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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 often 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 diet[1] (% 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	—

[1]The 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.

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

[3]Sodium selenate, 200 mg/kg.

[4]Contained 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.

[5]B-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 (± 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