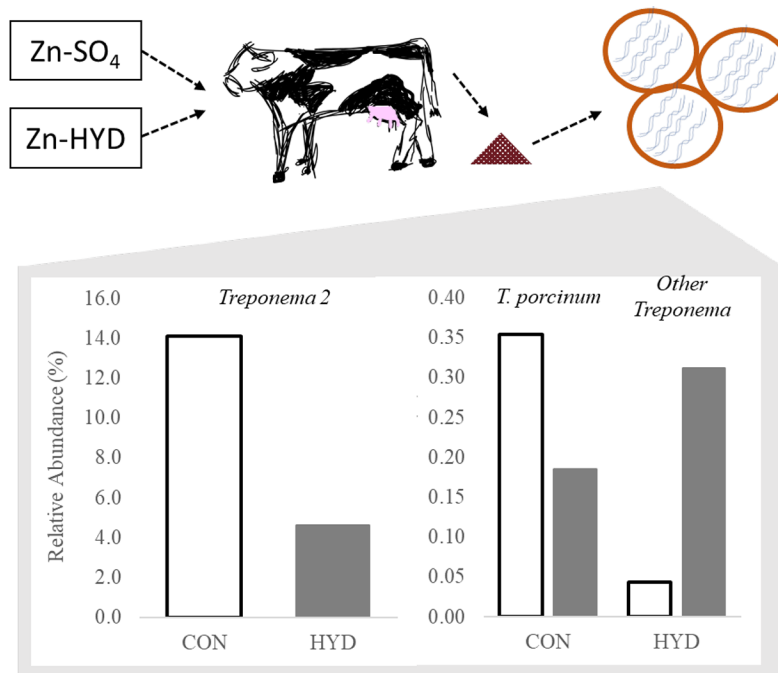


Effect of zinc source (zinc sulfate or zinc hydroxychloride) on relative abundance of fecal *Treponema* spp. in lactating dairy cows

B. A. Wenner,^{1*}† T. Park,² K. Mitchell,¹ S. K. Kvidera,^{3*} K. E. Griswold,³ E. A. Horst,⁴ and L. H. Baumgard⁴

Graphical Abstract

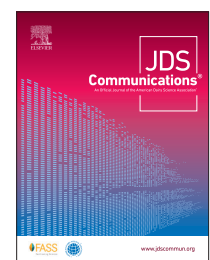


Summary

The present study describes the difference in fecal microbiome between cows fed supplemental zinc either in a sulfate or hydroxychloride form. Supplemental zinc hydroxychloride under ad libitum feeding conditions decreased *Treponema* spp., particularly those sequences within *Treponema 2*, by 67% compared with a similar diet with zinc sulfate, whereas other changes in the fecal microbiome were minimal. The shift in *Treponema* spp. from zinc source could reflect a fecal microbiome response to zinc availability, but it is unknown if that shift reflects a pathogenic risk to the ruminant.

Highlights

- Cows (n = 24) were fed typical lactating diets that only differed in zinc source inclusion.
- Fecal samples collected from cows were extracted for prokaryotic 16S gene DNA.
- Feeding zinc hydroxychloride decreased the *Treponema* spp. recovered from fecal samples compared with cows fed zinc sulfate.



¹Department of Animal Sciences, The Ohio State University, Columbus 43210, ²Department of Animal Science and Technology, Chung-Ang University, Anseong, Gyeonggi-do, Korea, ³Micronutrients USA LLC, Indianapolis, IN 46241, ⁴Department of Animal Science, Iowa State University, Ames 50011.

*Current location: Elanco Animal Health, Greenfield, IN 46140. †Corresponding author: wenner.20@osu.edu. © 2022, The Authors. Published by Elsevier Inc. and FASS Inc. on behalf of the American Dairy Science Association®. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>). Received April 06, 2022. Accepted May 23, 2022.

Effect of zinc source (zinc sulfate or zinc hydroxychloride) on relative abundance of fecal *Treponema* spp. in lactating dairy cows

B. A. Wenner,^{1*}†  T. Park,²  K. Mitchell,¹  S. K. Kvidera,^{3*} K. E. Griswold,³  E. A. Horst,⁴
and L. H. Baumgard⁴ 

Abstract: Previous research revealed a potential effect of dietary trace mineral source on both ruminal and fecal microbiota. However, the effect of Zn source, specifically, has not previously been considered. Based on reported post-ruminal solubility, we hypothesized that Zn hydroxychloride would decrease *Treponema* spp. fecal excretion relative to cows fed Zn sulfate. To test this hypothesis, lactating Holstein cows ($n = 24$; 685 ± 9 kg of body weight; 159 ± 8 d in milk; parity 3 ± 0.2) were randomly assigned to 1 of 2 dietary treatments: control (75 mg/kg Zn from ZnSO₄) or Zn hydroxychloride (HYD; 75 mg/kg IntelliBond Z; Micronutrients USA LLC). Single fecal grab samples were collected on d 1 before dietary treatments and on d 27 after dietary treatments were applied. Fecal microbial DNA was extracted and sequenced to establish taxonomy using a universal primer for the 16S rRNA gene. Supplementation of HYD decreased the relative abundance of *Treponema* 2 by 3-fold (14.7% vs. 4.9%). Poor sequencing resolution at the species level limited inference of *Treponema* spp. toward management or gut health implications of HYD supplementation. However, the inclusion of pathogenic species among *Treponema* spp. indicates a potential implication of HYD feeding to reduce environmental exposure of the dairy cow to *Treponema* spp.

Since the 1960s, Zn has been recognized as an essential micro-mineral in dairy cattle nutrition. More recently, the Zn source has been shown to have broader effects on animal health and metabolism beyond variation in bioavailability or cost (Weiss, 2017). Ionic salts such as ZnSO₄ are highly soluble and dissociate into elemental zinc (Zn²⁺) and sulfate (SO₄²⁻) ions quickly in an aqueous environment such as the rumen. In contrast, trace mineral sources with strong covalent bonds such as hydroxychloride trace minerals may prevent the metal from being released early and rapidly in the digestive tract (Goff, 2018).

Zinc-amino acid chelates have specifically been shown to shift rumen microbiomes compared with ZnSO₄ (Ishaq et al., 2019), whereas hydroxychloride trace minerals have been shown to positively affect fiber digestibility in both cows (Faulkner and Weiss, 2017; Miller et al., 2020) and sheep (VanValin et al., 2018). Both Zn-AA chelates and Zn hydroxychloride can have improved intestinal bioavailability compared with inorganic sources (Schlegel and Windisch, 2006; Shaeffer et al., 2017); this improved bioavailability may be due to the ability of covalently bonded Zn to avoid antagonistic reactions, resulting in less mineral binding to digesta contrasting with sulfate trace minerals (Caldera et al., 2019). More mineral availability throughout the lower digestive tract (Klasing and Naziripour, 2010) may positively or negatively affect microbial populations within the lower gut of cattle but remains to be fully investigated.

Given the role of Zn in *Treponema* metabolism (Hazlett et al., 2003) and the potential implication of *Treponema* spp. in bovine digital dermatitis (Klitgaard et al., 2014; Moreira et al., 2018),

our objective in the present study was to evaluate the effect of Zn source on fecal microbiota relative abundance. Based on a single study comparing glycinate mineral sources versus sulfates and their effect on fecal *Treponema* (Faulkner et al., 2017), we hypothesized that Zn hydroxychloride would shift the fecal microbiome away from *Treponema* spp. when replacing supplemental ZnSO₄.

All animal procedures were approved by the Iowa State University Institutional Animal Care and Use Committee as described previously in a companion study (Horst et al., 2020). Briefly, lactating Holstein cows ($n = 24$; 685 ± 9 kg of BW; 159 ± 8 DIM; parity 3 ± 0.2) were fed in an experiment consisting of 2 replications ($n = 12$ /replicate). Cows were randomly assigned to 1 of 2 dietary treatments: (1) control (CON; 75 mg/kg Zn from ZnSO₄; $n = 12$) or (2) Zn hydroxychloride (HYD; 75 mg/kg IntelliBond Z; Micronutrients USA LLC; $n = 12$). At the start of the study (d 0) cows were fed their respective treatment diets for 21 d. After the initial feeding phase, cows were moved to individual box stalls (4.57 × 4.57 m) for metabolic measurements relevant to the previous study (Horst et al., 2020). Cows were allowed 3 d to acclimate to housing conditions, then maintained in box stalls on their present diet ad libitum.

Throughout the experiment, all cows were fed a TMR formulated to meet or exceed the predicted requirements for energy, protein, minerals, and vitamins (NRC, 2001). While the diet is described in greater detail in Horst et al. (2020), it consisted of 30.3% corn silage, 23.5% alfalfa hay, 18.8% ground corn, 10.3% corn gluten pellets, 6.0% soybean meal (treated and untreated), 3.4% whole cottonseed, and 7.7% premix. Analysis of TMR and diet details

¹Department of Animal Sciences, The Ohio State University, Columbus 43210, ²Department of Animal Science and Technology, Chung-Ang University, Anseong, Gyeonggi-do, Korea, ³Micronutrients USA LLC, Indianapolis, IN 46241, ⁴Department of Animal Science, Iowa State University, Ames 50011.

*Current location: Elanco Animal Health, Greenfield, IN 46140. †Corresponding author: wenner.20@osu.edu. © 2022, The Authors. Published by Elsevier Inc. and FASS Inc. on behalf of the American Dairy Science Association®. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>). Received April 06, 2022. Accepted May 23, 2022.

are also available in the previously published study (Horst et al., 2020). Briefly, the control diet was 32.5% NDF, 16.2% CP, 24.6% starch, and 53.8% forage. Dietary Zn was 92.4 and 92.5 mg/kg, including 17.4 and 17.5 mg/kg baseline Zn within the diet for CON and HYD, respectively. Feed was provided once daily for all cows (0800 h) and feed intake averaged 26.5 kg/d (Horst et al., 2020).

On d 1 (pre-treatment) and d 27, a single fecal grab sample was obtained directly from each cow following the morning milking (0600 h) and before feeding. Individual grab samples were sub-sampled into 15-mL RNAase- and DNase-free conical tubes, then stored at -80°C within 1 h of sample collection until being shipped for analysis. Fecal DNA was extracted using the repeated bead beating and column method (Yu and Morrison, 2004) and DNA purified using Qiagen (Thermo Fisher Scientific Inc.) mini-stool kits. Researchers performing extractions were blind to treatment at the time of extraction and taxonomy assignment. Samples were compared with a 1 kb ladder for quality control and to assess DNA fragment length.

Amplicon libraries were prepared targeting the V4–V5 hypervariable region of the 16S rRNA gene using 515F (5'-GAGTGC-CAGCMGCCGCGGTAA-3') and 806R (5'-ACGGACTACH-VGGGTWTCTAAT-3') universal primers (Caporaso et al., 2011) with each library having a unique barcode for multiplexing at the Molecular and Cellular Imaging Center (The Ohio State University). Amplicon libraries were pooled and sequenced (2×300 bp paired-end sequencing) using the MiSeq platform (Illumina Inc.). Quality control (denoising, merging, removal of chimeras) was applied through QIIME2 (version 2019.10; Bolyen et al., 2019), similarly to Lee et al. (2021). The final quantity of quality amplicon sequencing variants (ASV) used in this analysis numbered 3,855,702 and were clustered into taxonomy based on 99% similarity using the Silva 16S reference database (NR 132 version; Quast et al., 2013).

Phyla, families, and genera present in at least 5 samples at a relative abundance of ASV greater than or equal to 0.5% were included in analysis. Alpha diversity indices were derived from rarified ASV (21,069 ASV per sample) including richness, Chao1, Shannon's index, Pielou's evenness, Good's coverage, and Faith's phylogenetic diversity. Phylogenetic investigation of communities by the reconstruction of unobserved studies (PICRUSt2; Douglas et al., 2020) was used to predict microbial metabolic functions from 16S ASV. Kyoto Encyclopedia of Genes and Genomes (KEGG) modules were reconstructed from predicted KEGG ortholog profiles in PICRUSt2 and represent microbial metabolic functions.

Microbial relative abundance data were analyzed using samples taken on d 1 as a covariate. The relative abundance data were not transformed because previous transformations of relative abundance were not effective in improving distribution normality (Faulkner et al., 2017). Using R project, relative abundances and predicted KEGG ortholog pathways were compared using the model where Y_{ij} is the dependent variable, μ is the overall population mean, N_i is the fixed effect of i th mineral treatment ($i = \text{CON, HYD}$), b represents the covariate of baseline relative abundance during the adaptation period, and ϵ_{ij} is the residual error, assumed independent and normal. Sequencing data are available upon request from the corresponding author. A Kenward-Roger degrees of freedom correction was applied to protect against type 1 error. Alpha diversity measures were compared using the same model

except without the covariate adjustment for the pre-treatment sampling. Beta diversity was visualized using principal coordinate analysis based on comparisons using Bray-Curtis distance matrices via the emperor plugin (Vázquez-Baeza et al., 2013) implemented within QIIME2. The microbial community differences were statistically analyzed using PERMANOVA test implemented in QIIME2.

A general description of the community recovered from the fecal samples at the phyla level is summarized in Figure 1. The top 97.2% of ASV relative abundance on average was dominated by *Firmicutes* (48.4%), *Bacteroidetes* (37.7%), *Spirochaetes* (7.7%), and *Actinobacteria* (3.3%). Whereas *Firmicutes* and *Bacteroidetes* dominated the microbial community, *Bacteroidetes* were proportionally lower than expected from previous work (Faulkner et al., 2017), where they were reported at 45.8% and 42.1%, respectively, but is more in line with the greater ratio of *Firmicutes*:*Bacteroidetes* recently seen in other cow studies (Huang et al., 2020; Li et al., 2020) evaluating fecal microbiota. Recovery of sequences within *Spirochaetes* was nearly 6-fold greater in the present study than previously reported (Faulkner et al., 2017; Huang et al., 2020); thus, it may be reasonable to scale inferred expected changes in *Spirochaetes*. There were no differences in α -diversity measures between CON and HYD ($P \geq 0.11$, Table 1) nor in β -diversity assessed by Bray-Curtis ($P = 0.11$).

The only genus within *Spirochaetes* with relative abundance greater than 0.5% was *Treponema 2*. With the exception of uncultured *Barnesiellaceae* (average 0.45% relative abundance, $P = 0.03$, data not shown), *Treponema 2* was the only genus significantly affected by Zn source. Relative abundance of *Treponema 2* was decreased 3-fold in cows fed HYD ($P = 0.05$; Table 1). Although most other prokaryotic genera remained static, the dramatic shift in *Treponema 2* supports our hypothesis that a shift in Zn source would decrease *Treponema* spp. within the fecal microbiome. The only other previous study to quantify a fecal microbial response to mineral source, Faulkner et al. (2017) found that a shift from sulfate to glycinate-based Cu, Mn, and Zn did not significantly decrease *Treponema* recovered from feces. However, a shift in Zn source only from sulfate to glycinate source did decrease *Treponema* spp. by 4-fold.

In vitro dosing of ZnSO_4 by Martinez and Church (1970), as well as Eryavuz and Dehority (2009), demonstrated that rumen microbes are sensitive to high concentrations of Zn, whereas Zinicola et al. (2015) reported the rumen as a downstream contributor of *Treponema* to the lower digestive tract of cows and ultimately to the fecal slurry. Given the demonstrated effect of mineral source on NDF digestibility (Faulkner and Weiss, 2017), it is possible that the fecal microbiome response measured in our current study could be a response to a shift in nutrient outflow exiting the rumen, especially as *Treponema* spp. are known fiber degraders. A less soluble Zn source within the rumen could have increased ruminal fiber digestibility and therefore decreased opportunity for a niche fiber degrader such as *Treponema* spp. (Bekele et al., 2011) within the lower digestive tract. However, ruminal digestibility of NDF was not reported in Horst et al. (2020). Further, a recent study found most microbiome effects within the rumen were undetectable within the fecal microbiome (Noel et al., 2019); thus, we expect the effect of Zn source to be localized to conditions within the lower digestive tract rather than a result of microbial flow from

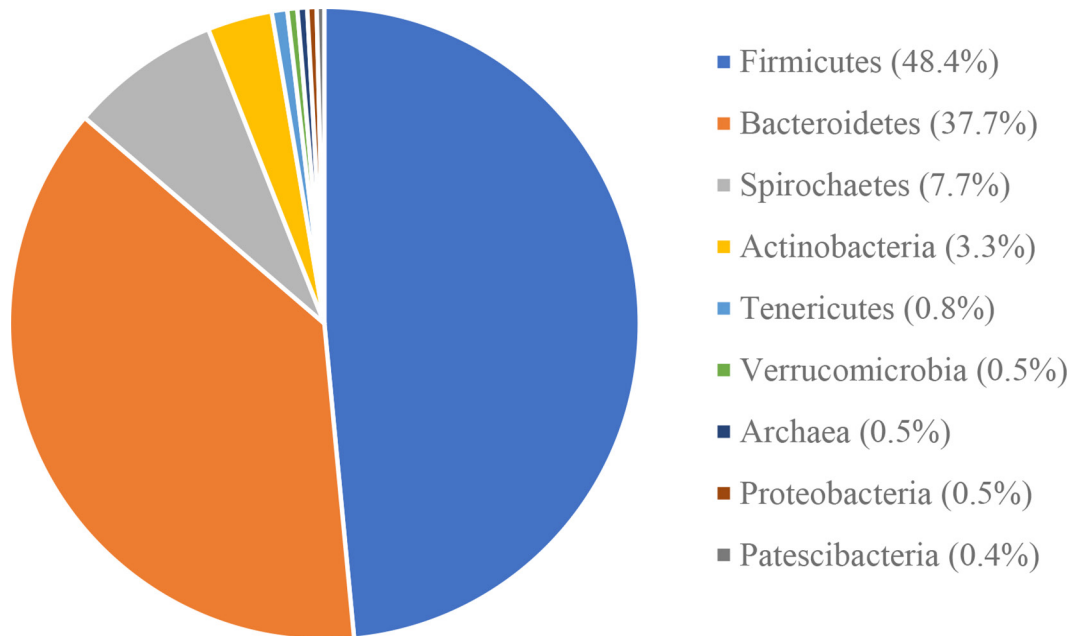


Figure 1. Average (across treatments) fecal microbial phyla relative abundance for all phyla in excess of 0.5% relative abundance from lactating dairy cows fed either 75 mg/kg supplemental ZnSO₄ or 75 mg/kg supplemental Zn hydroxychloride.

the rumen. We consider a rumen outflow effect unlikely due to the lack of variation among 47 other leading genera recovered from the fecal samples, including other cellulolytics.

Little is known about microbial sensitivity to mineral source within the lower gastrointestinal tract, particularly in ruminants where rumen interactions can confound mineral availability in the lower digestive tract. Given the difference in source between the current study (Zn hydroxychloride) and previous work using glycinate minerals (Faulkner et al., 2017), it is also possible that a competitive response of *Treponema* to sulfate sources—a common control in both studies—or their insoluble mineral complexes postabomasum, or both, better describes the similarities with 2 alternative mineral sources decreasing *Treponema* spp. relative

abundance compared with compared with sulfate mineral sources. It has been known for some time that Zn is required by *Treponema* spp. (Hazlett et al., 2003) and other pathogenic members of *Spirochaetes*, such as *Borellia burgdorferi* (Boylan et al., 2003). Although it could be argued that a more intestinally bioavailable Zn source would also favor the microbes inhabiting the lower digestive tract, it is more likely that *Treponema* spp. hold a competitive advantage within the lower digestive tract where insoluble Zn complexes are poorly available except to those microbes capable of scavenging mineral complexes. However, in the present study, data on absorption of Zn are unavailable and it is possible that both diets passed enough Zn to the lower digestive tract that solubility was a moot issue. We propose that when a greater proportion of Zn

Table 1. Relative abundance of 16S rRNA gene sequences for recovered *Treponema* spp. and diversity indices for fecal microbiome sequences of cows fed either Zn sulfate or Zn hydroxychloride

Amplicon sequencing variant	CON ¹	HYD	SE	P-value ²
<i>Treponema</i>	14.7	4.9	3.1	0.05
<i>Treponema 2</i>	14.1	4.6	3.0	0.05
<i>Treponema porcinum</i>	0.354	0.043	0.103	0.06
Uncultured <i>Treponema</i>	0.185	0.312	0.056	0.13
Gut metagenome <i>Treponema</i>	0.009	0.017	0.005	0.24
Diversity metric				
Richness	567	700	66	0.16
Chao1	573	714	67	0.16
Shannon's diversity	7.50	7.96	0.21	0.13
Inverse Simpson	16.3	19.5	1.3	0.11
Pielou's evenness	0.826	0.848	0.016	0.33
Good's coverage	0.999	0.999	0.0004	0.25
Faith's phylogenetic diversity	30.8	34.7	1.7	0.12

¹Treatments consisted of either 75 mg/kg supplemental ZnSO₄ (CON) or 75 mg/kg supplemental Zn hydroxychloride (HYD).

²P-values reported for the effects of HYD.

within digesta is bound in insoluble complexes, less is absorbed within the small intestine, and a greater opportunity exists for *Treponema* spp. to uptake Zn to meet their requirement within the large intestine.

Radolf et al. (2016) note heavy reliance on Fe within *Treponema pallidum*, a known human pathogen, that correlates to PICRUSt2 data in the present study, indicating a 50% reduction in Fe(III) transport pathway predictions (KEGG M00190, $P = 0.04$, data not shown). A decrease in relative abundance of iron transport systems in HYD might alter the gut microbial composition toward less pathogenic bacterial populations residing in the gut (Lee et al., 2008; Zimmermann et al., 2010). Additionally, the inhibition of *Borrelia burgdorferi* (a spirochete implicated in Lyme disease) by high Mn that was experimentally countered with increasing Zn dosage (Troxell et al., 2013) is further evidence toward a hypothesis that mineral availability (either to the cow or lack thereof) could influence the prevalence of *Treponema* spp. within the lower intestinal tract. Genome studies in *Treponema pallidum*, a pathogenic *Treponema* known for syphilis, indicate a requirement for Zn (Hazlett et al., 2003; Houston et al., 2012; Radolf et al., 2016) linked to specific transporters and transcription activators. This requirement for related species makes it increasingly unlikely that *Treponema* spp. in the present study decreased in the lower digestive tract as a response to purported increased Zn availability from chelated or hydroxychloride mineral sources. Ultimately, whether or not the prevalence of *Treponema* spp. is responding to increased availability of a Zn source for absorption in the small intestine or responding to a decreased concentration of insoluble Zn was not directly addressed within this study.

High-throughput 16S sequencing approaches with a universal primer have limited resolution at the species level, including the present fecal microbiome data. In the present study, there were only 4 *Treponema* species-level ASV identified and their proportions relative to total *Treponema* spp. are presented in Table 1. The only significant decrease in *Treponema* was for those sequences within unresolved sequences of *Treponema 2* ($P = 0.05$). Although no sequences were specifically identified that matched pathogenic strains of *Treponema*, more than 20 species are contained within *Treponema 2* including potential pathogens (Edwards et al., 2020). Thus, although not a sole causative factor for bovine digital dermatitis (Klitgaard et al., 2017), the potential hoof health benefit of decreased *Treponema* spp. fecal excretion cannot be ruled out. *Treponema* spp. within manure slurry may pose a likely indictment for bovine digital dermatitis (Klitgaard et al., 2014; Zinicola et al., 2015) but more recent papers cast doubt on whether fecally excreted *Treponema* spp. are a causative agent for bovine digital dermatitis (Klitgaard et al., 2017; Moreira et al., 2018) or just guilty by association. The exposure of the foot to microbes within manure slurry and evidence of mineral-related response of the fecal microbiome leads to questions on the potential role of bacteria shed in feces toward foot lesion development.

Our data demonstrate a clear linkage between a supplemental Zn source with limited rumen availability and decreased fecal populations of *Treponema* spp. The lack of specific opportunistic pathogen sequence recovery within the current study limits inference on the impact these changes may play in development of bovine digital dermatitis. However, the reduction in *Treponema* highlights the systemic influence of a mineral source bioavailability on micro-

bial populations in the lower digestive tract, regardless of animal health implications. More specific research needs to be conducted to consider the potential effect of sulfates on the fecal microbiome to isolate Zn bioavailability from other chemical mechanisms of influence on fecal populations.

References

- Bekele, A. Z., S. Koike, and Y. Kobayashi. 2011. Phylogenetic diversity and dietary association of rumen *Treponema* revealed using group-specific 16S rRNA gene-based analysis. *FEMS Microbiol. Lett.* 316:51–60. <https://doi.org/10.1111/j.1574-6968.2010.02191.x>.
- Bolyen, E., J. R. Rideout, M. R. Dillon, N. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodriguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvall, C. F. Edwards, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. R. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. J. Jiang, B. D. Kaehler, K. Bin Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolk, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y. X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ull-Hasan, J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 37:852–857. <https://doi.org/10.1038/s41587-019-0209-9>.
- Boylan, J. A., J. E. Posey, and F. C. Gherardini. 2003. *Borrelia* oxidative stress response regulator, BosR: A distinctive Zn-dependent transcriptional activator. *Proc. Natl. Acad. Sci. USA* 100:11684–11689. <https://doi.org/10.1073/pnas.2032956100>.
- Caldera, E., B. Weigel, V. N. Kucharczyk, K. S. Sellins, S. L. Archibeque, J. J. Wagner, H. Han, J. W. Spears, and T. E. Engle. 2019. Trace mineral source influences ruminal distribution of copper and zinc and their binding strength to ruminal digesta. *J. Anim. Sci.* 97:1852–1864. <https://doi.org/10.1093/jas/skz072>.
- Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA* 108(supplement_1):4516–4522. <https://doi.org/10.1073/pnas.1000080107>.
- Douglas, G. M., V. J. Maffei, J. R. Zaneveld, S. N. Yurgel, J. R. Brown, C. M. Taylor, C. Huttenhower, and M. G. Langille. 2020. PICRUSt2 for prediction of metagenome functions. *Nat. Biotechnol.* 38:685–688. <https://doi.org/10.1038/s41587-020-0548-6>.
- Edwards, J. E., S. A. Shetty, P. van den Berg, F. Burden, D. A. van Doorn, W. K. Pellikaan, J. Dijkstra, and H. Smidt. 2020. Multi-kingdom characterization of the core equine fecal microbiota based on multiple equine (sub)species. *Anim. Microbiome* 2:6. <https://doi.org/10.1186/s42523-020-0023-1>.
- Eryavuz, A., and B. A. Dehority. 2009. Effects of supplemental zinc concentration on cellulose digestion and cellulolytic and total bacterial numbers in vitro. *Anim. Feed Sci. Technol.* 151:175–183. <https://doi.org/10.1016/j.anifeeds.2009.01.008>.
- Faulkner, M. J., and W. P. Weiss. 2017. Effect of source of trace minerals in either forage- or by-product-based diets fed to dairy cows: 1. Production and macronutrient digestibility. *J. Dairy Sci.* 100:5358–5367. <https://doi.org/10.3168/jds.2016-12095>.
- Faulkner, M. J., B. A. Wenner, L. M. Solden, and W. P. Weiss. 2017. Source of supplemental dietary copper, zinc, and manganese affects fecal microbial relative abundance in lactating dairy cows. *J. Dairy Sci.* 100:1037–1044. <https://doi.org/10.3168/jds.2016-11680>.

- Goff, J. P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid-base and antioxidant status, and diet considerations to improve mineral status. *J. Dairy Sci.* 101:2763–2813. <https://doi.org/10.3168/jds.2017-13112>.
- Hazlett, K. R. O., F. Rusnak, D. G. Kehres, S. W. Bearden, C. J. La Vake, M. E. La Vake, M. E. Maguire, R. D. Perry, and J. D. Radolf. 2003. The *Treponema pallidum* tro operon encodes multiple metal transporter, a zinc-dependent transcriptional repressor, and a semi-autonomously expressed phosphoglycerate mutase. *J. Biol. Chem.* 278:20687–20694.
- Horst, E. A., E. J. Mayorga, M. Al-Qaisi, S. Rodriguez-Jimenez, B. M. Goetz, M. A. Abeyta, P. J. Gorden, S. K. Kvidera, and L. H. Baumgard. 2020. Evaluating effects of zinc hydroxychloride on biomarkers of inflammation and intestinal integrity during feed restriction. *J. Dairy Sci.* 103:11911–11929. <https://doi.org/10.3168/jds.2020-18860>.
- Houston, S., R. Hof, L. Honeyman, J. Hassler, and C. E. Cameron. 2012. Activation and proteolytic activity of the *Treponema pallidum* metalloprotease, pallilysin. *PLoS Pathog.* 8:e1002822. <https://doi.org/10.1371/journal.ppat.1002822>.
- Huang, S., S. Ji, F. Wang, J. Huang, G. M. Alugongo, and S. Li. 2020. Dynamic changes of the fecal bacterial community in dairy cows during early lactation. *AMB Express* 10:167. <https://doi.org/10.1186/s13568-020-01106-3>.
- Ishaq, S. L., C. M. Page, C. J. Yeoman, T. W. Murphy, M. L. Van Emon, and W. C. Stewart. 2019. Zinc AA supplementation alters yearling ram rumen bacterial communities but zinc sulfate supplementation does not. *J. Anim. Sci.* 97:687–697. <https://doi.org/10.1093/jas/sky456>.
- Klasing, K. C., and A. Naziripour. 2010. Effect of dietary copper source and level on GI copper levels and ileal *E. coli* survival in broiler chicks. *J. Dairy Sci.* 93(E-Suppl.1):498.
- Klitgaard, K., M. W. Nielsen, H. C. Ingerslev, M. Boye, and T. K. Jensen. 2014. Discovery of bovine digital dermatitis-associated treponema spp. in the dairy herd environment by a targeted deep-sequencing approach. *Appl. Environ. Microbiol.* 80:4427–4432. <https://doi.org/10.1128/AEM.00873-14>.
- Klitgaard, K., M. L. Strube, A. Isbrand, T. K. Jensen, and M. W. Nielsen. 2017. Microbiota analysis of an environmental slurry and its potential role as a reservoir of bovine digital dermatitis pathogens. *Appl. Environ. Microbiol.* 83:e00244-17. <https://doi.org/10.1128/AEM.00244-17>.
- Lee, C., J. E. Copelin, T. Park, K. E. Mitchell, J. L. Firkins, M. T. Socha, and D. Luchini. 2021. Effects of diet fermentability and supplementation of 2-hydroxy-4-(methylthio)-butanoic acid and isoacids on milk fat depression: 2. Ruminant fermentation, fatty acid, and bacterial community structure. *J. Dairy Sci.* 104:1604–1619. <https://doi.org/10.3168/jds.2020-18950>.
- Lee, S. H., P. Shinde, J. Choi, M. Park, S. Ohh, I. K. Kwon, S. I. Pak, and B. J. Chae. 2008. Effects of dietary iron levels on growth performance, hematological status, liver mineral concentration, fecal microflora, and diarrhea incidence in weanling pigs. *Biol. Trace Elem. Res.* 126(S1):57–68. <https://doi.org/10.1007/s12011-008-8209-5>.
- Li, H., R. Li, H. Chen, J. Gao, Y. Wang, Y. Zhang, and Z. Qi. 2020. Effect of different seasons (spring vs summer) on the microbiota diversity in the feces of dairy cows. *Int. J. Biometeorol.* 64:345–354. <https://doi.org/10.1007/s00484-019-01812-z>.
- Martinez, A., and D. C. Church. 1970. Effect of various mineral elements on in vitro rumen cellulose digestion. *J. Anim. Sci.* 31:982–990. <https://doi.org/10.2527/jas1970.315982x>.
- Miller, M. D., J. S. Lanier, S. K. Kvidera, H. M. Dann, C. S. Ballard, and R. J. Grant. 2020. Evaluation of source of corn silage and trace minerals on lactational performance and total-tract nutrient digestibility in Holstein cows. *J. Dairy Sci.* 103:3147–3160. <https://doi.org/10.3168/jds.2019-17716>.
- Moreira, T. F., E. J. Facury Filho, A. U. Carvalho, M. L. Strube, M. W. Nielsen, K. Klitgaard, and T. K. Jensen. 2018. Pathology and bacteria related to digital dermatitis in dairy cattle in all year round grazing system in Brazil. *PLoS One* 13:e0193870. <https://doi.org/10.1371/journal.pone.0193870>.
- Noel, S. J., D. W. Olijhoek, F. Mclean, P. Lovendahl, P. Lund, and O. Højberg. 2019. Rumen and fecal microbial community structure of Holstein and Jersey dairy cows as affected by breed, diet, and residual feed intake. *Animals (Basel)* 9:498. <https://doi.org/10.3390/ani9080498>.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th ed. National Academy Press.
- Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* 41(D1):D590–D596. <https://doi.org/10.1093/nar/gks1219>.
- Radolf, J. D., R. K. DeKa, A. Anand, D. Šmajš, M. V. Norgard, and X. F. Yang. 2016. *Treponema pallidum*, the syphilis spirochete: Making a living as a stealth pathogen. *Nat. Rev. Microbiol.* 14:744–759. <https://doi.org/10.1038/nrmicro.2016.141>.
- Schlegel, P., and W. Windisch. 2006. Bioavailability of zinc glycinate in comparison with zinc sulphate in the presence of dietary phytate in an animal model with Zn labelled rats. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 90:216–222. <https://doi.org/10.1111/j.1439-0396.2005.00583.x>.
- Shaeffer, G. L., K. E. Lloyd, and J. W. Spears. 2017. Bioavailability of zinc hydroxychloride relative to zinc sulfate in growing cattle fed a corn-cottonseed hull-based diet. *Anim. Feed Sci. Technol.* 232:1–5. <https://doi.org/10.1016/j.anifeedsci.2017.07.013>.
- Troxell, B., M. Ye, Y. Yang, S. E. Carrasco, Y. Lou, and X. F. Yang. 2013. Manganese and zinc regulate virulence determinants in *Borrelia burgdorferi*. *Infect. Immun.* 81:2743–2752. <https://doi.org/10.1128/IAI.00507-13>.
- VanValin, K. R., O. N. Genter-Schroeder, R. N. Carmichael, C. P. Blank, E. L. Deters, S. J. Hartman, E. K. Niedermayer, S. B. Laudert, and S. L. Hansen. 2018. Influence of dietary zinc concentration and supplemental zinc source on nutrient digestibility, zinc absorption, and retention in sheep. *J. Anim. Sci.* 96:5336–5344. <https://doi.org/10.1093/jas/sky384>.
- Vázquez-Baeza, Y., M. Pirrung, A. Gonzalez, and R. Knight. 2013. EMPeror: A tool for visualizing high-throughput microbial community data. *Gigascience* 2:16. <https://doi.org/10.1186/2047-217X-2-16>.
- Weiss, W. P. 2017. A 100-year review: From ascorbic acid to zinc – Mineral and vitamin nutrition of dairy cows. *J. Dairy Sci.* 100:10045–10060. <https://doi.org/10.3168/jds.2017-12935>.
- Yu, Z., and M. Morrison. 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. *Biotechniques* 36:808–812. <https://doi.org/10.2144/04365ST04>.
- Zimmermann, M. B., C. Chassard, F. Rohner, E. K. N’Goran, C. Nindjin, A. Dostal, J. Utzinger, H. Ghattas, C. Lacroix, and R. F. Hurrell. 2010. The effects of iron fortification on the gut microbiota in African children: A randomized controlled trial in Cote d’Ivoire. *Am. J. Clin. Nutr.* 92:1406–1415. <https://doi.org/10.3945/ajcn.110.004564>.
- Zinicola, M., F. Lima, S. Lima, V. Machado, M. Gomez, D. Döpfer, C. Guard, and R. Bicalho. 2015. Altered microbiomes in bovine digital dermatitis lesions, and the gut as a pathogen reservoir. *PLoS One* 10:e0120504. <https://doi.org/10.1371/journal.pone.0120504>.

Notes

- B. A. Wenner  <https://orcid.org/0000-0002-2333-1297>
 T. Park  <https://orcid.org/0000-0002-4480-4524>
 K. Mitchell  <https://orcid.org/0000-0002-9769-3960>
 K. E. Griswold  <https://orcid.org/0000-0003-3024-7939>
 L. H. Baumgard  <https://orcid.org/0000-0002-3077-5996>

This project was funded by Micronutrients USA LLC (Indianapolis, IN). We are additionally grateful to L. Moraes, formerly within the Department of Animal Sciences at The Ohio State University, for his advice on statistical analysis.

K. E. Griswold is an employee of the funding source for this work. The authors have not stated any other conflicts of interest.