Evaluation of source of corn silage and trace minerals on rumen characteristics and passage rate of Holstein cows

M. D. Miller,¹ J. S. Lanier,^{2*} S. K. Kvidera,² H. M. Dann,¹ C. S. Ballard,¹ and R. J. Grant^{1†}

¹William H. Miner Agricultural Research Institute, Chazy, NY 12921

²Micronutrients USA LLC, Indianapolis, IN 46241

ABSTRACT

The effects of source of corn silage and trace mineral on rumen fermentation, turnover, and particle passage rates were evaluated with 8 ruminally cannulated Holstein cows averaging 83 (standard error = 5) days in milk in a replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments and 28-d periods. The diets consisted (dry basis) of 55% conventional (CON) or brown midrib-3 (BM3) corn silage, 2% chopped wheat straw, and 43% grain mix with either sulfate (STM) or hydroxy (HTM) source of Cu, Zn, and Mn trace minerals. The targeted supplemental amount of Cu, Zn, and Mn was 194, 1,657, and 687 mg/d, respectively. The dietary treatments were (1) CON-STM, (2) CON-HTM, (3) BM3-STM, and (4) BM3-HTM. Dietary nutrient composition of BM3 diets averaged 32.1% amylase neutral detergent fiber on an organic matter basis (aNDFom) and 6.9% undigested neutral detergent fiber at 240 h of *in vitro* fermentation (uNDF240om; % of dry matter), and CON diets averaged 36.2% aNDFom and 8.6% uNDF240om (% of dry matter). Data were summarized by period and analyzed as a replicated Latin square design with fixed model effects for corn silage, trace mineral, corn silage and trace mineral interaction, period within replicated square, and replicated square using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Cow within replicate was a random effect. Daily mean, standard deviation, minimum, and maximum for rumen pH were unaffected by corn silage or trace mineral source. Cows fed the CON diets had greater rumen acetate percentage than cows fed the BM3 diets (65.7 vs. 64.7 molar %). In contrast, cows fed the BM3 diets had greater rumen propionate percentage than cows fed the CON diets (21.4 vs. 20.4 molar %). Total volatile fatty acid concentration was lower for cows fed STM versus

HTM in BM3 diets, but not for the cows fed the CON diets. Cows fed the BM3 diets had faster turnover rate and shorter turnover time for uNDF240om than cows fed the CON diets (3.12 vs. 2.86%/h and 33.3 vs. 36.5 h, respectively). Cows fed the BM3 diets had a faster passage rate of small and medium corn silage neutral detergent fiber particles than cows fed the CON diets (5.73 vs. 5.37%/h and 4.74 vs. 4.31%/h, respectively). We observed a corn silage by source of trace mineral interaction on organic matter and uNDF240om rumen pool size and organic matter turnover. Overall, source of corn silage had a pronounced influence on rumen dynamics presumably related to greater *in vitro* neutral detergent fiber digestibility and lower uNDF240om content of BM3 corn silage that allowed for faster turnover of indigestible neutral detergent fiber and greater passage rate of corn silage particles. In contrast, the source of trace mineral had much less significant effects on rumen fermentation, turnover, and particle passage rates. Corn silage-based diets intended to enhance rumen fiber fermentation, turnover, and passage are more affected by source and digestibility of neutral detergent fiber than source of dietary trace minerals.

Key words: trace mineral, corn silage, passage rate, rumen pH

INTRODUCTION

Forages are the major feedstuff that provides fiber for dairy cows, and both physical and chemical characteristics will affect rumen fermentation, digestibility, and energy value (Allen and Mertens, 1988). Corn silage provides digestible and physically effective fiber, as well as starch, which has led to increased inclusion of this forage in dairy cow diets (Hassanat et al., 2017). In fact, corn silage is the most commonly fed forage in the United States and its production has increased more than 30% since the 1980s (Grant and Adesogan, 2018).

Brown midrib corn silage has a gene mutation, either brown midrib-1 or brown midrib-3, that reduces the lignin content and enhances NDF digestibility compared with non-brown midrib hybrids (Oba and Allen, 1999;

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*Current address: Purina Animal Nutrition, Raleigh, NC 27615.

†Corresponding author: grant@whminer.com

Hassanat et al., 2017). In a corn silage hybrid study replicated over 3 yr, brown midrib-3 hybrids had greater NDF digestibility, lower indigestible NDF (**iNDF**), and higher potentially digestible NDF ($\text{NDF} - \text{iNDF}$) than either brown midrib-1 or non-brown midrib hybrids (Miller et al., 2018). In previous studies, lactating cows fed brown midrib-3 corn silage had lower rumen fill and greater DMI than cows fed diets with conventional corn silage due to a lower **iNDF** fraction and greater fiber passage rates from the rumen for brown midrib-3 corn silage (Oba and Allen, 1999, 2000a,b; Hassanat et al., 2017). Undigested NDF residue on an OM basis (**uNDF240om**) is a laboratory measure of **iNDF** and has been related to NDF digestion and passage kinetics, gut fill and intake, and physical effectiveness of fiber (Nousiainen et al., 2003; Cotanch et al., 2014; Raffrenato et al., 2018).

In contrast to corn silage, relatively little research has focused on the role of trace mineral source on rumen fiber fermentability, turnover, and DMI. Rumen microorganisms require some trace minerals for proper function, although the release of free metal from the mineral in the rumen could reduce its bioavailability to the cow. Different sources of trace minerals have varying stabilities in the rumen and may affect rumen fermentation. Importantly, hydroxy trace minerals (**HTM**) are less soluble in the rumen compared with sulfate sources (Torre et al., 1991; Cao et al., 2000; Spears et al., 2004; Genther and Hansen, 2015; Caldera et al., 2019). Faulkner and Weiss (2017) reported that feeding sulfate sources of Cu, Mn, and Zn minerals decreased total-tract NDF digestibility compared with hydroxy Cu, Mn, and Zn minerals in forage- and byproduct-based dairy cattle diets. Feeding dairy heifers organic trace minerals of Cu, Zn, Mn, Se, and Co resulted in lower rumen pH and greater total VFA concentrations compared with dairy heifers fed sulfate trace minerals (**STM**; Pino and Heinrichs, 2016). Taken together, these results suggest that source of trace mineral affects rumen fermentation.

However, to date no study has assessed the interaction between source of forage fiber and the source of trace minerals on rumen fermentation, turnover, and particle passage rates in diets fed to lactating dairy cows. Therefore, the objective of this study was to evaluate the effect of source of corn silage (brown midrib-3 or conventional) and source of trace minerals (hydroxy or sulfate) on rumen characteristics and particle passage rate in lactating Holstein cows. Our hypothesis was that brown midrib-3 corn silage would positively affect rumen fermentation and fiber turnover characteristics, and STM would negatively affect rumen fermentation and fiber turnover characteristics with this negative ef-

fect being greater in diets containing conventional corn silage.

MATERIALS AND METHODS

Experimental Design, Diets, and Management of Cows

This paper is a companion study to Miller et al. (2020), and focuses on rumen characteristics and particle passage rate using a subset of 8 ruminally cannulated, multiparous cows.

All experimental procedures involving animals were approved by the William H. Miner Agricultural Research Institute Animal Care and Use Committee. The study was conducted at the William H. Miner Agricultural Research Institute (Chazy, NY) in the Charles J. Sniffen Dairy Research and Education Complex. Eight ruminally cannulated, multiparous lactating Holstein cows averaging 83 ± 5 (SE) DIM at the beginning of the experiment were blocked by cannulation status, DIM, and milk production and were used in a replicated 4×4 Latin square design with a 2×2 factorial arrangement of treatments. The study had 28-d periods. Each square was conducted concurrently, with the first 17 d serving as an adaption period, and the last 11 d serving as the collection period. Cows were enrolled on the study 14 d before period 1 began and were fed a TMR with no supplemental trace minerals or monensin (Elanco Animal Health, Greenfield, IN).

Dietary treatments (Table 1) were formulated to contain either conventional (**CON**; Doeblar 554GRQ, Doeblar's PA Hybrids, Williamsport, PA) or brown midrib-3 corn silage (**BM3**; Mycogen F2F499, Dow AgroSciences LLC, Indianapolis, IN) and either a sulfate (**STM**) source of Cu, Zn, and Mn or a hydroxy source of trace minerals (**HTM**; IntelliBond C, Z, and M, Micronutrients USA LLC, Indianapolis, IN). The diets were formulated to contain 54.5% (DM basis) of either BM3 or CON corn silage so that the differences in fiber fractions among the diets were determined primarily by the source of corn silage. The targeted supplemental amount of Cu, Zn, and Mn was 194, 1,657, and 687 mg/d, respectively. The 4 dietary treatments were (1) CON silage and sulfate trace minerals (**CON-STM**), (2) CON silage and hydroxy trace minerals (**CON-HTM**), (3) BM3 silage and sulfate trace minerals (**BM3-STM**), and (4) BM3 silage and hydroxy trace minerals (**BM3-HTM**). Diets were formulated using Cornell Net Carbohydrate Protein System biology (AMTS.Cattle.Professional, version 4.8, Agricultural Modeling & Training systems, LLC, Groton, NY). Inputs used for diet formulation included 29.9 kg/d of

DMI, 54 kg/d of milk with 3.60% fat and 3.05% true protein, and 750 kg of BW. Cows were fed the diets as TMR for ad libitum intake (approximately $1.15 \times$ expected intake) once daily (fed at 1400 h; Calan Data Ranger, American Calan Inc., Northwood, NH) in a tiestall barn equipped with individual feed boxes.

Data Collection, Sample Procedures, and Analytical Methods

Forages, diets, and orts were collected 3 times per week during d 1 to 17 and daily for d 18 to 28. Grain mixes were collected once per week during d 1 to 17 and daily from d 18 to 28. A portion of each sample was dried in a forced-air oven at 105°C for 24 ± 6 h for DM determination. Diets were adjusted for changes in DM content of the feed ingredients when a feed ingredient DM value was outside the range of the DM mean ± 1.2 standard deviations or a new feed ingredient source was used or delivered.

Starting on d 18 of each period, a portion of the feed ingredients, diets, and orts collected daily was stored frozen at -20°C , and then was composited by period by combining equal volumes of the daily as-fed samples. The composites of feed ingredients were analyzed for chemical composition (CPM Plus, Cumberland Valley Analytical Services Inc., Waynesboro, PA). Analyses included DM, ash (method 942.05; AOAC International, 2012), OM (method 942.05; AOAC International, 2012), CP (method 990.03; AOAC International, 2012), soluble protein according to Krishnamoorthy et al. (1982), fat (method 2003.05; AOAC International, 2012), ADF (method 973.18; AOAC International, 2012), NDF using α -amylase, sodium sulfite, and ash-corrected (Van Soest et al., 1991), ADL (Goering and Van Soest, 1970), starch according to Hall (2009), sugar as ethanol-soluble carbohydrates according to DuBois et al. (1956), and minerals (method 985.01; AOAC International, 2012).

Fiber characteristics and NDF digestibility at 30 h were determined on the forage and grain composite samples (Cumberland Valley Analytical Services Inc.). Undigested NDF at 30-, 120-, and 240-h time points (uNDF30om, uNDF120om, uNDF240om, respectively) for forages and undigested NDF at 12-, 72-, and 120-h time points (uNDF12om, uNDF72om, and uNDF120om) for grains were assessed using an in vitro rumen fermentation system (Raffrenato et al., 2018; Cumberland Valley Analytical Services Inc.). Starch digestibility at 7 h (Hall, 2009; Cumberland Valley Analytical Services Inc.) was determined on the period composite samples of corn silage and grain mixes. A portion of the forage and diet composite samples was used to determine particle size distribution on an as-fed

basis using a Penn State Particle Separator (Lammers et al., 1996) modified to include a 4-mm screen. A portion of the period composite for forage and grain mixes was also used to determine particle size distribution on a DM basis (55°C) by dry vertical sieving (Ro-Tap testing sieve shaker model B; W. S. Tyler Combustion Engineering Inc., Mentor, OH). The physical effectiveness factor (**pef**) was defined as the fraction of particles retained on the 1.18-mm screen or greater (for dry sieving) or the 4.0-mm screen and greater (for as-fed sieving). Physically effective NDF (**peNDF**) of a forage or diet was calculated as the product of its NDF content and its pef (Mertens, 1997).

Rumen pH was measured in ruminally cannulated cows ($n = 8$) with an indwelling rumen pH/ORP/REDOX measurement system (Penner et al., 2006; LRCpH, Dascor, Escondido, CA) at 1-min intervals for a 96-h period starting on d 23 of each period. Rumen pH measurements were averaged over a 10-min period within day and summarized as mean pH, minimum pH, maximum pH, pH range, the area that the pH curve was below a pH of 5.8 (**AUC**), and minutes per day that pH was below 5.5 or 5.8 (Beauchemin and Yang, 2005).

Samples of rumen fluid (approximately 500 mL) were collected from beneath the rumen digesta mat at 4-h intervals for 24 h starting at the beginning of d 26 of each period. Cows were sampled based on their square assignment. Feeding and the associated sampling times were staggered by 10 min between squares of cows to allow for collection of samples at desired times. Samples were strained through 4 layers of cheesecloth. A portion of each sample of rumen fluid (approximately 40 mL) was frozen and stored at -20°C until analysis for VFA concentration (Bulletin 856B, Supelco Inc., Bellefonte, PA). Volatile fatty acid concentration was determined by GC with use of a Varian CP-3800 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with a flame-ionization detector and an 80/120 Carbowax B-DA/4% Carbowax 20M column (Supelco Inc.). Ten mL of rumen fluid was added to 100 μL of concentrated HCl and frozen at -20°C until analysis of rumen $\text{NH}_3\text{-N}$ concentration (Chaney and Marbach, 1962).

Rumen contents of cows ($n = 8$) were evacuated manually through the rumen cannula after daily feeding on d 27 and at the end of d 28. To ensure that cows experienced the same interval of time between rumen evacuations, cows were divided into 2 groups of 4 cows each based on square. The first group was evacuated 3.5 h after feeding on d 27 (1730 h) and 20.5 h after feeding on d 28 (1030 h). The second group of cows was evacuated 4.5 h after feeding on d 27 (1830 h) and 19.5 h after feeding on d 28 (0930 h). The mass and volume of rumen contents were determined. Dur-

Table 1. Ingredient composition (expressed as a percentage of DM) of diets containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with either sulfate trace minerals (STM) or hydroxy trace minerals (HTM) fed to lactating Holstein cows

Item	Diet			
	CON		BM3	
	STM	HTM	STM	HTM
Conventional corn silage (CON)	54.56	54.56	—	—
Brown midrib corn silage (BM3)	—	—	54.55	54.55
Straw	2.27	2.27	2.27	2.27
Beet pulp (pellets)	6.82	6.82	6.82	6.82
Corn meal	2.67	2.67	2.67	2.67
Concentrate mix				
Soy hull	7.31	7.31	3.07	3.07
Protein supplement ¹	6.59	6.59	5.27	5.27
Soybean meal	5.30	5.30	6.67	6.67
Corn meal	1.71	1.71	5.60	5.60
Canola meal	2.69	2.69	4.02	4.02
99% Sugar	1.41	1.41	1.41	1.41
Dried distillers grains	1.34	1.34	—	—
Fat supplement 1 ²	1.21	1.21	1.21	1.21
Calcium carbonate	1.10	1.11	1.10	1.11
Protein supplement ³	0.86	0.86	1.17	1.17
Molasses	0.82	0.82	0.82	0.82
Sodium sesquicarbonate	0.73	0.73	0.73	0.73
Potassium carbonate ⁴	0.59	0.59	0.59	0.59
Fat supplement 2 ⁵	0.59	0.59	0.59	0.59
Salt	0.38	0.38	0.38	0.38
Urea	0.37	0.37	0.37	0.37
Magnesium oxide	0.31	0.31	0.31	0.31
Monocalcium phosphate	0.12	0.12	0.12	0.12
Selenium yeast 0.06% ⁶	0.05	0.05	0.05	0.05
Calcium chloride	0.04	0.04	0.04	0.04
Vitamin E ⁷	0.03	0.03	0.03	0.03
Fly larvicide ⁸	0.03	0.03	0.03	0.03
Methionine analog ⁹	0.02	0.02	0.04	0.04
Vitamin E ¹⁰	0.02	0.02	0.02	0.02
Rumen-protected methionine ¹¹	0.01	0.01	0.01	0.01
Copper sulfate	0.004	—	0.004	—
Manganese sulfate	0.012	—	0.012	—
Zinc sulfate	0.017	—	0.017	—
Hydroxy Cu ¹²	—	0.002	—	0.002
Hydroxy Zn ¹²	—	0.009	—	0.009
Hydroxy Mn ¹²	—	0.011	—	0.011
Vitamin A ¹³	0.0003	0.0003	0.0003	0.0003
Vitamin D ¹⁴	0.0002	0.0002	0.0002	0.0002
Cobalt carbonate	0.0002	0.0002	0.0002	0.0002
Potassium iodide	0.0001	0.0001	0.0001	0.0001
Total	100	100	100	100

¹Aminomax Pro, Afgriotech, LLC, Watertown, NY.²BergaFat, Berg + Schmidt America, LLC, Libertyville, IL.³PGI Amino Enhancer, Poulin Grain, Newport, VT.⁴DCAD Plus, Arm & Hammer Animal Nutrition, Ewing, NJ.⁵Energizer Gold, IFFCO (Malaysia) SDN.BHD, Sharjah, United Arab Emirates.⁶Co-operative Feed Dealers Inc., Conklin, NY.⁷Contained 500,000 IU of vitamin E/kg % of DM.⁸Clarifly, 0.67%, Central Garden and Pet Company, Schaumburg, IL.⁹MetaSmart, Adisseo USA Inc., Alpharetta, GA.¹⁰Contained 8,816 IU of vitamin E/kg % of DM.¹¹Smartamine, Adisseo USA Inc.¹²Intellibond C, Z, M, Intellibond, Micronutrients USA LLC, Indianapolis, IN.¹³Contained 1,000,000 IU of vitamin A/g % of DM.¹⁴Contained 500,000 IU of vitamin D/g % of DM.

ing the evacuation, approximately 10% of the rumen contents were subsampled and squeezed through a nylon screen (1-mm pore size) to separate solid and liquid phases, and each phase was weighed. Aliquots (approximately 300 g) from both the solid and liquid phases were collected, frozen at -20°C , dried at 55°C , ground (solids: 1-mm screen, Wiley mill; Arthur H. Thomas, Philadelphia, PA; liquid: 2-mm screen, UDY Cyclone Sample Mill; UDY Corp., Fort Collins, CO), and recombined based on the proportion of DM of each phase. The recombined rumen contents were analyzed for ash (modified method 942.05; AOAC International, 2012; 4 h at 600°C), aNDFom (as described previously), uNDF240om, and starch (Hall, 2009; Raffrenato et al., 2018; Cumberland Valley Analytical Services Inc.). The remaining rumen contents were returned to the cow within 60 min of initiating the evacuation.

Rumen pool size of OM, NDF, uNDF240om, and starch was calculated as the product of the DM mass of the rumen contents and the nutrient content of the rumen contents. Rumen turnover rate (%/h) of OM, NDF, uNDF240om, and starch was calculated as $[100 \times (\text{intake of nutrient}/\text{rumen pool of nutrient})/24]$ (Voelker Linton and Allen, 2008). Nutrient intake was calculated using DMI from d 27 and 28 and the nutrient content of the diets from d 18 to 28. Rumen turnover time (h) was calculated as $1/[\text{rumen turnover rate (\%/h)}/100]$.

Rates of passage of liquid, small, medium, and large particles were determined by chemical marker. Before start of the study, approximately 150 kg of conventional and brown midrib-3 corn silages were collected and dried in a forced-air oven at 55°C for 24 h. Dry silages were hand shaken through a 3.2-mm screen to separate the large and small particles. The dry silages that did not pass the 3.2-mm screen were soaked in a barrel of tap water, approximately 2 kg at a time, and all floating particles were skimmed off the top and retained, with the heavier kernels of starch sinking to the bottom and not being retained. The fibrous fraction was skimmed from the barrel and the silage particles that passed a 3.2-mm screen were boiled in a solution of 3% sodium lauryl sulfate for 1 h, and were agitated at intervals of approximately 10 min. After boiling, the fibrous silage particles were placed in fine mesh bags and rinsed with warm tap water in a washing machine on rinse and spin cycles 6 to 8 times. This material was dried in a forced-air oven for 24 h at 55°C and separated by dry vertical sieving (Ro-Tap testing sieve shaker model B; W. S. Tyler Combustion Engineering Inc.) with 19.00-, 13.20-, 9.50-, 6.70-, 4.75-, 3.35-, 2.36-, 1.18-, 0.60-, and 0.30-mm sieves for 5 min. Particle sizes were classified according to Mertens (2005), whereby particles passing through the 1.18-mm screen but retained on the 0.30-mm screen were labeled small

particles, particles retained on the 1.18-mm screen but passing through the 4.75-mm screen were combined and labeled medium particles, and all particles retained on the 4.75- to 19.00-mm screens were combined and labeled as large particles. Particles passing through the 0.30-mm screen were discarded. Small, medium, and large particles of conventional and brown midrib-3 corn silage NDF were soaked in rare earth acetates of Sm, Yb, and Pr, respectively, at a concentration of 50 mg of rare earth to 1,000 mg of corn silage NDF particles for 48 h according to the methods of Ellis and Beaver (1984), with the exception that a solution of 0.1 M acetic acid was used to rinse particles for 3 h after marking with the rare earth acetate.

Cows were dosed with 310 g of the appropriate large and medium silage NDF particles, and 100 g of small conventional or brown midrib-3 corn silage NDF, depending on the source of corn silage in the treatment diet, at time of fresh feed delivery on d 18. All particles were combined, hydrated with tap water to achieve approximately 50% DM, mixed with 3 L of as-fed TMR, and offered to the cows before feeding. Any portion of the dose not consumed within 20 min was manually administered through the rumen cannula and mixed with the top mat of rumen contents. Additionally, a Na Co-EDTA solution was prepared according to Udén et al. (1980) for determination of liquid passage rate. Twenty grams of Na Co-EDTA was dissolved in 500 mL of reverse osmosis-treated H_2O and pulse dosed into the rumen before feeding on d 18.

Fecal samples were collected from the rectum at 0, 3, 6, 9, 12, 18, 24, 30, 36, 42, 48, 60, 72, 84, 96, 120, 144, and 168 h after dosing. Fecal samples were dried at 55°C for 48 h, ground (1-mm screen, Wiley Mill, Arthur H. Thomas), and analyzed using inductively-coupled plasma atomic emission spectroscopy (Cornell College of Agriculture and Life Sciences Nutrient Analysis Laboratory, Ithaca, NY). Solid and liquid passage rates were calculated according to Grovum and Williams (1973) using the `lm` function of R version 3.5.2 (R Core Team, 2014).

Statistical Analysis

Repeated measurements of performance data from the collection period (i.e., rumen pH, rumen VFA, rumen digestion kinetics, and passage rate) were reduced to period means for each cow before statistical analysis. Data with a normal distribution (rumen pH, rumen VFA, rumen digestion kinetics, and passage rate) were analyzed as a replicated Latin square design (Latin rectangle) with fixed effects of corn silage, trace mineral, corn silage and trace mineral interaction, period within replicated square, and replicated square

using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Cow within replicate was a random effect.

Data that followed a nonnormal distribution (i.e., pH range, AUC <5.8, pH <5.8, pH <5.5, propionate concentration, starch turnover rate, particle passage rate, and mean retention time of large particles) were analyzed using an alternative distribution (negative binomial with log link function) that optimized the chi-squared statistic using the GLIMMIX procedure of SAS. Data were analyzed as a replicated Latin square design (Latin rectangle) with fixed effects of corn silage, trace mineral, corn silage and trace mineral interaction, period within replicated square, and replicated square. Cow within replicate was a random effect. Data from this analysis are reported based on the mean using the ilink function.

Least squares means were separated using the Tukey's procedure for MIXED and GLIMMIX procedure when a significant *F*-test ($P \leq 0.05$) was detected. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Dietary Ingredient and Nutrient Composition

The dietary ingredient and nutrient composition are discussed thoroughly in Miller et al. (2020) and will be briefly described here. The NDF content averaged 41.9 and 39.1% for CON and BM3 corn silage, respectively (Tables 2 and 3). The CON corn silage had an unexpected change in starch content during periods 3 and 4 compared with periods 1 and 2 of the study (29.6 vs. 35.6% of DM), and consequently starch content was lower for CON than the BM3 corn silage (32.8 vs. 36.0% of DM). The BM3 corn silage had a 9.7 percentage-unit greater NDF digestibility at 30 h and a 2.8 percentage-unit lower uNDF240om content compared with the CON corn silage (68.4 vs. 58.7% of aNDFom; 7.8 vs. 10.6% of DM, respectively). These relative differences in NDF content and digestibility were expected based on previous literature comparing brown midrib-3 and conventional corn silage hybrids (Oba and Allen, 2000b; Hassanat et al., 2017; Miller et al., 2018).

The calculated chemical composition and fiber fractions of the treatment diets (based on analysis of individual ingredients) are shown in Tables 4 and 5. More detailed information for individual feed ingredients may be found in Miller et al. (2020). The CON diets had numerically greater aNDFom and lower starch content than the brown midrib diets due to the greater aNDFom and lower starch content of the CON corn

silage (36.2 vs. 32.1% of DM; 21.9 vs. 26.2% of DM, respectively). The NDF digestibility at 30 h was numerically greater for the BM3 diets compared with the CON diets and the uNDF240om was numerically lower for the BM3 diets compared with the CON diets (62.0 vs. 55.7% of aNDFom; 6.9 vs. 8.6% of DM, respectively). As expected, the pef among the diets was similar and the peNDF content of the CON diets was greater than BM3 diets (24.1% vs. 21.9%, respectively), which can be attributed to differences in aNDFom content in each diet and not particle size distribution. The overall TMR composition primarily reflected compositional differences between the 2 sources of corn silage.

The supplemental and total dietary trace mineral concentrations were numerically similar among diets as concentrations of trace minerals were kept constant across diets, but the source was either STM or HTM and differed among diets, as shown in Table 6. The concentrations of trace minerals were chosen to be similar to on-farm diets for high-producing dairy cows and purposely exceeded NRC (2001) guidelines.

In summary, we anticipated that the response to diet would be a function of source of corn silage and trace minerals. Although we had intended for the silage to differ mainly in NDF digestibility, the unexpected variation in starch content during the course of the study means that any measured effect of corn silage will be a combination of NDF and starch effects.

Intake, Milk, and BW Responses

The subset of 8 cannulated, multiparous cows used for the rumen measures had DMI, milk, and BW responses to diet that were similar to the entire group of 14 cows reported in Miller et al. (2020). Specifically, the same 1-kg increase in DMI occurred for cows fed the BM3-HTM diet versus the other 3 diets (29.2 versus 28.2 kg/d; $P < 0.16$) just as in Miller et al. (2020), but with the smaller number of cows the effect was not significant. Milk yield was greater for cows fed the BM3-HTM diet relative to the other diets (49.5 versus 47.2 kg/d; $P < 0.02$), which agrees with the responses observed by Miller et al. (2020) for the larger group of 14 cows.

Overall, the cannulated cows fed the CON and BM3-STM diets had 0.7 kg greater DMI compared with the entire group of 14 cows fed the CON and BM3-STM diets (28.2 vs. 27.5 kg/d, respectively). The cannulated cows fed the BM3-HTM diet had 0.6 kg greater DMI compared with the entire group of 14 cows fed the BM3-HTM (29.2 vs. 28.6 kg/d). The cannulated cows had a greater average BW of 688 kg compared with the entire group BW of 665 kg. The cannulated cows fed the BM3 diets had 0.6 kg greater ECM yield compared

Table 2. Data (mean $\pm$ SD) characterizing the analyzed chemical composition of feed ingredients fed to lactating Holstein cows

Item	CON ¹ corn silage	BM3 ¹ corn silage	Straw	Beet pulp	Corn meal
DM, ² %	30.6 $\pm$ 1.7	33.8 $\pm$ 1.2	88.5 $\pm$ 0.7	91.4 $\pm$ 0.5	86.3 $\pm$ 0.3
CP, % of DM	7.4 $\pm$ 0.3	7.3 $\pm$ 0.1	4.3 $\pm$ 0.2	8.1 $\pm$ 0.2	8.0 $\pm$ 0.1
Soluble protein, % of CP	57.8 $\pm$ 2.0	49.6 $\pm$ 5.1	36.7 $\pm$ 2.6	10.4 $\pm$ 2.5	14.1 $\pm$ 1.7
Ammonia, % of CP	11.3 $\pm$ 5.1	12.5 $\pm$ 1.8	—	—	—
ADF, % of DM	25.7 $\pm$ 3.0	22.4 $\pm$ 1.1	59.1 $\pm$ 2.2	30.8 $\pm$ 1.2	2.5 $\pm$ 0.2
aNDFom, ³ % of DM	41.9 $\pm$ 2.8	39.1 $\pm$ 1.4	78.0 $\pm$ 2.3	33.9 $\pm$ 1.1	9.6 $\pm$ 1.0
30-h aNDF digestibility, % of aNDFom	58.7 $\pm$ 1.5	68.4 $\pm$ 2.4	33.7 $\pm$ 0.6	—	—
Lignin, % of DM	3.0 $\pm$ 0.0	2.0 $\pm$ 0.1	11.1 $\pm$ 0.6	5.8 $\pm$ 0.1	0.9 $\pm$ 0.2
Starch, % of DM	32.8 $\pm$ 4.1	36.0 $\pm$ 2.3	1.4 $\pm$ 1.7	0.5 $\pm$ 0.2	72.7 $\pm$ 0.5
7-h starch digestibility, % of starch	81.2 $\pm$ 8.1	80.6 $\pm$ 7.7	—	—	55.1 $\pm$ 3.2
Sugar (ESC ⁴), % of DM	0.6 $\pm$ 0.1	0.5 $\pm$ 0.2	0.9 $\pm$ 0.4	8.3 $\pm$ 0.7	2.0 $\pm$ 0.1
Crude fat, % of DM	2.74 $\pm$ 0.20	3.27 $\pm$ 0.57	1.01 $\pm$ 0.17	1.14 $\pm$ 0.29	3.72 $\pm$ 0.02
Ash, % of DM	3.8 $\pm$ 0.6	3.7 $\pm$ 0.2	7.7 $\pm$ 0.5	10.9 $\pm$ 1.0	1.3 $\pm$ 0.2
Ca, % of DM	0.36 $\pm$ 0.08	0.29 $\pm$ 0.04	0.17 $\pm$ 0.01	1.99 $\pm$ 0.1	0.02 $\pm$ 0.01
P, % of DM	0.24 $\pm$ 0.02	0.24 $\pm$ 0.02	0.07 $\pm$ 0.01	0.13 $\pm$ 0.01	0.29 $\pm$ 0.01
Mg, % of DM	0.16 $\pm$ 0.02	0.16 $\pm$ 0.01	0.07 $\pm$ 0.01	0.29 $\pm$ 0.01	0.11 $\pm$ 0.01
K, % of DM	0.93 $\pm$ 0.15	0.96 $\pm$ 0.01	0.87 $\pm$ 0.10	0.31 $\pm$ 0.02	0.42 $\pm$ 0.01
S, % of DM	0.10 $\pm$ 0.01	0.12 $\pm$ 0.01	0.08 $\pm$ 0.01	0.38 $\pm$ 0.02	0.1 $\pm$ 0.01
Na, % of DM	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.01 $\pm$ 0.00	0.05 $\pm$ 0.02	0.01 $\pm$ 0.01
Chloride ion, % of DM	0.12 $\pm$ 0.03	0.15 $\pm$ 0.03	0.08 $\pm$ 0.05	0.01 $\pm$ 0.02	0.06 $\pm$ 0.01
Fe, mg/kg	165 $\pm$ 61	132 $\pm$ 16	217 $\pm$ 92	1,146 $\pm$ 72	29 $\pm$ 3
Cu, mg/kg	4.5 $\pm$ 1.0	5.0 $\pm$ 0.0	3.0 $\pm$ 0.0	12.3 $\pm$ 1.0	2.5 $\pm$ 0.6
Mn, mg/kg	24.8 $\pm$ 3.6	19.5 $\pm$ 0.6	24.3 $\pm$ 6.7	104.3 $\pm$ 5.5	5.5 $\pm$ 0.6
Zn, mg/kg	22.8 $\pm$ 3.5	22.8 $\pm$ 3.0	10.5 $\pm$ 0.6	29.8 $\pm$ 3.6	22.3 $\pm$ 1.0
Lactic acid, % of DM	4.6 $\pm$ 2.1	5.4 $\pm$ 1.0	—	—	—
Acetic acid, % of DM	2.9 $\pm$ 1.1	3.5 $\pm$ 0.8	—	—	—
Propionic acid, % of DM	0.1 $\pm$ 0.1	0.3 $\pm$ 0.1	—	—	—
Butyric acid, % of DM	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	—	—	—
Total VFA, % of DM	7.5 $\pm$ 3.1	8.9 $\pm$ 1.1	—	—	—
pH	3.91 $\pm$ 0.02	3.96 $\pm$ 0.11	—	—	—

¹Conventional corn silage (CON) or brown midrib corn silage (BM3).²For DM analysis, n = 44 for CON and BM3 corn silage and straw; n = 8 for beet pulp and corn meal.³aNDFom = amylase neutral detergent fiber on an organic matter basis.⁴Ethanol-soluble carbohydrates.

with the entire group fed the BM3 diets (48.1 vs. 47.6 kg/d), whereas the cannulated cows fed the CON diets had 0.9 kg greater ECM yield compared with the entire group fed the CON diets (47.1 vs. 46.2 kg/d).

In summary, the cannulated cows used in this study had slightly greater DMI, BW, and milk yield than the entire group of 14 cows reported in Miller et al. (2020),

but the pattern of response to diet was similar between the studies.

Rumen pH and VFA

Rumen pH data are presented in Table 7. Minutes below rumen pH of 5.8 showed a tendency for a corn

Table 3. Data (mean $\pm$ SD) characterizing the fiber fractions of feed ingredients fed to lactating Holstein cows

Item	CON ¹ corn silage	BM3 ¹ corn silage	Straw	Beet pulp	Corn meal
aNDFom, ² % of DM	41.9 $\pm$ 2.8	39.1 $\pm$ 1.4	78.0 $\pm$ 2.3	33.9 $\pm$ 1.1	9.6 $\pm$ 1.0
Undigested NDFom ³ at 12 h, % of DM	—	—	—	12.9 $\pm$ 4.8	7.7 $\pm$ 0.6
Undigested NDFom at 30 h, % of DM	17.3 $\pm$ 1.6	12.3 $\pm$ 0.8	51.7 $\pm$ 1.4	—	—
Undigested NDFom at 72 h, % of DM	—	—	—	6.2 $\pm$ 2.0	3.0 $\pm$ 0.4
Undigested NDFom at 120 h, % of DM	11.4 $\pm$ 1.3	8.3 $\pm$ 0.6	39.9 $\pm$ 1.8	5.5 $\pm$ 1.8	2.2 $\pm$ 0.2
Undigested NDFom at 240 h, % of DM	10.6 $\pm$ 0.7	7.8 $\pm$ 0.3	38.8 $\pm$ 0.9	—	—
Potentially digestible NDF, ⁴ % of aNDFom	74.8 $\pm$ 0.8	80.1 $\pm$ 1.0	50.2 $\pm$ 1.5	—	—

¹Conventional corn silage (CON) or brown midrib corn silage (BM3).²Amylase neutral detergent fiber on an organic matter basis.³NDFom = neutral detergent fiber on an organic matter basis.⁴Potentially digestible NDF = (aNDFom – undigested NDFom at 240 h)/aNDFom.

Table 4. Calculated diet composition based on chemical analysis of ingredients in diets containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with either sulfate trace minerals (STM) or hydroxy trace minerals (HTM) fed to lactating Holstein cows

Item	Diet			
	CON		BM3	
	STM	HTM	STM	HTM
DM, ¹ %	43.5 ± 2.0 ²	43.7 ± 1.6	45.9 ± 1.2	46.4 ± 1.3
CP, % of DM	15.0 ± 0.4	15.3 ± 0.3	15.6 ± 0.5	15.4 ± 0.2
Soluble protein, % of CP	40.3 ± 1.9	41.0 ± 2.0	36.3 ± 4.0	35.6 ± 3.4
ADF, % of DM	23.4 ± 1.8	23.3 ± 1.8	19.7 ± 0.7	19.6 ± 0.9
aNDFom, ³ % of DM	36.3 ± 2.1	36.0 ± 2.1	32.1 ± 0.9	32.0 ± 0.9
Lignin, % of DM	3.4 ± 0.1	3.4 ± 0.1	2.7 ± 0.1	2.8 ± 0.1
Starch, % of DM	21.8 ± 2.4	21.9 ± 2.4	26.2 ± 1.3	26.1 ± 1.2
Sugar (ESC ⁴), % of DM	4.1 ± 0.3	4.1 ± 0.1	4.0 ± 0.3	3.9 ± 0.2
Crude fat, % of DM	3.0 ± 0.4	3.0 ± 0.4	3.2 ± 0.6	3.3 ± 0.5
Ash, % of DM	8.12 ± 0.33	8.06 ± 0.43	7.91 ± 0.28	7.74 ± 0.62
Ca, % of DM	1.14 ± 0.07	1.17 ± 0.02	1.07 ± 0.05	1.05 ± 0.10
P, % of DM	0.35 ± 0.01	0.36 ± 0.0	0.36 ± 0.01	0.35 ± 0.01
Mg, % of DM	0.41 ± 0.04	0.44 ± 0.02	0.41 ± 0.03	0.40 ± 0.03
K, % of DM	1.40 ± 0.11	1.40 ± 0.10	1.38 ± 0.03	1.38 ± 0.03
S, % of DM	0.23 ± 0.01	0.23 ± 0.01	0.25 ± 0.01	0.23 ± 0.01
Na, % of DM	0.49 ± 0.05	0.49 ± 0.02	0.49 ± 0.04	0.46 ± 0.03
Chloride ion, % of DM	0.39 ± 0.01	0.39 ± 0.02	0.41 ± 0.05	0.39 ± 0.02
Fe, mg/kg of DM	306 ± 33	310 ± 36	275 ± 14	274 ± 14
Cu, mg/kg of DM	18 ± 0.7	17 ± 1.1	17 ± 1.2	16 ± 0.8
Mn, mg/kg of DM	61 ± 4	68 ± 2	60 ± 8	64 ± 5
Zn, mg/kg of DM	101 ± 20	93 ± 3	107 ± 30	88 ± 6

¹Diets were analyzed for DM at 105°C; n = 44 for each diet. For all other analyses, n = 4.²Mean ± SD.³aNDFom = amylase neutral detergent fiber on an organic matter basis.⁴Ethanol-soluble carbohydrates.**Table 5.** Data (mean ± SD) characterizing the fiber fractions and particle size of diets containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with either sulfate trace minerals (STM) or hydroxy trace minerals (HTM) fed to lactating Holstein cows

Item	Diet			
	CON		BM3	
	STM	HTM	STM	HTM
aNDFom, ¹ % of DM	36.3 ± 2.1	36.0 ± 2.1	32.1 ± 0.9	32.0 ± 0.9
30-h aNDF digestibility, % of aNDFom	56.0 ± 2.4	55.3 ± 4.4	61.9 ± 2.1	62.0 ± 2.0
Undigested NDFom ² at 30 h, % of DM	15.9 ± 0.2	16.0 ± 0.8	12.2 ± 0.5	12.1 ± 0.4
Undigested NDFom at 120 h, % of DM	9.3 ± 0.5	9.4 ± 0.6	7.6 ± 0.2	7.5 ± 0.3
Undigested NDFom at 240 h, % of DM	8.6 ± 0.3	8.6 ± 0.4	6.9 ± 0.2	6.9 ± 0.1
Potentially digestible NDF, ³ % of aNDFom	76.3 ± 1.0	76.1 ± 0.9	78.4 ± 0.5	78.2 ± 0.6
pef (PSPS) ⁴	0.67 ± 0.01	0.66 ± 0.01	0.66 ± 0.01	0.65 ± 0.01
peNDF (PSPS), ⁵ % of DM	24.3	23.8	21.2	20.8
pef (Ro-Tap) ⁶	0.67 ± 0.02	0.66 ± 0.02	0.68 ± 0.02	0.69 ± 0.02
peNDF (Ro-Tap), ⁷ % of DM	24.3	23.8	21.8	22.1

¹aNDFom = amylase NDF on an organic matter basis.²NDFom = neutral detergent fiber on an organic matter basis.³Potentially digestible NDF = (aNDFom – undigested NDFom at 240 h)/aNDFom.⁴pef (PSPS) = physical effectiveness factor measured with the Penn State Particle Separator (Lammers et al., 1996); % of as-fed sample ≥ 4.0 mm.⁵peNDF (PSPS) = physically effective neutral detergent fiber [pef × aNDFom; calculated according to Mertens (1997)] using the Penn State Particle Separator.⁶pef (Ro-Tap) = physical effectiveness factor with Ro-Tap (Mentor, OH), % of DM ≥ 1.18 mm.⁷peNDF (Ro-Tap) = physically effective neutral detergent fiber [pef × aNDFom; calculated according to Mertens (1997)] using the Ro-Tap.

Table 6. Analyzed concentrations and amounts of supplemental and dietary Cu, Mn, and Zn in diets containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with either sulfate trace minerals (STM) or hydroxy trace minerals (HTM) fed to lactating Holstein cows

Item	Diet			
	CON		BM3	
	STM	HTM	STM	HTM
Supplemental mineral concentration, ¹ mg/kg of DM				
Cu	10.6	10.5	9.2	8.9
Mn	41.5	41.2	41.2	39.6
Zn	64.3	63.8	63.2	60.8
Total dietary concentration, mg/kg of DM				
Cu	17.5	17.1	17.1	16.4
Mn	60.5	68.4	59.9	63.5
Zn	100.9	93.2	107.2	87.9
Total dietary mineral, mg/d				
Cu	493	469	468	458
Mn	1,671	1,877	1,650	1,830
Zn	2,767	2,567	2,943	2,517
Supplemental mineral, % of total dietary mineral				
Cu	60.6	61.6	53.9	54.0
Mn	68.6	60.3	68.8	62.4
Zn	63.7	68.5	58.9	69.1

¹Concentrations based on treatment average DMI.

silage by trace mineral interaction ($P = 0.09$). However, for all diets the time that rumen pH was below 5.8 was less than the concern threshold for SARA of 180 to 240 min/d as reported by Zebeli et al. (2012). The generally low level of SARA in our study was not surprising given the relatively high dietary forage content of approximately 55% of ration DM. Stone (2004) reported that diets with peNDF content between 21 and 23% of DM would have a marginal risk of SARA, and the treatment diets in this study averaged 23% peNDF. Daily mean, standard deviation, minimum, and maximum rumen pH were unaffected ($P > 0.10$) by corn silage or trace mineral source. The measures of SARA (AUC <5.8 and pH <5.5, min/d) were also unaffected ($P > 0.10$) by corn silage or trace mineral source. In agreement with our findings, Genther and Hansen (2015) and Caldera et al. (2019) found no effect of trace mineral on rumen pH in cannulated beef steers.

In contrast with our findings, Pino and Heinrichs (2016) observed that Holstein heifers fed diets with organic trace minerals had lower rumen pH than did heifers fed diets with STM. Two potentially important differences between Pino and Heinrichs (2016) and our present study were the source of organic trace mineral (proteinates and yeast vs. HTM), and the animal model (growing dairy heifers vs. lactating dairy cows). To our knowledge, no previous study has evaluated the effect of HTM compared with STM on rumen pH in lactating dairy cows. It is possible that an effect of source of trace minerals fed to lactating cows might have been observed with lower forage diets that would have pre-

disposed the rumen to lower pH. Future research should assess the interaction between source of trace minerals and fiber content of the diet when fed to lactating cows.

Rumen VFA data are presented in Table 8. The interactions of time of rumen fluid collection relative to feeding for corn silage, trace mineral, and corn silage by trace mineral were not statistically significant ($P > 0.10$) for all measures and are not reported in Table 8. For all VFA measures, time was significant ($P < 0.01$), which was expected because digestion throughout the day is dependent on substrate availability and influenced by delivery of fresh TMR and distribution of eating bouts throughout the day (Dijkstra et al., 2012).

A tendency for an interaction ($P = 0.07$) between corn silage and trace mineral source was observed for total VFA concentration. The total VFA concentration was lower for the cows fed the STM compared with HTM in the BM3 diets (116.0 vs. 121.5 mM), whereas the total VFA concentration was not affected by source of trace minerals in the CON diets (116.1 mM). This was likely due to higher DMI, and consequently greater fermentable substrate, for cows fed the BM3-HTM compared with cows fed the BM3-STM as reported in a companion paper (Miller et al., 2020).

Cows fed the CON diets had greater ($P = 0.02$) acetate concentration than cows fed the BM3 diets (65.7 vs. 64.7 molar %), which was possibly due to higher NDF content of the CON diets. Oba and Allen (2000a) reported that diets with higher NDF content had greater concentrations of rumen acetate compared with lower NDF diets. In contrast, cows fed the BM3

Table 7. Least squares means of rumen pH for lactating Holstein cows (n = 8) fed a diet containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with either sulfate trace minerals (STM) or hydroxy trace minerals (HTM)

Item	Diet							P-value
	CON			BM3				
	STM	HTM	STM	HTM	SE	Corn silage (CS)	Trace mineral (TM)	
24-h mean pH	6.16	6.17	6.14	6.12	0.04	0.27	0.79	0.71
24-h SD of pH	0.25	0.22	0.22	0.24	0.02	0.93	0.57	0.15
24-h minimum pH	5.59	5.66	5.64	5.53	0.07	0.48	0.79	0.15
24-h maximum pH	6.61	6.60	6.57	6.57	0.03	0.23	0.84	0.77
24-h range (maximum pH – minimum pH)	0.99	0.91	0.91	1.01	—	0.87	0.87	0.15
	(0.87 to 1.15 ¹)	(0.81 to 1.05 ¹)	(0.81 to 1.05 ¹)	(0.90 to 1.17 ¹)				
AUC ² <5.8, pH units × min	21.1	11.5	14.2	18.4	—	0.92	0.65	0.27
	(6.7 to 50.3 ¹)	(5.2 to 38.9 ¹)	(4.1 to 32.6 ¹)	(7.7 to 57.5 ¹)				
pH <5.5, min/d	20	24	12	27	—	0.71	0.32	0.53
	(7.0 to 55.6 ¹)	(8.6 to 66.1 ¹)	(4.0 to 34.7 ¹)	(9.9 to 74.7 ¹)				
pH <5.8, min/d	118	79	66	151	—	0.92	0.53	0.09
	(46 to 298 ¹)	(31 to 200 ¹)	(26 to 168 ¹)	(60 to 381 ¹)				

¹95% CI reported because original data were not normally distributed and were analyzed using an alternative distribution.²AUC = area under the curve.**Table 8.** Least squares means of rumen VFA profiles for lactating Holstein cows (n = 8) fed a diet containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with either sulfate trace minerals (STM) or hydroxy trace minerals (HTM)

Item	Diet							Hour ¹	
	CON				BM3		P-value		
	STM	HTM	STM	HTM	SE	Corn silage (CS)			Trace mineral (TM)
Total VFA, mM	116.1	116.1	116.0	121.5	2.9	0.09	0.08	0.07	<0.01
VFA, molar %									
Acetate (A)	65.6	65.8	64.9	64.5	0.5	0.02	0.69	0.37	<0.01
Propionate (P)	20.5	20.3	21.0	21.8	—	0.01	0.49	0.22	<0.01
	(19.4 to 21.6 ²)	(19.2 to 21.4 ²)	(19.9 to 22.1 ²)	(20.7 to 23.0 ²)					
Butyrate (B)	10.9	11.0	11.2	10.9	0.3	0.56	0.61	0.41	<0.01
Isobutyrate	0.60	0.61	0.55	0.54	0.02	<0.01	0.84	0.25	<0.01
Valerate	1.76	1.70	1.66	1.65	0.05	0.08	0.46	0.52	<0.01
Isovalerate	0.57	0.58	0.52	0.51	0.03	<0.01	0.94	0.46	<0.01
A:P	3.22	3.27	3.11	2.98	0.10	0.02	0.60	0.25	<0.01
(A+B):P	3.75	3.81	3.65	3.48	0.12	0.03	0.57	0.22	<0.01
Ammonia, mg/dL	5.35	6.46	5.66	5.25	0.79	0.50	0.60	0.26	<0.01

¹Interaction terms hour × CS, hour × TM, and hour × CS × TM were not statistically significant and are not reported in this table.²95% CI reported because original data were not normally distributed and were analyzed using an alternative distribution.

diets had greater ($P = 0.01$) propionate concentrations than cows fed the CON diets (21.4 vs. 20.4 molar %), which was likely due to higher starch content of BM3 diets. These responses in rumen acetate and propionate track with established relationships between dietary carbohydrate (NDF and starch) content and rumen fermentation as exemplified by Oba and Allen (2003) who reported higher propionate concentrations when cows were fed high-starch compared with low-starch diets. Butyrate was unaffected ($P > 0.10$) by source of corn silage or trace mineral.

Cows fed the CON diets had greater ($P < 0.01$) concentrations of isobutyrate and isovalerate compared with cows fed the BM3 diets (0.61 vs. 0.55 molar %; 0.58 vs. 0.52 molar %, respectively). In agreement with our findings, earlier studies have reported that cows fed diets with conventional corn silage had greater concentrations of isobutyrate and isovalerate compared with cows fed diets with brown midrib-3 corn silage (Greenfield et al., 2001; Gehman et al., 2008; Hassanat et al., 2017). Gehman et al. (2008) proposed that the lower concentrations of isobutyrate and isovalerate when cows were fed brown midrib-3 corn silage are due to enhanced rumen fiber fermentation and use of branched-chain VFA by cellulolytic bacteria.

Cows fed the CON diets had a tendency for greater ($P = 0.08$) rumen concentrations of valerate compared with cows fed the BM3 diets (1.73 vs. 1.66 molar %). Reflecting the small but significant changes in acetate and propionate concentrations, cows fed the CON diets had greater ($P < 0.05$) acetate to propionate ratio and acetate plus butyrate to propionate ratio compared with the cows fed the BM3 diets (3.25 vs. 3.04; 3.78 vs. 3.57). Rumen ammonia concentration was unaffected ($P > 0.10$) by corn silage type or trace mineral source. In agreement with our findings, Pino and Heinrichs (2016) reported no effect of source of trace mineral on acetate, propionate, isobutyrate, valerate, and isovalerate concentrations, but did report that heifers fed a diet with organic trace minerals had a higher butyrate concentration than heifers fed a diet with STM. They suggested that this was due to shorter eating time for heifers fed the organic trace minerals. However, in a companion paper we did not observe a trace mineral effect on eating time (Miller et al., 2020). As previously discussed, the likely differences between Pino and Heinrichs (2016) and our current study are the source of organic trace mineral (proteinates and yeast vs. HTM) and the animal model (growing dairy heifers vs. lactating dairy cows). Similar to rumen pH, to our knowledge, no previous study has evaluated the effect of HTM compared with STM on rumen fermentation and VFA profiles in lactating dairy cows.

Rumen Digesta Pools and Turnover

Rumen digesta characteristics, pools, and turnover are presented in Table 9. Cows fed the CON diets had greater ($P < 0.05$) rumen digesta volume and mass compared with cows fed the BM3 diets (124 vs. 119 L; 105 vs. 101 kg). Rumen density was unaffected ($P > 0.10$) by corn silage or trace mineral source. The differences among treatments for rumen digesta volume and mass were small and most likely not biologically important as rumen density (i.e., mass/volume) was not different ($P > 0.10$) among treatments.

A significant interaction ($P < 0.01$) between corn silage and trace mineral source was observed for rumen OM pool size, indicating that the difference among trace mineral sources was greater in the BM3 versus CON diets. A significant interaction ($P = 0.04$) between corn silage and trace mineral source was observed for OM turnover rate with the difference being greater among the trace mineral sources for the BM3 diets. The OM turnover time had a tendency for an interaction ($P = 0.10$) between corn silage and trace mineral source. The OM turnover time was longer for cows fed the BM3-HTM diet compared with cows fed the BM3-STM diet (12.5 vs. 11.5 h), whereas the OM turnover time was not greatly affected by trace minerals in the CON diets (12.8 vs. 12.7 h). Overall, the smaller rumen OM pool for cows fed the BM3-STM diets was due to a faster turnover rate compared with cows fed the BM3-HTM diets.

The rumen pool of NDF was unaffected ($P > 0.10$) by corn silage or trace mineral source. A similar study investigating the effects of conventional and brown midrib-3 corn silage in low- and high-forage diets found no measurable difference in rumen NDF pool between a high-forage diet formulated with either conventional or brown midrib-3 corn silage (Cotanch et al., 2012a). The NDF turnover rate and time were unaffected ($P > 0.10$) by corn silage or trace mineral source.

A tendency for an interaction ($P = 0.10$) between corn silage and trace mineral source was observed for rumen uNDF240om pool size, reflecting the greater difference between HTM and STM for cows fed the CON diets. This effect on rumen uNDF240om pool size could be due to the different fiber characteristics of the CON and BM3 diets. For example, forage-based diets have resulted in greater differences in total-tract NDF digestibility between sulfate and HTM than byproduct-based diets fed to lactating dairy cows (Faulkner and Weiss, 2017). In our study, cows fed the CON diets had a larger ($P < 0.01$) rumen uNDF240om pool than cows fed the BM3 diets (3.45 vs. 2.61 kg). This was expected as CON diets had greater uNDF240om con-

Table 9. Least squares means of rumen digesta characteristics, pool sizes, and turnover for lactating Holstein cows (n = 8) fed a diet containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with either sulfate trace minerals (STM) or hydroxy trace minerals (HTM)

Item	Diet									
	CON				BM3				P-value	
	STM	HTM	STM	HTM	SE	Corn silage (CS)	Trace mineral (TM)	CS × TM		
Rumen digesta volume, L	126	122	118	119	5	0.01	0.45	0.35		
Rumen digesta mass, kg	106	103	100	101	4	0.02	0.66	0.24		
Rumen density, kg/L	0.84	0.85	0.85	0.85	0.01	0.43	0.53	0.85		
Rumen pool, kg										
OM	13.2	12.6	11.9	13.1	0.6	0.09	0.30	<0.01		
NDF	8.27	7.86	8.00	7.88	0.33	0.51	0.17	0.45		
uNDF240om ¹	3.53	3.37	2.54	2.67	0.20	<0.01	0.91	0.10		
Starch	0.24	0.22	0.36	0.41	0.03	<0.01	0.58	0.28		
Rumen turnover rate, %/h										
OM	8.01	8.28	8.96	8.19	0.46	0.08	0.30	0.04		
NDF	4.92	4.96	4.85	4.81	0.24	0.52	0.99	0.81		
uNDF240om	2.83	2.89	3.21	3.03	0.22	0.05	0.65	0.34		
Starch	131.3 (109.3 to 157.6 ²)	166.3 (138.7 to 199.4 ²)	116.1 (96.5 to 139.5 ²)	87.0 (72.2 to 104.9 ²)	—	<0.01	0.70	<0.01		
Rumen turnover time, h										
OM	12.8	12.7	11.5	12.5	0.7	0.06	0.24	0.10		
NDF	20.8	20.9	21.1	21.2	1.0	0.70	0.89	0.99		
uNDF240om	36.6	36.4	32.6	33.9	2.7	0.04	0.71	0.60		
Starch	0.96	0.91	1.20	1.36	0.08	<0.01	0.55	0.24		

¹uNDF240om = undigested neutral detergent fiber at 240 h of in vitro fermentation on an organic matter basis.²95% CI reported because original data were not normally distributed and were analyzed using an alternative distribution.

tent than the BM3 diets. Cows fed the BM3 diets had faster ($P = 0.05$) turnover rate and shorter ($P = 0.04$) turnover time for uNDF240om than cows fed the CON diets (3.12 vs. 2.86%/h; 33.3 vs. 36.5 h, respectively). In agreement, a similarly designed study with lactating cows fed corn silage-based diets reported a faster iNDF turnover rate for cows fed diets with brown midrib-3 corn silage compared with cows fed diets with conventional corn silage (Oba and Allen, 2000b).

Cows fed the BM3 diets also had a larger rumen starch pool than cows fed the CON diets (0.38 vs. 0.23 kg; $P < 0.01$). Rumen starch pool reflected the difference in starch content between the BM3 and CON diets. An interaction ($P < 0.01$) between corn silage and trace mineral source was observed for starch turnover rate, indicating that the difference among trace mineral sources was greater for cows fed the CON diets. Cows fed the BM3 diets had longer ($P < 0.01$) turnover time for starch compared with cows fed the CON diets (0.94 vs. 1.3 h). This longer turnover time could be due to a larger rumen starch pool and lower total-tract starch digestibility for cows fed the BM3 diets compared with the cows fed the CON diets. Ferraretto and Shaver (2015) reported cows fed brown midrib-3 corn silage-based diets had lower total-tract starch digestibility compared with cows fed conventional corn silage-based diets due to greater kernel vitreousness.

Passage Rate of Corn Silage Particles

Passage rate and mean retention time of marked corn silage particles are presented in Table 10. Liquid passage rate and mean retention time were unaffected ($P > 0.10$) by corn silage or trace minerals. Cows fed the BM3 diets had faster ($P < 0.05$) passage rate of small and medium corn silage NDF particles than cows fed the CON diets (5.73 vs. 5.37%/h; 4.74 vs. 4.31%/h, respectively), but passage was unaffected by trace mineral source ($P > 0.10$). For cows fed the BM3 diets, small and medium corn silage NDF particles spent 1.3 and 2.3 h less time in the rumen than similarly sized NDF particles for cows fed the CON diets (17.9 vs. 19.2 h, $P = 0.03$; 21.4 vs. 23.7 h, $P < 0.01$, respectively). The passage rate of large corn silage NDF particles and their mean retention time were unaffected ($P > 0.10$) by either the source of corn silage or trace mineral in the diet.

As expected, as particle size of corn silage NDF increased from small to large, the mean retention time of these particles increased. Similarly, Cotanch et al. (2012b) reported that, as forage particle size increased, so did mean retention time for cows fed conventional and brown midrib-3 corn silage in low- and high-forage diets. In the study by Cotanch et al. (2012b), cows fed

the brown midrib-3 corn silage diets had faster passage rate and shorter mean retention time for small and medium corn silage NDF particles compared with cows fed the conventional corn silage diets. Likewise, Cotanch et al. (2012b) reported that cows fed high-forage diets with brown midrib-3 corn silage had shorter mean retention time for medium corn silage particles than cows fed high-forage diets with conventional corn silage diets. Our results agree with studies comparing conventional and brown midrib-3 corn silage diets where the differences in DMI and rumen fill were attributable to a lower indigestible fiber fraction and greater fiber passage rates (Oba and Allen, 1999, 2000a,b; Hassanat et al., 2017).

We observed no effect of trace mineral on passage rates of the small, medium, or large corn silage NDF particles. This was surprising because the cows fed the HTM diets had a tendency ($P = 0.10$) for greater total-tract digestibility of aNDFom than the cows fed STM diets (56.9 vs. 54.9% of DM; Miller et al., 2020). The release of free metals in the rumen could have negative effects on cellulolytic bacteria or bind to undigested fractions (e.g., fiber fractions) that pass from the rumen (Torre et al., 1991; Genter and Hansen, 2015; Faulkner et al., 2017). If the free minerals were binding to corn silage particles and affecting their fermentability, then these minerals possibly could alter the buoyancy of the particle, and potentially affect the passage of undigested fiber particles from the rumen.

The process of marking the corn silage particles with rare earth could also be a reason for not observing a trace mineral effect. The particles underwent an extensive process to be marked including neutral detergent extraction, soaking in a rare earth solution for 48 h, and then rinsing with a weak acid for 3 h. In theory, the majority of the available binding sites should have had a rare earth attached, which would limit the free metals from disassociated trace mineral complexes from binding. Further research is needed to identify the mechanism of how trace minerals affect total-tract aNDFom digestibility as described in the companion paper (Miller et al., 2020).

CONCLUSIONS

Source of corn silage influenced rumen dynamics as the greater in vitro NDF digestibility and lower uNDF240om content allowed for a faster turnover rate of iNDF and faster passage rates of small and medium corn silage particles. Source of trace mineral did not have a great effect on rumen characteristics and particle passage rate although we observed a corn silage by trace mineral interaction on OM and uNDF240om pool size and OM turnover. It appears that any influence of

Table 10. Passage rate and mean retention time of liquid, and small, medium, and large corn silage particle data from lactating Holstein cows (n = 8) fed a diet containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with either sulfate trace minerals (STM) or hydroxy trace minerals (HTM)

Item	Diet						P-value	
	CON			BM3				
	STM	HTM	STM	HTM	SE	Corn silage (CS)		Trace mineral (TM)
Liquid								
Passage rate, %/h	7.66	7.51	7.54	7.97	0.3	0.55	0.61	0.29
Mean retention time, h	13.3	13.5	13.5	12.8	0.6	0.69	0.57	0.39
Small ¹ corn silage particles								
Passage rate, %/h	5.45	5.29	5.78	5.67	0.3	0.03	0.37	0.87
Mean retention time, h	18.8	19.5	17.8	17.9	1.1	0.03	0.45	0.53
Medium ² corn silage particles								
Passage rate, %/h	4.27	4.34	4.79	4.70	0.2	<0.01	0.94	0.51
Mean retention time, h	23.9	23.5	21.1	21.6	0.9	<0.01	0.96	0.49
Large ³ corn silage particles								
Passage rate, %/h	3.97	4.01	4.15	3.86	—	0.94	0.24	0.11
	(3.63 to 4.41 ⁴)	(3.67 to 4.47 ⁴)	(3.77 to 4.66 ⁴)	(3.55 to 4.27 ⁴)				
Mean retention time, h	25.2	24.9	23.9	25.8	—	0.89	0.66	0.55
	(21.7 to 29.3 ⁴)	(21.5 to 28.9 ⁴)	(20.5 to 27.9 ⁴)	(22.3 to 29.9 ⁴)				

¹Small = ≥0.03 mm and <1.18 mm.

²Medium = ≥1.18 mm and <4.75 mm.

³Large = ≥4.75 mm and <19.00 mm.

⁴95% CI reported because original data were not normally distributed and were analyzed using an alternative distribution.

the source of trace mineral on intake or digestibility is not primarily effected through changes in rumen pH, VFA ratios, digesta turnover, or particle passage. More research is needed to identify the mechanism of how trace minerals influence DMI and total-tract aNDFom digestibility.

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ORCIDS

- M. D. Miller <https://orcid.org/0000-0002-1448-6289>
 J. S. Lanier <https://orcid.org/0000-0003-3043-7820>
 H. M. Dann <https://orcid.org/0000-0002-7372-7011>
 C. S. Ballard <https://orcid.org/0000-0002-1371-2256>
 R. J. Grant <https://orcid.org/0000-0001-5797-9916>