (2) supplemental Cu, Zn, and Mn in the glycinate form (B-Traxim, Pancosma, Geneva, Switzerland); or (3) Cu and Mn from sulfates with Zn glycinate. Target total diet Cu, Zn, and Mn concentrations for all treatments were 20, 80, and 80 mg/kg, respectively. Supplemental minerals were fed daily as a top dress (0.40 kg/d).

Diets were fed once daily with a target refusal rate of 2% of delivered feed and cows were milked twice daily at approximately 0200 and 1400 h (milk yields were measured electronically). In the tie stalls, individual feed delivery and feed refusal amounts were weighed and recorded daily. Cows were weighed on 2 consecutive mornings (approximately 4 h after feeding) at the end of the supplementation period.

Feed Samples and Production

Forages and concentrates were sampled weekly ($n = 2$) for future nutrient analysis and weekly dietary DM

(100°C for 24 h) adjustments. A subsample of each top dress mix was sampled immediately following preparation at the feed mill, and an additional sample was taken from each of the individual bags for each treatment when the top dress samples were being weighed out for delivery ($n = 4$ samples/treatment). All feedstuffs were sent to Cumberland Valley Analytical Services (Hagerstown, MD) for nutrient analysis using wet chemistry methods (AOAC International, 2000). For mineral analyses, samples were ashed (1 h at 535°C), digested in 15% nitric acid, diluted, and assayed by inductively coupled plasma emission spectroscopy. Milk (a.m. and p.m.) was sampled on d 7 of the experimental period and analyzed for milk fat, true protein, lactose (B2000 Infrared Analyzer, Bentley Instruments, Chaska MN), and MUN (Skalar SAN Plus segmented flow analyzer, Skalar Inc., Norcross, GA) by DHI Cooperative Inc. (Columbus, OH).

Fecal Sampling and DNA Analyses

Two fecal grab samples (~8 g/sample) were collected approximately 3 and 9 h postfeeding from each cow on d 0 (i.e., just before the start of mineral supplementation) and d 16 ($n = 4$ total collected samples/cow). Samples were collected into separate 15-mL RNase- and DNase-free conical tubes, immediately placed on ice, and frozen at -80°C within 1 h of sample collection for future analysis. Immediately before fecal sampling, the shoulder length collection sleeves were sprayed with ethanol. New sleeves were used for each cow. Prior to DNA extraction, fecal samples were composited within collection day (d 0 or 16) by weight (4 g/sample). Fecal DNA was extracted (Yu and Morrison, 2004) and nucleic acids were purified before sequencing using the QIAamp DNA Stool Mini Kit protocol (Qiagen Inc., Valencia, CA). Illumina sequencing by MiSeq platform (Illumina Inc., San Diego, CA) was performed by the

Molecular and Cellular Imaging Center (The Ohio State University, Ohio Agricultural and Research and Development Center, Wooster) using reagent kit V3 with 2×300 -bp pair-ended sequencing. The V4-V5 hypervariable region of the 16S rRNA gene was targeted with a universal primer and 300-bp pair-ended approach (Caporaso et al., 2010). Data processing was performed using QIIME 1.9.0 (Caporaso et al., 2010). The specific processing steps were as follows. Raw FASTQ data were demultiplexed and quality filtered to a Phred score of 20, with a minimum sequence length of 200 nts, with a total of 733,710 high-quality reads. Operational taxonomic units (OTU) were chosen in a 2-step process. First, sequences were clustered into OTU at 97% similarity using UCLUST followed by de novo OTU picking, then assigned taxonomy using the 97_SILVA_111 rep set (v123, <http://www.arb-silva.de/>; Edgar, 2013; Quast et al., 2013). Phyla-, family-, and genus-level operational taxonomic units below 0.5% relative abundance were not reported here to condense results. The OTU table was analyzed for α and β diversity (Shannon-Weiner and Bray-Curtis dissimilarity, respectively) using R (package vegan, <http://cran.r-project.org/web/packages/vegan/>).

Statistical Analysis

Production data and DMI were analyzed using PROC MIXED of SAS (v9.3, SAS Institute Inc., Cary, NC). The model included the fixed effects of treatment (2 df) and residual error. Relative abundance data were analyzed by analysis of covariance using d 0 fecal relative microbial relative abundance as a covariate for d 16 data. The model included the fixed effects of treatment (2 df) and d 0 covariate (1 df), and residual error. Mean separation of significant effects was performed using the PDIFF option in SAS. Relative abundance data were not normally distributed. Data transformations ($\log_{10}$,

Table 2. Nutrient composition of the basal diet and feedstuffs¹

Item	Basal diet	Corn silage	Alfalfa silage	Alfalfa hay	Concentrate
DM, %	59.3	30.0	38.3	89.8	89.1
NDF, %	32.9	40.8	44.8	54.8	17.3
Starch, %	24.1	31.7	0.6	0.3	37.3
CP, %	17.3	6.7	20.9	19.2	21.3
Ca, %	1.0	0.26	1.20	1.36	1.38
P, %	0.4	0.25	0.33	0.30	0.56
Mg, %	0.3	0.15	0.26	0.21	0.31
K, %	1.9	1.35	3.11	3.19	1.20
Fe, mg/kg	237	113	390	290	213
Cu, mg/kg	9 (21) ²	7	13	11	9
Zn, mg/kg	29 (73)	19	30	27	29
Mn, mg/kg	32 (55)	27	49	26	32

¹The basal diet was the same for all treatments (Table 1). Mineral treatments were top dressed (Table 3).

²Values in parentheses are total diet concentrations.

ARCSIN, and LOGIT) were performed and the results were compared with those of nontransformed data. Data transformation decreased standard errors, but did not influence the occurrence of statistically significant ($P \leq 0.10$) observations. Therefore, data are presented as relative abundance without transformation.

RESULTS

Body weight and production measures were similar ($P \geq 0.14$) between all treatments (Table 3). Sequencing results returned an average of 1,590 OTU per sample. No difference was detected between treatments for richness (1,570, 1,440, and 1,490) or Shannon-Weiner index (5.64, 5.21, and 5.44) for sulfate, sulfate with glycinate, and glycinate mineral treatments, respectively. Bray-Curtis dissimilarity, expressed in a nonmetric multidimensional scaling plot (not shown), did not significantly cluster by treatment ($P > 0.10$). The DNA sequencing analysis identified 14 different phyla in the fecal samples, which is greater than previous observations (Mao et al., 2012). *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Spirochaetes*, and *Actinobacteria* comprised the largest populations of phyla observed in feces (Figure 1) similar to previous observations in dairy cattle (Mao et al., 2012), beef cattle (Shanks et al., 2011), and other mammalian species (Ley et al., 2008). Trace mineral treatment had no effect ($P \geq 0.14$) on phyla (Table 4) and family (Table 5) fecal microbial relative abundance with the exception of bacteria in phylum *Spirochaetes* ($P = 0.08$) and family *Spirochaetaceae* ($P = 0.08$).

Fecal relative abundance of *Spirochaetes* was similar between treatments before trace mineral supplementation (Supplemental Table S1; <https://doi.org/10.3168/jds.2016-11680>). *Spirochaetes* relative abundance was less for cattle fed Cu and Mn sulfate with glycinate Zn compared with cows fed sulfate minerals ($P = 0.03$), but was similar ($P = 0.14$) to cows fed glycinate minerals only (Table 5). *Spirochaetes* fecal relative abundance was similar ($P = 0.15$) between cows consuming

exclusively glycinate and sulfate mineral sources (Table 5). *Spirochaetaceae* was the only family identified within the *Spirochaetes* phylum; therefore, treatment means and differences were the same at the phyla and family level (Tables 4 and 5). Similar to *Spirochaetes* and *Spirochaetaceae*, relative abundance for the genus *Treponema* within *Spirochaetaceae* was less for cows consuming Cu and Mn sulfate with glycinate Zn ($P = 0.03$), than for those fed exclusively sulfate minerals, but was similar ($P = 0.22$) to cows fed exclusively glycinate minerals (Figure 2). Six *Treponema* OTU were identified at low relative abundance (average = 0.3%, 5 uncultured and *Treponema zioleckii*), but none were different between treatments (data not shown, $P > 0.10$).

DISCUSSION

All mineral treatments were fed to modestly exceed (approximately $2\times$ Cu and Zn) Cu, Zn, and Mn requirements (NRC, 2001). Absorption coefficients for glycinate minerals are unknown; therefore, the absorption coefficient values for Cu (0.05) and Zn (0.20; NRC, 2001) were assumed to be similar between mineral sources, which may or may not be true. Manganese supplied in excess of the NRC (2001) requirement was not calculated because the Mn requirement is underestimated (Weiss and Socha, 2005; Hansen et al., 2006). Target dietary concentrations for Cu, Zn, and Mn were 20, 80, and 80 mg/kg, respectively. Target concentrations were chosen with the intent of supplying equal concentrations of Cu, Zn, and Mn from the basal diet and supplemental minerals (Table 2). Supplemental Cu, Zn, and Mn provided approximately 57, 60, and 55% of total dietary mineral concentrations, respectively, for the mineral treatments.

The effects of source of trace mineral supplementation on production measures are inconsistent (Rabiee et al., 2010) and were not primary objectives in the current study. These data are presented as a means to characterize the cows in the current study.

Table 3. Effects of mineral treatment on production

Item	Treatment ¹			SEM	P-value
	Sulfate	Glycinate	Sulfate + glycinate Zn		Treatment
BW, kg	721	730	732	18.7	0.90
DMI, kg/d	25.5	25.7	26.2	0.88	0.85
Milk, kg/d	40.3	37.3	38.8	2.30	0.66
Milk fat, %	3.31	3.53	3.75	0.15	0.14
Milk protein, %	2.85	2.95	3.01	0.09	0.48
Milk lactose, %	4.82	4.72	4.84	0.04	0.15

¹Sulfate and glycinate treatments provided all supplemental Cu, Zn, and Mn from sulfate or glycinate sources, respectively. The sulfate + glycinate Zn treatment provided supplemental Cu and Mn from sulfate sources and Zn from glycinate.

Table 4. Effects of feeding different Cu, Zn, and Mn treatments on microbial phyla relative abundance (in excess of 0.5%) in feces from lactating dairy cows (covariate adjusted using d 0 abundance)

Item	Treatment ¹			SEM	P-value
	Sulfate	Glycinate	Sulfate + glycinate Zn		Treatment
<i>Bacteroidetes</i>	47.77	43.25	45.24	4.30	0.75
<i>Firmicutes</i>	42.40	48.47	42.18	5.25	0.61
<i>Proteobacteria</i>	1.81	0.92	5.83	1.82	0.14
<i>Spirochaetes</i>	3.16 ^a	2.21 ^{ab}	0.78 ^b	0.74	0.08
<i>Actinobacteria</i>	0.87	1.36	1.60	0.50	0.58
Unassigned	1.44	1.38	1.71	0.14	0.21
<i>Tenericutes</i>	0.74	0.74	0.57	0.10	0.40
<i>Euryarchaeota</i>	0.73	0.59	0.85	0.27	0.77
<i>Fibrobacteres</i>	0.59	0.13	0.34	0.29	0.50
Candidate division TM7	0.21	0.17	0.12	0.04	0.37
<i>Cyanobacteria</i>	0.19	0.08	0.12	0.08	0.60
<i>Lentisphaerae</i>	0.08	0.07	0.07	0.03	0.98
<i>Verrucomicrobia</i>	0.06	0.18	0.12	0.05	0.21
Candidate division SR1	0.04	<0.01	0.05	0.02	0.27

^{a,b}Means within a row differ ($P < 0.05$).

¹Sulfate and glycinate treatments provided all supplemental Cu, Zn, and Mn from sulfate or glycinate sources, respectively. The sulfate + glycinate Zn treatment provided supplemental Cu and Mn from sulfate sources and Zn from glycinate.

Fecal Microbial Phyla

Figure 1. Average (across treatments) fecal microbial phyla relative abundance in excess of 0.5% relative abundance for lactating dairy cattle fed no supplemental Cu, Zn, or Mn and then fed supplemental Cu, Zn, and Mn for 16 d. Color version available online.

Table 5. Effects of feeding different Cu, Zn, and Mn treatments on bacterial family relative abundance (in excess of 0.5%) in feces from lactating dairy cows (covariate-adjusted using d 0 abundance)

Item	Treatment ¹			SEM	P-value Treatment
	Sulfate	Glycinate	Sulfate + glycinate Zn		
<i>Bacteroidetes</i>					
<i>Prevotellaceae</i>	23.26	17.80	26.00	6.08	0.61
<i>Rikenellaceae</i>	9.85	11.96	8.07	1.73	0.28
<i>Bacteroidales</i> S24–7	6.46	3.39	3.79	1.31	0.19
<i>Bacteroidaceae</i>	2.81	4.63	3.27	1.23	0.55
<i>Bacteroidales</i> RF16	2.31	2.69	1.82	0.62	0.61
BS11 gut group	1.83	0.88	0.62	0.77	0.51
Other <i>Bacteroidales</i>	0.99	0.80	0.66	0.18	0.46
<i>Porphyromonadaceae</i>	0.25	0.47	0.32	0.11	0.36
Family incertae sedis	0.19	0.30	0.20	0.08	0.57
Uncultured <i>Bacteroidales</i>	0.21	0.05	0.08	0.06	0.22
18B5 gut group	<0.01	0.10	0.03	0.04	0.14
<i>Firmicutes</i>					
<i>Ruminococcaceae</i>	20.19	28.15	20.87	5.06	0.45
<i>Lachnospiraceae</i>	13.76	11.10	11.85	1.62	0.49
<i>Christensenellaceae</i>	3.08	3.96	3.18	0.65	0.55
<i>Veillonellaceae</i>	1.44	1.52	2.63	0.69	0.40
Family XIII incertae sedis	1.46	1.15	0.99	0.36	0.62
<i>Clostridiaceae</i>	0.11	0.47	0.54	0.31	0.58
Other <i>Clostridiales</i>	0.54	0.75	0.57	0.19	0.70
<i>Erysipelotrichaceae</i>	0.49	0.50	0.70	0.10	0.23
<i>Anaeroplasmataceae</i>	0.32	0.29	0.18	0.12	0.67
<i>Streptococcaceae</i>	0.01	0.01	0.02	0.01	0.79
<i>Peptostreptococcaceae</i>	0.13	0.29	0.20	0.07	0.30
<i>Proteobacteria</i>					
<i>Succinivibrionaceae</i>	1.57	0.75	5.60	1.79	0.14
<i>Alcaligenaceae</i>	0.04	0.03	0.03	0.01	0.97
<i>Comamonadaceae</i>	0.02	0.03	<0.01	0.02	0.70
<i>Spirochaetes</i>					
<i>Spirochaetaceae</i>	3.16 ^a	2.20 ^{ab}	0.78 ^b	0.74	0.08
<i>Actinobacteria</i>					
<i>Bifidobacteriaceae</i>	0.76	1.31	1.45	0.47	0.56
<i>Coriobacteriaceae</i>	0.11	0.05	0.15	0.06	0.51
<i>Tenericutes</i>					
Uncultured RF9	0.31	0.32	0.22	0.06	0.46
<i>Fibrobacteres</i>					
<i>Fibrobacteraceae</i>	0.59	0.13	0.34	0.29	0.50
Candidate division TM7					
Uncultured rumen bacteria	0.10	0.14	0.08	0.02	0.16
Other candidate division TM7	0.05	0.02	0.04	0.02	0.37
<i>Cyanobacteria</i>					
Other <i>Chloroplast</i>	0.11	0.03	0.04	0.04	0.34
<i>Lentisphaerae</i>	0.08	0.07	0.07	0.03	0.98
Uncultured rumen bacteria	0.01	0.01	0.01	0.01	0.99
<i>Verrucomicrobia</i>					
<i>Verrucomicrobiaceae</i>	0.05	0.18	0.12	0.05	0.19

^{a,b}Means within a row differ ($P < 0.05$).

¹Sulfate and glycinate treatments provided all supplemental Cu, Zn, and Mn from sulfate or glycinate sources, respectively. The sulfate + glycinate Zn treatment provided supplemental Cu and Mn from sulfate sources and Zn from glycinate.

Spirochaetaceae are free-living or host-associated, obligate, or anaerobic spiral-shaped bacteria that have high motility (Karami et al., 2014). *Spirochaetes* such as *Treponema* are unique because they have a rapidly rotating axial flagellum that allows them to move freely and invade and colonize host tissues, and are known to cause diseases such as Lyme disease and syphilis (Karami et al., 2014). In cattle, *Treponema* bacteria have been associated with bovine digital dermatitis

(Brandt et al., 2011; Evans et al., 2011, 2012). Amplified *Treponema* DNA strains *T. pedis* sp. Nov., *T. medium* ssp. *bovis*, and *T. phagedenis* ssp. *vaccae* were identified in 51.0, 37.7, and 4.4% of skin lesions in dairy cows diagnosed with bovine digital dermatitis (Brandt et al., 2011). *Treponema* species associated with bovine digital dermatitis have been observed in the rumen, feces, slurry, and skin lesions (Klitgaard et al., 2014; Zinicola et al., 2015) and may be transferred by touch

Figure 2. Treatment effects ($P = 0.10$) on fecal *Treponema* relative abundance (in excess of 0.5%) in lactating dairy cows fed supplemental minerals for 16 d (covariate-adjusted using d 0 abundance). Sulfate and glycinate treatments provided all supplemental Cu, Zn, and Mn from sulfate or glycinate sources, respectively. The sulfate + glycinate Zn treatment provided supplemental Cu and Mn from sulfate sources and Zn from glycinate. ^{a,b}Mean relative abundance differs ($P < 0.05$) between treatments. Error bars represent SEM. Color version available online.

(Evans et al., 2012). The bovine gut has been suggested to be a primary reservoir of bacteria related to digital dermatitis (Zinicola et al., 2015).

Increased keratinization in response to feeding supplemental Zn (Moore et al., 1989; Kessler et al., 2003), as well as other trace nutrients (Tomlinson et al., 2004), has long been the suspected mechanism for trace nutrients improving hoof health. Data from our experiment suggest an additional mode of action. Zinc glycinate may improve hoof health by reducing hoof exposure to the *Treponema* bacterial species associated with bovine digital dermatitis via reduced fecal excretion of the bacteria.

Zinc is an essential nutrient to bacterial metabolism and increases extracellular stability when bound to the exterior bacterial cell wall (Durand and Kawashima, 1980). *Campylobacter jejuni*, a commensal bacterium in chickens, is unable to replicate or colonize in the gastrointestinal tract when the bacteria is lacking Zn transporter ZnuA (Gielda and DiRita, 2012). Extremely high Zn (3,100 mg/kg) supplementation from Zn oxide to weanling pigs increased intestinal IgA and reduced mesenteric lymph nodes, possibly by altering cytokine profiles in gut-associated lymphatic tissue, and neutralization of bacteria (Broom et al., 2006).

The mechanism of action for the observed treatment differences in bacterial fecal relative abundance is unclear. Why cows fed Cu and Mn from sulfates with organic Zn had less fecal relative abundance of *Spirochaetes* bacteria compared with the all glycinate

treatment is also unclear. Copper can be toxic to rumen bacteria (Durand and Kawashima, 1980) and Cu sulfate footbaths are used on dairy farms to treat hoof disease. Increased concentrations of soluble, reduced Cu may have been present in the intestine when Cu sulfate was fed compared with glycinate Cu, and these effects combined with Zn glycinate provided the optimal environment to reduce fecal abundance of *Spirochaetes* bacteria. Further experimentation is needed to elucidate the mode of action. However, no intestinal observations were made, and fecal excretion of bacteria may not mimic intestinal abundance.

CONCLUSIONS

Feeding Cu and Mn sulfate with Zn glycinate at modest supplemental concentrations decreased fecal relative abundance of *Treponema* spp. within phylum *Spirochaetes*. *Treponema* spp. have been associated with the occurrence of bovine digital dermatitis, and decreasing excretion may improve hoof health. The mechanisms of action for reduced relative abundance, why the combination of mineral sources had the greatest response compared with exclusively sulfate or glycinate sources, or if less excretion of the bacteria species means less colonization are unclear. However, this study identified a new potential mode of action for the positive effects organic Zn can have on bovine digital dermatitis. Further investigation into the health effects and mechanism of action of these observations is warranted.

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