Effect of source of trace minerals in either forage- or by-product-based diets fed to dairy cows: 2. Apparent absorption and retention of minerals

M. J. Faulkner,¹ N. R. St-Pierre,² and W. P. Weiss³

Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster 44691

ABSTRACT

Eighteen multiparous cows were used in a split-plot replicated Latin square with two 28-d periods to evaluate the effects of source of supplemental Cu, Zn, and Mn (sulfates or hydroxy) on apparent absorption of minerals when fed in either a forage- or by-product-based diet. The by-product diets were formulated to have greater concentrations of NDF and lesser concentrations of starch, and specific ingredients were chosen because they were good sources of soluble fiber and β -glucans, which bind trace minerals in nonruminants. We hypothesized that hydroxy trace minerals would interact less with digesta and have greater apparent absorption compared with sulfate minerals, and the difference in apparent absorption would be greater for the by-product diet compared with the forage-based diet. During the 56-d experiment, cows remained on the same fiber treatment but source of supplemental trace mineral was different for each 28-d period; thus, all cows were exposed to both mineral treatments. During each period cows were fed no supplemental Cu, Zn, or Mn for 16 d, followed by 12 d of feeding supplemental minerals from either sulfate or hydroxy sources. Supplemental minerals for each of the mineral sources fed provided approximately 10, 35, and 32 mg/kg of supplemental Cu, Zn, and Mn, respectively, for both fiber treatments. Total Cu, Zn, and Mn dietary concentrations, respectively, were approximately 19, 65, and 70 mg/kg for the forage diets and 21, 85, and 79 for the by-product diets. Treatment had no effect on dry matter intake (24.2 kg/d) or milk production (34.9 kg/d). Cows consuming the by-product diets had greater Zn (1,863 vs. 1,453 mg/d) and Mn (1,790 vs. 1,588 mg/d) intake compared with cows fed forage diets, but apparent Zn absorption was similar between treatments. Manganese apparent absorption was greater for the

by-product diets compared with the forage diets (16 vs. 11%). A fiber by mineral interaction was observed for Cu apparent absorption, as cows fed hydroxy minerals with forage diets had greater apparent absorption compared with cows fed sulfate minerals; however, the opposite was observed with the by-product diets. Source of supplemental trace minerals and type of fiber in diets affected availability of Cu and Mn and should be considered in ration formulation.

Key words: mineral, fiber, by-product, digestibility

INTRODUCTION

As animal production intensifies and environmental constraints increase, chemical and physical forms of minerals and potential interactions of minerals with other feedstuffs must be considered during diet formulation. Substitution of forage fiber with nonforage fiber by-products in dairy rations has been successful (Clark and Armentano, 1993) and can be economically beneficial in certain situations. However, inclusion of small-particle by-products can decrease ruminal pH and the consistency of the rumen mat. These can decrease the rate and extent of fiber digestion and increase the rate of fiber passage (Grant, 1997). Increased flow of undigested fiber fractions increases the binding of divalent minerals, such as Cu and Zn, within the gastrointestinal tract, reducing mineral availability in nonruminants (Drews et al., 1979; van der Aar et al., 1983); however, this has not been studied in ruminants. Binding of polyvalent metal ions to fiber is enhanced in low-pH environments (Torre et al., 1991), similar to that found in the abomasum and proximal small intestine. Increased flow of undigested fiber in by-product diets could increase cationic binding with minerals to a greater extent than observed with forage-based diets.

Decreasing the disassociation of metals in the rumen limits potential antagonism with other digesta, such as fiber fractions, and could increase bioavailability of trace minerals. Hydroxy Cu and Mn (IntelliBond; Micronutrients USA LLC, Indianapolis, IN) are less soluble in the rumen compared with sulfate sources (Genther and Hansen, 2015); however, data are not consistent regarding differences in Zn solubility between

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¹Current address: VitaPlus, Madison WI 53713.

²Current address: Perdue AgriBusiness, Salisbury, MD 21804.

³Corresponding author: weiss.6@osu.edu

sulfate and hydroxy sources (Cao et al., 2000; Genther and Hansen, 2015). The decreased rumen solubility of hydroxy Cu and Mn make these a good experimental model for evaluating the potential antagonistic effects of fiber on apparent mineral absorption in ruminants. The primary objective of our study was to determine whether apparent Cu, Zn, and Mn apparent absorption and retention in lactating dairy cows are affected by the interaction between forage or by-product diets and trace minerals.

MATERIALS AND METHODS

Cows and Treatments

All animal procedures were approved by The Ohio State University Institutional Animal Care Committee. Prior to the experiment, all cows used in the experiment were fed diets that contained 15 to 20 mg/kg of total Cu, 50 to 60 mg/kg of total Zn, and 40 to 50 mg/kg of total Mn for several months and should have had adequate body stores of those trace minerals.

Eighteen Holstein cows (7, 6, and 5 in second, third, and fourth lactation, respectively) were assigned to 3 groups of 6 cows based on DIM (153 ± 36 DIM at the start of the experiment). Groups consisted of 6 cows because only 6 stalls designed for total collection of feces and urine are available at our research center. Therefore, within a group and period, treatment replications were not balanced (i.e., within each period, 2 treatments were fed to 2 cows each and 2 treatments were applied to only 1 cow each). However, over the entire experiment, replication was balanced. Within a group, 3 cows were randomly assigned to either a forage-based diet or a by-product-based diet, and remained on the diet throughout the 56-d experiment. Then, each cow was assigned to 1 of 2 mineral treatment sequences. Hence, the experimental design is a split-plot Latin square with two 28-d periods. Each of the 28-d periods consisted of a 16-d phase where no supplemental Cu, Zn, or Mn were fed, followed by a 12-d phase of feeding either sulfate or hydroxy (IntelliBond C, Z, and M; Micronutrients USA LLC) supplemental minerals. Diet ingredients and nutrient composition are in Tables 1, 2, and 3. Forage and by-product diets were formulated to provide different concentrations of NDF and starch (Table 2). To achieve differing NDF and starch concentrations, a portion of corn silage, alfalfa silage, and all corn grain from the forage diet were replaced with rolled oats, dried corn gluten, dried beet pulp, and soy hulls in the by-product diet (Table 1). These ingredients (Table 2) were chosen to increase dietary concentrations of soluble fiber (specifically, β -glucans and pectin), which have negatively influenced min-

eral absorption in nonruminants (Torre et al., 1991). Supplemental trace minerals were provided in a top dress (a mix of the appropriate concentrate plus appropriate amount of either sulfate or hydroxyl Cu, Mn, and Zn) fed at 0.2 kg/d. This rate supplied dietary concentrations of 10, 35, and 30 mg/kg of diet DM of supplemental Cu, Zn, and Mn based on average DMI. Actual concentrations of supplemental minerals were similar and close to the target (Table 3). As expected, concentrations of trace minerals in basal diets differed because they were based on different ingredients. Additional information regarding materials and methods are reported in the companion paper (Faulkner and Weiss, 2017).

Cows were housed in individual tiestalls and fed once daily with a target refusal rate of 5% delivered feed. Cows were milked twice daily at approximately 0200 and 1400 h (milk yields were measured electronically). Individual feed delivery and refusal amounts were weighed and recorded daily.

Digestion Collection and Feed Samples

On d 24 of each period (d 8 of feeding supplemental minerals) cows were moved to specifically designed stalls and total output of milk, urine, and feces was measured for 4 d (Weiss et al., 2009). Urine and milk collection containers were acid-washed (50% sulfuric acid) before each collection period, and fecal and feed samples were stored in new plastic bags. Urine (not acidified), milk, orts, and feeds were sampled daily (kept refrigerated) and composited (within cow) over the 4 d. Water intake was measured and recorded daily during the collection periods by water meters connected to each individual water bowl. Silage, fecal, and refusal samples were frozen, lyophilized, and ground through a 1-mm screen (Wiley mill; Arthur H. Thomas Co., Philadelphia, PA). Concentrates were ground but not dried. Feed, orts, and fecal samples were sent to Cumberland Valley Analytical Services (Hagerstown, MD) for mineral analyses using standard wet chemistry methods (AOAC International, 2000; CVAS, 2014). Mineral samples were ashed (1 h at 535°C), digested in 15% nitric acid, diluted, and assayed by inductively coupled plasma emission spectroscopy. Methods for feed analyses were described in a companion manuscript (Faulkner and Weiss, 2017).

On d 28, blood samples were collected from the tail vein into trace mineral free vacutainers (BD Vacutainer, Franklin Lakes, NJ) for serum and into heparinized tubes for whole blood assays. Whole blood was assayed in duplicate for hemoglobin (Kit Cat. No. MAK115, Sigma-Aldrich, St. Louis, MO) and superoxide dismutase (**SOD**) activity (Ransod SD 125, Randox Laboratories, Crumlin, UK). Blood was allowed to clot;

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

Ingredient	Forage		By-product	
	Sulfate	Hydroxy	Sulfate	Hydroxy
Corn silage	44.0	44.0	20.0	20.0
Alfalfa silage	20.0	20.0	15.0	15.0
Ground corn	18.5	18.5	—	—
Soybean meal, 48% CP	14.9	14.9	8.5	8.5
Rolled oats	—	—	14.1	14.6
Dried corn gluten feed	—	—	11.0	11.0
Dried beet pulp	—	—	15.0	15.0
Soy hulls	—	—	14.1	14.1
Animal/vegetable fat	0.51	0.51	0.65	0.65
Limestone	0.69	0.69	0.47	0.47
Magnesium oxide	0.16	0.16	0.07	0.07
Dicalcium phosphate	0.25	0.25	0.13	0.13
Iodized salt	0.48	0.48	0.42	0.42
Selenium premix ²	0.15	0.15	0.15	0.15
Vitamin mix ³	0.37	0.37	0.37	0.37
Cu sulfate	0.0003	—	0.0003	—
Zn sulfate	0.0008	—	0.0008	—
Mn sulfate	0.0008	—	0.0008	—
Hydroxy Cu ⁴	—	0.0001	—	0.0001
Hydroxy Zn ⁴	—	0.0005	—	0.0005
Hydroxy Mn ⁴	—	0.0006	—	0.0006

¹Forage- or by-product-based diets with Cu, Zn, and Mn from sulfate or hydroxy mineral sources.

²Sodium selenate, 200 mg/kg.

³Contained 735 kIU of vitamin A/kg, 270 kIU of vitamin D/kg, 4,400 IU of vitamin E/kg and 135 mg of biotin (Rovimix Biotin, DSM Nutritional Products, Heerlen, the Netherlands)/kg.

⁴IntelliBond C, Z, M (IntelliBond; Micronutrients USA LLC, Indianapolis, IN). Based on assays, the products contained approximately 59, 57, and 45% Cu, Zn, and Mn, respectively.

Table 2. Analyzed nutrient composition of diets and forages without supplemental Cu, Zn, and Mn

Item	Diet ¹		Corn silage	Alfalfa silage	Forage concentrate	By-product concentrate
	Forage	By-product				
DM, %	55.0	71.6	35.4	39.8	87.3	90.1
OM, %	93.7	92.8	96.7	88.7	92.9	92.5
NDF, %	25.3	36.4	32.4	36.7	10.2	37.6
Hemicellulose, ² %	10.9	14.1	15.6	9.0	6.1	14.8
β-Glucan, %	0.06	0.29	0.05	0.02	0.09	0.42
NDSF, ³ %	4.40	7.97	2.47	8.74	4.34	9.48
CP, %	16.3	17.2	7.0	21.2	24.8	19.4
Starch, %	35.4	17.6	39.3	1.6	49.4	14.7
LCFA, ⁴ %	3.66	3.54	2.86	2.64	5.22	4.0
Ca, %	0.94	1.00	0.26	1.0	1.3	0.95
P, %	0.34	0.40	0.21	0.31	0.36	0.37
Mg, %	0.25	0.30	0.16	0.26	0.27	0.28
K, %	1.58	1.53	1.00	3.84	1.03	1.16
S, %	0.16	0.20	0.09	0.27	0.18	0.22
Na, %	0.22	0.30	0.03	0.02	0.46	0.60
Fe, mg/kg	257	453	70	460	205	500
Mo, mg/kg	1.81	1.60	0.61	1.29	3.55	1.98
Cu, mg/kg	9	11	6	13	10	12
Zn, mg/kg	30	50	23	28	39	64
Mn, mg/kg	38	47	34	65	29	48

¹Forage- or by-product-based diets with Cu, Zn, and Mn from sulfate or hydroxy mineral sources.

²Hemicellulose = NDF – ADF.

³Neutral detergent-soluble fiber by difference (Hall et al., 1999).

⁴LCFA = long-chain fatty acids.

serum was then harvested ($1,000 \times g$ for 10 min at 4°C) and frozen (-20°C). Serum, urine, and milk samples were assayed for Cu, Zn, and Mn by Michigan State University Veterinary Diagnostic Laboratory (East Lansing, MI) using inductively coupled plasma MS (Wahlen et al., 2005).

Calculations and Statistical Analyses

Apparent absorption $[(\text{intake} - \text{fecal})/\text{intake}]$ and apparent retention $(\text{intake} - \text{fecal} - \text{milk} - \text{urine})$ of Cu, Zn, and Mn were calculated for each cow during each of the two 4-d collection periods. Concentrations of all minerals in the water consumed were low (data not shown) and intakes of Cu, Zn, and Mn from water were negligible (<1.0 mg/d) and were not included in retention or absorption measures. All data were analyzed using PROC MIXED of SAS (v9.3; SAS Institute Inc., Cary, NC). One cow fed the by-product treatment with a mineral sequence of sulfate then hydroxy minerals was removed from the experiment after period 1 for reasons not related to treatment. Copper data from 1 group for 1 period was removed because all Cu apparent retention values were extremely negative (average = -159 mg/d) across all treatments. For unknown reasons, average fecal Cu (mg/kg and mg/d) were approximately 30% greater for the removed period compared with the average of all other 5 group-periods. All data other than Cu were used from this period. Blood indices and digestion data were evaluated using a model that included the fixed effects of fiber (1 df), mineral source (1 df), their interaction (1 df), and the random effects of group (2 df), group $\times$ fiber (2 df), period (1 df), period $\times$ group (3 df), cow within group $\times$ fiber, and residual error.

RESULTS AND DISCUSSION

Production measures and macronutrient digestibility are discussed elsewhere (Faulkner and Weiss, 2017). Briefly, production measures, with the exception of milk fat and MUN, were not affected by treatment ($P \geq 0.26$). Cows fed the forage diets had greater milk fat and MUN than cows fed the by-product diets. Digestibility of DM was greater ($P < 0.01$) for forage diets (70.1 vs. 65.5%), but NDF digestibility was greater ($P < 0.01$) for by-product diets (50.5 vs. 44.5%).

Total-Tract Apparent Mineral Absorption and Balance

Milk production and DMI (Table 4) during the 4-d collection period were not affected by treatment. Water intake tended ($P = 0.10$) to be greater for cows fed sulfate minerals (Faulkner and Weiss, 2017). Because by-product diets had greater concentrations of basal minerals, intakes of Cu, Zn, and Mn were greater ($P < 0.01$ to 0.07) for cows fed by-product diets compared with cows fed forage diets (Table 4). Mineral source did not affect intake of Cu or Zn but Mn intake by cows fed sulfate minerals averaged 4.5% greater ($P < 0.01$) than for cows fed hydroxy minerals.

Concentrations of Cu and Mn in milk were not affected by treatments; however, cows fed hydroxy minerals had lower ($P < 0.02$) concentrations of Zn in milk (3.7 vs. 3.9 mg/kg) compared with cows fed sulfate minerals (Table 5). This resulted in about a 7% decrease in the amount of Zn excreted in milk daily ($P < 0.01$; Table 4). Milk Cu and Mn secretion was $<0.01\%$ of intake, whereas the amount of Zn excreted in milk was $\sim 8.0\%$ of Zn intake; the reason cows fed sulfates partitioned

Table 3. Concentrations of supplemental and total dietary Cu, Zn, and Mn (mg/kg of diet DM)

Item	Treatment ¹			
	Forage		By-product	
	Sulfate	Hydroxy	Sulfate	Hydroxy
Supplemental minerals concentrations ²				
Cu	10	9	11	9
Zn	34	37	36	34
Mn	33	32	34	31
Total dietary concentrations				
Cu	19	18	22	20
Zn	64	67	86	84
Mn	71	70	81	78
Supplemental mineral, % of total dietary mineral				
Cu	53	50	50	45
Zn	53	55	42	41
Mn	46	46	42	40

¹Forage- or by-product-based diets with Cu, Zn, and Mn from sulfate or hydroxy mineral sources.

²Concentrations based on treatment average DMI.

more Zn into milk is unknown. Over reasonable ranges in dietary Zn, concentrations of Zn in the diet and milk are not related (Castillo et al., 2013), but excessively high concentrations of dietary Zn (540 mg/kg of diet DM) can increase milk concentrations (Sobhanirad et al., 2010). Source of Zn (sulfate vs. different forms of organic Zn) did not affect Zn concentration in bovine colostrum (Muehlenbein et al., 2001; Formigoni et al., 2011) or milk (Sobhanirad et al., 2010). However, Cope et al. (2009) reported an interaction ($P < 0.06$) between Zn source (oxide or organic) and dietary concentration of Zn (approximately 41 and 63 mg/kg of diet DM). At greater concentrations of dietary Zn, Zn concentrations in milk were greater for cows fed ZnO, but the opposite was true at the lesser dietary concentration.

Urinary loss of trace minerals was $<2\%$ of intake (Table 4). Loss of urinary Cu was not affected by treat-

ment, but cows fed by-product diets had greater urinary excretion of Zn ($P < 0.01$) and Mn ($P < 0.03$) than cows fed the forage-based diets (Table 4). Urinary loss of Zn and Mn was less than 2 mg/d, and the average difference between fiber treatments was 0.6 mg/d or less. Relative to the amount of Zn and Mn apparently absorbed, the treatment effects on urinary excretion were minor. When urinary losses of Zn and Mn were expressed relative to their intakes, treatment effects were reduced (Table 4). Mean urinary Cu excretion was greater than reported in other studies (Buckley, 1991; Gustafson, 2000), but Cu intakes by cows in those studies were 20 to 44% less than our study, which could explain the differences.

Treatment effects on apparent absorption were not consistent across the minerals examined (Table 4). No treatment effects or interactions were found for Zn, but

Table 4. Effects of fiber and trace mineral sources on mineral apparent absorption and retention

Item	Treatment ¹				SEM ²	<i>P</i> <		
	Forage		By-product			Fiber (F)	Mineral (M)	F × M
	Sulfate	Hydroxy	Sulfate	Hydroxy				
Intake								
DM, kg/d	24.6	24.2	24.0	23.8	1.3	0.74	0.65	0.85
Cu, mg/d	410	390	450	420	18	0.07	0.14	0.78
Zn, mg/d	1,440	1,460	1,900	1,820	63	0.01	0.38	0.15
Mn, mg/d	1,610	1,570	1,840	1,740	60	0.01	0.04	0.34
Milk, kg/d	35.5	35.1	34.7	33.4	3.2	0.41	0.29	0.62
Cu, mg/d	1.5	1.4	1.3	1.2	0.2	0.52	0.11	0.65
Zn, mg/d	147	137	127	120	12	0.23	0.01	0.68
Mn, mg/d	0.9	0.9	0.9	1.0	0.1	0.88	0.91	0.68
Cu, % of intake	0.37	0.35	0.29	0.30	0.053	0.34	0.77	0.32
Zn, % of intake	10.2	9.36	6.70	6.71	0.711	0.005	0.07	0.08
Mn, % of intake	0.059	0.058	0.05	0.055	0.0058	0.36	0.59	0.38
Urine, kg/d	21.8	23.1	19.6	18.1	1.6	0.03	0.90	0.12
Cu, mg/d	6.3	5.1	6.5	5.5	0.8	0.67	0.14	0.92
Zn, mg/d	1.0	1.2	1.9	1.5	0.2	0.01	0.37	0.10
Mn, mg/d	0.3	0.4	0.9	0.7	0.2	0.03	0.77	0.22
Cu, % of intake	1.6	1.3	1.4	1.4	0.23	0.91	0.39	0.58
Zn, % of intake	0.07	0.08	0.10	0.08	0.01	0.19	0.74	0.24
Mn, % of intake	0.02	0.03	0.05	0.04	0.01	0.07	0.95	0.30
Feces, kg/d of DM	7.5	7.1	8.2	8.1	0.6	0.14	0.44	0.52
Cu, mg/d	406	372	409	424	27	0.21	0.62	0.26
Zn, mg/d	1,072	1,124	1,383	1,357	62	0.01	0.68	0.25
Mn, mg/d	1,445	1,395	1,522	1,487	82	0.19	0.33	0.87
Cu, % of intake	98.4	94.9	91.4	100.1	3.93	0.74	0.34	0.05
Zn, % of intake	76.7	74.4	74.3	72.6	1.90	0.28	0.14	0.80
Mn, % of intake	89.5	89.0	83.1	85.3	3.37	0.02	0.67	0.53
Apparent absorption, %								
Cu	1.6	5.1	8.6	−0.1	3.9	0.73	0.34	0.05
Zn	25.4	23.3	27.4	25.7	1.9	0.25	0.15	0.80
Mn	10.5	11.0	16.9	14.7	3.4	0.02	0.67	0.53
Apparent retention, mg/d								
Cu	−3	11	29	−5	16	0.49	0.38	0.05
Zn	219	199	389	346	35	0.01	0.20	0.66
Mn	167	167	315	255	55	0.01	0.43	0.45

¹Forage- or by-product-based diets with Cu, Zn, and Mn from sulfate or hydroxy mineral sources.

²Data were unbalanced due to one missing observation; therefore, the greatest SEM values were reported.

Table 5. Effects of fiber type and trace mineral source on concentration of minerals excreted

Item	Treatment ¹				SEM ²	<i>P</i> <		
	Forage		By-product			Fiber (F)	Mineral (M)	F × M
	Sulfate	Hydroxy	Sulfate	Hydroxy				
Milk, kg/d	35.5	35.1	34.7	33.4	3.2	0.41	0.29	0.62
Cu, mg/kg	0.04	0.04	0.04	0.04	0.01	0.58	0.25	0.87
Zn, mg/kg	4.17	3.90	3.70	3.61	0.30	0.32	0.02	0.21
Mn, mg/kg	0.03	0.03	0.03	0.03	0.01	0.76	0.81	0.34
Feces, kg/d of DM	7.5	7.1	8.2	8.1	0.6	0.14	0.44	0.52
Cu, mg/kg of DM	55.1	52.1	51.7	52.0	2.0	0.46	0.33	0.26
Zn, mg/kg of DM	147.9	159.1	169.7	168.2	7.0	0.07	0.17	0.10
Mn, mg/kg of DM	196.8	197.3	189.0	185.6	7.3	0.28	0.81	0.74
Ca, g/kg of DM	22.7	23.4	21.0	19.9	0.7	0.01	0.69	0.11
P, g/kg of DM	7.4	7.8	7.0	7.1	0.3	0.05	0.19	0.37
Mg, g/kg of DM	7.4	7.6	6.6	6.7	0.1	0.01	0.05	0.19
K, g/kg of DM	11.5	9.5	9.0	10.2	0.8	0.30	0.66	0.06
Urine, kg/d	21.8	23.1	19.6	18.1	1.6	0.03	0.90	0.12
Cu, mg/kg	0.31	0.22	0.33	0.33	0.01	0.10	0.29	0.35
Zn, mg/kg	0.05	0.05	0.10	0.08	0.01	0.01	0.53	0.53
Mn, mg/kg	0.01	0.02	0.05	0.04	0.01	0.02	0.84	0.37

¹Forage- or by-product-based diets with Cu, Zn, and Mn from sulfate or hydroxy mineral sources.

²Data were unbalanced due to one missing observation; therefore, the greatest SEM values were reported.

fiber source affected ($P < 0.05$) apparent absorption of Mn and an interaction ($P < 0.05$) between fiber and mineral source was found for Cu. One potential reason for the differences between the minerals is that they may have different binding affinities for fiber (Ghodrat et al., 2017). Using an in vitro assay, Cu usually had greater binding affinity than Zn, but Ghodrat et al. (2017) did not include Mn.

A fiber by mineral source interaction ($P < 0.05$) was observed for Cu apparent absorption and apparent retention (Table 4). For cows fed the forage diets, apparent Cu retention was greater when hydroxy minerals were supplemented compared with sulfate minerals, but the opposite response was observed for cows fed by-product diets. Greater Cu apparent absorption for cows fed the forage diet with hydroxy minerals compared with the forage diet with sulfate minerals may be caused by less ruminal antagonism with the less rumen-soluble hydroxy Cu (Genther and Hansen, 2015). The by-product diets had greater NDF digestibility (50 vs. 44%) but, because those diets were greater in NDF concentration, output of undigestible NDF was also greater (4.4 vs. 3.4 kg/d; Faulkner and Weiss, 2017). When apparent Cu absorption (mg/d) was regressed on undigested NDF intake, the slope for the by-product diets was not different from zero ($P > 0.60$), whereas the slope of the forage diets ($-8.3/\text{kg}$ of undigested NDF) was different from zero ($P < 0.02$). These data could mean that undigested fiber from forages had an antagonistic effect of Cu absorption, whereas the undigested fiber from by-products does not. However, caution should be taken

when interpreting those regressions, as they are based on a limited number of observations and numerous factors other than undigested NDF differed between the forage and by-product diets.

Dry matter from the by-product diets was less digestible than the forage diets (70 vs. 66%; Faulkner and Weiss, 2017). Poorer solubility of hydroxy Cu and increased passage of undigested DM could explain the apparent absorption data for the by-product diet. Copper absorption primarily occurs in the small intestine (Wang et al., 2011) via high-affinity copper transporters (Wapnir, 1998; Kim et al., 2008; Han et al., 2009) following the reduction of soluble Cu by metalloredutase enzymes (Ohgami et al., 2006). Decreased DM digestibility for the by-product diet (Faulkner and Weiss, 2017) would increase the passage of undigested DM through the intestine, reducing the amount of time Cu spends in the intestine. The hydroxy Cu may not have had adequate time to solubilize and be reduced, which are necessary steps for absorption by Cu transporters at intestinal absorption sites. The by-product diet also contained higher concentrations of Fe (Table 2), which can antagonize Cu (Chase et al., 2000). However, the difference in Fe concentrations between diets was less than what has been shown to affect Cu status, and the Fe in the diets in our study likely were less reactive than the Fe sulfate used by Chase et al. (2000).

Treatment did not affect apparent Zn absorption (Table 4), but cows fed by-product diets had greater ($P < 0.01$) apparent retention of Zn. The greater retention was probably caused in part by greater intake, but,

Table 6. Effects of fiber type and trace mineral source on selected macromineral apparent absorption

Item	Treatment ¹				SEM ²	<i>P</i> <		
	Forage		By-product			Fiber (F)	Mineral (M)	F × M
	Sulfate	Hydroxy	Sulfate	Hydroxy				
Intake, g/d								
P	84	82	95	94	5	0.06	0.64	0.92
Mg	62	61	70	69	4	0.07	0.58	0.98
K	379	374	374	369	18	0.82	0.59	0.99
Fecal excretion, g/d								
P	55	56	58	59	4	0.69	0.67	0.77
Mg	54	55	54	54	4	0.93	0.92	0.79
K	85	69	73	85	9	0.80	0.74	0.05
Apparent absorption, %								
P	34.2	32.4	39.5	38.9	2.4	0.04	0.41	0.67
Mg	12.3	11.2	22.9	21.6	2.0	0.01	0.25	0.94
K	77.7	81.8	80.4	77.3	2.0	0.66	0.76	0.05

¹Forage- or by-product-based diets with Cu, Zn, and Mn from sulfate or hydroxy mineral sources.

²Data were unbalanced due to one missing observation; therefore, the greatest SEM values were reported.

when expressed as a percent of Zn intake, Zn retention by cows fed by-product diets was still greater ($P < 0.01$; data not shown).

Apparent Mn absorption ($P = 0.02$) and retention ($P < 0.01$) were greater for cows consuming the by-product diets compared with the forage diets, but were not affected by mineral source or the fiber by mineral interaction ($P < 0.54$). Apparent Mn absorption for the forage diets (approximately 11%) was greater than that measured in late-gestation dairy cows (Weiss and Socha, 2005). A possible reason for the difference is that cows in our study were consuming about 44% more Mn and Weiss and Socha (2005) fed Mn methionine and Mn sulfate rather than hydroxy Mn.

Macromineral Absorption

Trace mineral source did not affect apparent absorption of selected macrominerals, but cows fed the by-product diets had greater ($P < 0.05$) apparent absorption of P and Mg (Table 6). An interaction between fiber and trace mineral source was observed ($P < 0.05$) for apparent absorption of K. The major factors affecting Mg apparent absorption were concentration of dietary K and source of Mg (Weiss, 2004; Schonewille et al., 2008). The concentration of K was similar between diets, but ingredients and concentration of MgO differed greatly between the forage and by-product diets. Magnesium oxide provided twice as much Mg in the forage diets than in the by-product diets (Table 1), and, contrary to NRC (2001), Mg in basal feeds is more available than Mg from MgO (Davenport et al., 1990; Jittakhot et al., 2004). Several factors can increase apparent absorption of P including decreased P intake and concentration of dietary NDF (Klop et al.,

2013) and increased digestibility of diet DM (Weiss and Wyatt, 2004). The availability of P also differs among ingredients (Feng et al., 2016). The greater apparent absorption of P for the by-product diets contradicts many of these relationships. The by-product diet had lower DM digestibility (Faulkner and Weiss, 2017), had greater NDF concentrations (Table 2), and cows fed those diets tended to have greater ($P < 0.06$) P intake. The ingredients in the by-product diets could have had greater P availability than those in the forage diets, or equations generated mostly from forage-based diets (e.g., Weiss and Wyatt, 2004; Klop et al., 2013) do not fit when cows are fed low-forage, higher-by-product diets.

A fiber × mineral interaction was observed for apparent absorption of K (Table 6). Greater K apparent absorption was observed when cows were fed the forage diets with hydroxy minerals compared with sulfates, but the opposite was observed with the by-product diets. Feed K exists as simple ions readily available for absorption (Emanuele and Staples, 1990; Ledoux and Martz, 1991). The main route of K excretion is through urine, and is under primary regulation by aldosterone, and can be further affected by blood acid-base balance (McGuirk and Butler, 1980). Urinary K excretion was not measured, however decreased fecal excretion of K by cows fed the forage diet with hydroxy minerals and by-product diet with sulfate minerals likely led to greater urinary K excretion (Table 6).

Red Blood Cell SOD Activity and Blood Serum Concentrations

No treatment differences were observed for serum Cu, Zn, Mn, or Fe concentrations (Table 7); however,

Table 7. The effects of fiber and mineral source on enzyme activity and serum mineral concentrations

Blood measure	Treatment ¹				SEM ²	<i>P</i> <		
	Forage		By-product			Fiber (F)	Mineral (M)	F × M
	Sulfate	Hydroxy	Sulfate	Hydroxy				
Cu, mg/L	0.75	0.79	0.83	0.82	0.07	0.57	0.61	0.50
Zn, mg/L	1.08	1.16	1.10	1.14	0.08	1.00	0.10	0.47
Mn, µg/L	2.64	2.44	2.46	2.34	0.36	0.67	0.66	0.91
Fe, mg/L	1.65	1.61	1.58	1.56	0.16	0.75	0.63	0.88
Mo, µg/L	187	229	47	49	22.7	0.01	0.03	0.06
Hemoglobin, mg/dL	103	104	114	112	3.31	0.01	0.57	0.16
Superoxide dismutase activity/g of hemoglobin	3,234	3,150	3,049	3,237	170.5	0.76	0.54	0.12

¹Forage- or by-product-based diets with Cu, Zn, and Mn from sulfate or hydroxy mineral sources.

²Data were unbalanced due to one missing observation; therefore, the greatest SEM values were reported.

cows consuming hydroxy minerals tended to have greater serum Zn concentrations ($P = 0.10$). Concentrations of Cu, Zn, and Mn in serum are usually poor measures of adequacy and are not sensitive to changes in dietary supply (Sol Morales et al., 2000; Mullis et al., 2003; Hansen et al., 2006). With the exception of a trend toward higher Zn concentrations ($P < 0.10$) when hydroxy minerals were fed, serum minerals were not affected by treatment (Table 7).

Fiber, mineral, and interaction effects were observed for serum Mo (Table 7). Concentrations of serum Mo were greater for cows consuming the forage diets compared with by-product diets ($P < 0.01$) and sulfate

minerals compared with hydroxy ($P = 0.03$). Dietary concentration of Mo was slightly greater for the forage diets than the by-product diets (1.81 vs 1.60 mg/kg); however, it is unlikely that a 12% increase in dietary Mo from the forage diets increased serum Mo concentrations >400%. The reason for the difference in serum Mo is not known, but it could be caused by differences in Mo availability between dietary ingredients. Decreased rumen soluble hydroxy Cu compared with sulfate sources may have lessened Cu and thiomolybdate binding in the rumen, thereby increasing thiomolybdate (i.e., Mo) absorption. Hemoglobin concentrations were within the normal range but for unknown reasons were higher (P

Figure 1. Relationship between apparent absorption of Cu and superoxide dismutase (SOD) activity in red blood cells in lactating dairy cows fed forage (blue, open symbols) or by-product (red, closed symbols) based diets with sulfate (triangles) or hydroxy (squares) supplemental Cu, Zn, and Mn. Treatment did not affect slope or intercept and the common slope and intercept are different from zero ($P < 0.05$; root mean squared error = 400.8). Color version available online.

= 0.01) for cows fed the forage diets compared with the by-product diets. Superoxide dismutase activity in erythrocytes (i.e., SOD1) was not affected by fiber or mineral main effects but tended ($P = 0.12$) toward an interaction between fiber treatment and mineral source; with the forage diets, cows fed sulfate minerals had higher activity of SOD than those fed hydroxy minerals, but the opposite was found when by-product diets were fed (Table 7). This interaction is opposite of the interaction observed for apparent Cu absorption (Table 4). Activity of SOD was negatively associated ($P < 0.05$) with apparent Cu absorption (Figure 1). A weaker ($P < 0.07$) negative relationship was observed between SOD and apparent Zn absorption (data not shown). One explanation for this apparent paradoxical relationship between SOD and apparent absorption of Cu is that cows with higher SOD activity may reflect a state of excess Cu, and excess body Cu may lead to downregulation of Cu absorption (van den Berghe and Klomp, 2009).

CONCLUSIONS

Absorption and retention of Zn were not affected by diet or source of metal. The by-product-based diets had greater apparent Mn absorption than forage-based diets, which could reflect differences in absorption of Mn from the feedstuffs or could be caused by differences in Mn intake. Fiber source affected Cu absorption, but the effects were different depending on the source of supplemental trace minerals. Absorption of Cu from Cu sulfate, but not from hydroxy Cu, was negatively influenced by the forage-based diet, possibly because of differences in mineral interaction with digesta in the rumen. However, when a by-product-based diet was fed, absorption of Cu from hydroxy Cu was lesser than that from Cu sulfate possibly because of decreased solubility of hydroxy Cu at the interface between digesta and the enterocytes. Because DM digestibility of the by-product diets was less than that of the forage diets, the increased mass of digesta flowing through the intestine could potentially reduce access of Cu to the enterocyte. A paradoxical relationship of higher SOD activity with lesser apparent absorption of Cu may reflect regulation of Cu absorption and warrants further investigation.

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