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Influence of fiber degradability of corn silage in diets with lower and higher fiber content on lactational performance, nutrient digestibility, and ruminal characteristics in lactating Holstein cows. *By Miller et al., page xx.* Brown midrib-corn silage has greater fiber degradability and may allow for faster turnover of fiber from the rumen. We compared brown midrib-3 corn silage to conventional corn silage in diets with lower and higher fiber concentrations. In higher fiber diets, corn silage with lower fiber degradability limited dry matter intake and milk yield. In lower fiber diets, the fiber degradability of the corn silage had no effect on intake or milk yield. Lower fiber degradability corn silage fed at higher dietary levels has a rumen-filling effect and limits dry matter intake and milk production.

Influence of fiber degradability of corn silage in diets with lower and higher fiber content on lactational performance, nutrient digestibility, and ruminal characteristics in lactating Holstein cows

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ABSTRACT

The effect of neutral detergent fiber (NDF) degradability of corn silage in diets containing lower and higher NDF concentrations on lactational performance, nutrient digestibility, and ruminal characteristics in lactating Holstein cows was measured. Eight ruminally cannulated Holstein cows averaging 91 ± 4 (standard error) days in milk were used in a replicated 4 x 4 Latin square design with 21-d periods (7-d collection periods). Dietary treatments were formulated to contain either conventional (CON; 48.6% 24-h NDF degradability) or brown midrib-3 (BM3; 61.1% 24-h NDF degradability) corn silage and either lower NDF (LNDF) or higher NDF (HNDF) concentration (32.0 and 35.8% of ration dry matter, DM) by adjusting the dietary forage content (52 and 67% forage, DM basis). The dietary treatments were: 1) CON-LNDF, 2) CON-HNDF, 3) BM3-LNDF, and 4) BM3-HNDF. Data were analyzed as a factorial arrangement of diets within a replicated Latin square with the MIXED procedure of SAS with fixed effects of diet, period, and replicate. Cow within square was the random effect[RG1]. Cows fed the BM3 corn silage had higher dry matter intake (DMI) than cows fed the CON corn silage whereas cows fed the HNDF diet consumed less DM than cows fed the LNDF diet. An interaction between NDF degradability and NDF content resulted in the HNDF diet decreasing DMI with CON corn silage but not with BM3 silage. Cows fed the BM3 diets had greater energy-corrected milk yield, higher milk true protein content, and lower milk urea nitrogen concentration than cows fed CON diets. Additionally, cows fed the BM3 diets had greater total tract digestibility of organic matter and NDF than cows fed the CON diets. Compared to CON diets, the BMR diets accelerated ruminal NDF turnover. When incorporated into higher NDF diets, corn silage with greater in vitro 24-h NDF degradability and lower undegradable NDF at 240 h of in vitro fermentation (uNDF240) allowed for greater DMI intake than CON. In contrast, for lower NDF diets, NDF degradability of corn silage did not affect DMI which suggests that a threshold level of inclusion of higher NDF degradability corn silage is necessary to observe enhanced lactational performance. Results suggest that there is a maximum gut fill of dietary uNDF240 and that higher NDF degradability corn silage can be fed at greater dietary concentrations.

Key words: brown midrib-3 corn silage, fiber content, undigested fiber, dairy cattle

INTRODUCTION

Forages are the foundation of the dairy cow diet, and specifically corn silage has become the primary forage source for lactating dairy cows in North America (Martin et al., 2017). Forage fiber influences ruminal fermentation, nutrient digestibility, and turnover due to its physical and chemical characteristics. The physical and chemical properties of fiber become especially important as dietary forage inclusion increases (Allen and Mertens, 1988). Forage fiber is commonly quantified as NDF, and the degradability of NDF influences the animal response to any given level of dietary NDF (Oba and Allen, 2000a). In vitro or situ fermentation for 24 or 30 h is a common, single-point measure of NDF degradability (NDFD) that has been related to DMI and milk production in lactating dairy cows (Oba and Allen, 1999b). A meta-analysis by Nousiainen et al. (2009) comprising 92 studies found that forage NDF and NDFD were the two most important dietary factors when predicting OM digestibility in dairy cows.

Recently, a laboratory measure of undegradable NDF, termed undegradable NDF at 240 h of in vitro fermentation (uNDF240), has been found to be related to NDF degradation and passage kinetics, gut fill and DMI, and physical effectiveness of fiber (Nousiainen et al., 2003; Cotanch et al., 2014; Raffrenato et al., 2018). However, due to its novelty, few published studies have used the measure in relation to dairy cow performance (Fustini et al., 2017; Miller et al., 2020). Recently, Miller et al. (2020) showed that corn silage with a lower uNDF240 fraction allowed for greater DMI and milk yield. The corn silage used was brown midrib (BMR) which has a gene mutation that results in less lignin in the plant cell wall, lower uNDF240 content, and greater NDF degradability (Oba and Allen, 1999a; Hassanat et al., 2017; Miller et al., 2020). Generally, substituting a corn silage hybrid with higher NDFD for a hybrid with lower NDFD, on a DM basis, leads to greater DMI, faster fiber turnover from the rumen, greater total tract NDFD, and increased milk production (Oba and Allen, 1999; Hassanat et al., 2017). However, responses have been more variable when corn silage substitution occurs on an NDF basis due in part to variation in NDFD at similar NDF content (Oba and Allen, 2000abc; Hristov et al., 2020).

Oba and Allen (2000abc) remains the only published study to investigate the effects of conventional or brown midrib-3 corn silage in low or high NDF content diets. Their study was comprehensive in relating the brown midrib-3 mutation to animal response although it pre-dated the use of uNDF240 as a measure of undegradable NDF. Consequently, it would be useful to assess cow responses to higher and lower forage diets, containing conventional or brown midrib corn silage, using the newer measures of NDF undegradability in addition to more common measures of NDFD.

Therefore, the objective of this study was to evaluate the effect of lower or higher NDF degradability of corn silage (i.e., conventional or brown midrib-3), when substituted on an NDF basis, in diets containing lower and higher NDF concentration (i.e., forage percentage) and their interaction on lactational performance, nutrient digestibility, and ruminal fermentation and turnover characteristics in Holstein cows. Our hypothesis was that greater NDFD and lower uNDF240 content of brown midrib-3 corn silage would increase DMI and milk yield, nutrient digestibility, and ruminal fiber turnover similarly in lower and higher NDF diets compared to conventional corn silage with lower NDFD and higher uNDF240.

MATERIALS AND METHODS

Experimental Design, Diets, and Management of Cows

All experimental procedures involving animals were approved by the William H. Miner Agricultural Research Institute Animal Care and Use Committee. Eight ruminally fistulated multiparous Holstein cows averaging 91 ± 4 (standard error) DIM were housed in individual tie stalls at the William H. Miner Agricultural Research Institute (Chazy, NY). Cows were stratified by parity, milk production, and DIM and used in a replicated 4 x 4 Latin square design to evaluate the interaction of lower and higher NDFD of corn silage with lower and higher NDF concentrations. Each square consisted of four 21-d periods with 14 d of adjustment to treatment followed by 7 d of data collection. This design and length of experimental period has been shown previously to allow for measurement of animal response to corn silage NDFD and dietary NDF content (Oba and Allen, 2000a).

Dietary Formulation and Feeding Strategy

Dietary treatments (Table 1) were formulated to contain either conventional (CON;?) or brown midrib-3 corn silage (BM3?) and either lower or higher NDF concentration (32.0 and 35.8% of DM) by adjusting the forage percentage (52 and 67% forage, DM basis). The four dietary treatments were: 1) CON and lower NDF (CON-LNDF), 2) CON and higher NDF

(CON-HNDF), 3) BM3 and lower NDF (BM3-LNDF), and 4) BM3 and higher NDF (BM3-HNDF). Diets were formulated using the CPM-Dairy nutritional model (version 3.0; Cornell University, Ithaca, NY; University of Pennsylvania, Philadelphia, PA; and William H. Miner Agricultural Research Institute, Chazy, NY) to supply the required nutrients for a lactating Holstein cow weighing 685 kg with a BCS of 2.75, at 88 DIM, consuming 29 kg/d of DM, and producing 54.5 kg/d milk containing 3.8% fat and 3.2% true protein.

Diets were formulated such that, as corn silage content of the diet was increased, the corn grain inclusion was reduced resulting in lower (52% of DM) and higher (67% of DM) forage-to-concentrate ratios. Corn silages were substituted for each other on an NDF basis in an effort to achieve similar dietary NDF content within a level of dietary forage. Within the higher and lower NDF diets, the concentrate mix was adjusted to maintain similar concentrations of starch, other carbohydrate fractions, and protein fractions (Table 2). Diets were delivered as a TMR once daily (1500 h; Calan Data Ranger; American Calan Inc., Northwood, NH) allowing for ad libitum access to feed; free access to fresh water was provided.

Data Collection, Sampling Procedures, and Analytical Methods

Ingredient and diet sampling and analysis. Individual feed ingredients were collected weekly and dried in a forced-air oven at 105°C for 18 to 24 h (until weight remained unchanged) for DM determination to maintain consistent dietary DM content throughout the study. During the collection period (d 15 to 21), feed ingredients and diets were collected daily and a portion of each sample was dried in a forced-air oven at 105°C for 18 to 24 h for DM determination. The remaining portion of each sample collected was frozen at -20°C until composited by collection period and analyzed for chemical composition using AOAC International (2000; 2006) approved methods (CPM Plus; Cumberland Valley Analytical Services, Inc., Waynesboro, PA). In vitro NDFD of forage composite and dietary composite samples (1-mm grind; Wiley mill; Arthur H. Thomas, Philadelphia, PA) at 24-h was determined using an in vitro fermentation (Daisy^{II} Incubator; Ankom Technology Corp., Fairport, NY) with buffered media containing ruminal fluid (Goering and Van Soest, 1970). The uNDF240 for forages and dietary composite samples was assessed using a batch in vitro rumen fermentation system (Raffrenato et al., 2018).

Dietary ingredients and composite samples were used to determine particle size distributions and a physical effectiveness factor ($pef_{1.18}$; DM basis) by dry vertical sieving (Ro-Tap testing sieve shaker model B; W. S. Tyler Combustion Engineering Inc., Mentor, OH) using a 1.18-mm sieve (Mertens, 1997). The resultant pef value was utilized to calculate peNDF by multiplication of NDF content and pef using the procedure described by Mertens (1997).

Cow lactational response measures. Individual DMI was determined by recording feed offered and refused daily. Samples of diets and orts were collected daily (d 15 to 21) during each period and a portion of each sample was dried in a forced-air oven at 105°C to constant weight for DM determination.

Body weight was measured (Allweigh computerized scale; Allweigh Scale System Inc., Red Deer, AB, Canada) and BCS assigned in 0.25-unit increments on a 1 to 5 scale (Ferguson et al., 1994) for each cow 2 d prior to the beginning of the study and on d 18 of each period. Two trained individuals assigned BCS independently throughout the study.

Cows were milked 3 times daily (0430, 1230, and 2030 h). Milk yields were recorded electronically (ProVantage Information Management System; Bou-Matic, Madison, WI) on d 15 to 21 of each period. Milk samples from 6 consecutive milkings for each cow were collected on d 17 and 18 of each test period and were preserved (Bronolab-W II Liquid Preservative; D & F Control Systems, Inc., Dublin, CA). Samples were sent for analyses within two to three days of sampling and were analyzed for fat, true protein, lactose, solids nonfat, urea nitrogen, and somatic cells by mid-infrared procedures (Dairy One, Ithaca, NY; Foss 4000; Foss Technology, Eden Prairie, MN). After analysis, samples were composited mathematically by day in proportion to milk yield at each sampling within the day. Somatic cell count was transformed and analyzed as SCS according to Shook et al. (1993) using the equation: SCS = $\log_2(SCC/100) + 3$ where SCC is in units of 1,000 cells/mL. Energy-corrected milk was calculated using a formula modified to account for use of true protein instead of total protein (Tyrrell and Reid, 1965; Mark Stephenson, University of Wisconsin; https://dairymarkets.org/PubPod/Reference/Library/Energy%20Corrected%20Milk): (0.327 × kg of milk + 12.95 × kg of fat + 7.65 × kg of true protein).

Rumen VFA and pH. Ruminal pH was measured in 8 ruminally cannulated cows with an indwelling ruminal pH/ORP/REDOX measurement system (Penner et al., 2006; LRCpH; Dascor, Escondido, CA) at 30-s intervals over a 72-h period from 1500 h on d 16 to 1500 h on d 19. Ruminal pH measurements were averaged over a 10-min period within day and summarized as mean pH, minimum pH, maximum pH, variation in pH, minutes under pH of 5.5 and 5.8, and area that the pH curve was below pH of 5.8 (AUC; Beauchemin and Yang, 2005).

Samples of ruminal fluid (approximately 300 mL) were collected from beneath the ruminal digesta mat at 4-h intervals for 24 h on d 17 (1430) and d 18 (1830, 2230, 0230, 0630, and 1030) of each period, and strained through 4 layers of cheesecloth; pH was determined immediately. A portion of each sample of ruminal fluid (approximately 40 mL) was frozen and stored at -20°C until analysis for VFA concentration (Bulletin 856B; Supelco, Inc., Bellefonte, PA) by gas chromatography with use of a Varian CP-3800 gas chromatograph (Varian, Inc., Palo Alto, CA) equipped with a flame-ionization detector and an 80/120 Carbopack B-DA/4% Carbowax 20M column (Supelco, Inc., Bellefonte, PA). Ten mL of ruminal fluid was added to 1 drop (approximately 100 μL) of concentrated HCl and frozen at -20ºC for analysis of ruminal NH_3-N concentration according to the procedure of Chaney and Marback (1962).

Eating and ruminating responses. Chewing activity (eating and ruminating) was monitored using a 5-min scanning interval over a 72-h period (from 1500 h on d 17 to 1500 h on d 20) by direct visual observation. Total time (min) spent on each activity for each day was quantified by multiplying the total number of observations for that activity by 5 min. Number of bouts and the length of bout of eating were calculated. A bout was defined as at least two consecutive observations of eating behavior not interrupted by more than two observations of a different behavior using the procedure of Black et al. (2016).

Nutrient digestibility. Total tract digestibility of DM, OM, ADF, NDF, and potentially digestible NDF (pdNDF) was determined on d 16 to 20 of each test period. Fecal grab samples were collected on d 17 to 20 for each period so that every 3 h in a 24-h period were represented. Fecal samples from each cow were composited by combining approximately 100 g of feces from each time point (n = 8). Samples of diets and orts were collected on d 15 to 21. Samples of diets, orts, and feces were frozen at -20°C, dried in a forced air oven at 55°C for 48 h, and ground to pass through a 1-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA). Composite samples of diets (by period), orts (by cow by period), and feces (by cow by period) were analyzed for DM, ash, ADF, NDF, and lignin (Cumberland Valley Analytical Services, Inc., Waynesboro, PA). Undegraded NDF at 120 h (uNDF120) was used as an internal marker and was quantified as NDF content of diets, orts, and feces samples following an in vitro fermentation (Ankom Technology Corp., Fairport, NY) in buffered ruminal media (Goering and Van Soest, 1970) for 120 h. Total tract digestibility was calculated by the ratio technique using the concentrations of the nutrients and uNDF120 in the diet and feces (Maynard et al., 1979). The nutrient content of the diet used in the digestibility calculation was adjusted for each cow based on the nutrient composition of the diet offered and refused.

Rumen digesta measures and NDF and OM turnover. Ruminal contents were manually evacuated through the ruminal cannula after daily feeding on d 20 and prior to feeding on d 21 for determination of ruminal content mass and volume. To ensure that cows experienced the same interval of time between rumen evacuations, cows were divided into two groups of four cows, each based on square assignment. The first group was evacuated 3.5 h after feeding on d 20 (1830 h) and 3.5 h prior to feeding on d 21 (1130 h). The second group of cows was evacuated 4.5 h after feeding on d 20 (1930 h) and 4.5 h prior to feeding on d 21 (1030 h). During evacuation, approximately 10% of ruminal contents were subsampled and squeezed through a nylon screen (1-mm pore size) to separate solid and liquid phases (Voelker Linton and Allen, 2008). Each phase was weighed, and aliquots (300 g) from solid and liquid phases were then stored at -20ºC for further analysis. Solid and liquid phase samples were subsequently dried at 55ºC, ground (solids: 1-mm screen; Wiley mill; Arthur H. Thomas, Philadelphia, PA; liquid: 1-mm screen, UDY Cyclone Sample Mill; UDY Corp., Fort Collins, CO), and recombined based on DM proportion of each phase for analysis of ash (modified method 942.05; AOAC, 1990; 4 h at 600ºC) and NDF.

Ruminal pool size of OM and NDF was calculated as the product of ruminal content mass and nutrient content of ruminal contents. Ruminal turnover rate (%/h) of OM and NDF was calculated as [100 × (intake of nutrient/ruminal pool of nutrient)/24] (Voelker Linton and Allen, 2008). Nutrient intake was calculated using DMI from d 20 and 21 and corresponding composite sample nutrient content. Ruminal turnover time (h) was calculated as 1/(ruminal turnover rate (%/h)/100).

Statistical Analysis[RG2]

Ingredient and diet analyses were analyzed using the MEANS procedure of SAS (version 9.1, Statistical Analysis Systems Institute Inc., Cary, NC) and reported as the mean and standard error. Repeated measurements on performance, chewing, ruminal digesta characteristics and digestion kinetics, microbial nitrogen, and total tract nutrient digestibility were reduced to period means for each cow before statistical analysis. All data, except for ruminal pH and VFA, were analyzed as a replicated Latin square design with model effects for diet, period within square, and square using MIXED procedure of SAS with cow within replicate as a square effect. Ruminal pH and VFA were analyzed as a replicated Latin square design with repeated measures using the MIXED procedure of SAS. The model included the effects of diet, period within square, and square, time, and the interaction of diet and time with cow within square as a random effect. Significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Dietary and Ingredient Nutrient Composition

Chemical composition, in vitro NDFD, and particle size distribution of the ingredients are presented in Tables 2 and 3. The amylase-modified NDF (OM basis; aNDFom) content averaged 36.1 and 34.8% (DM basis) for CON and BM3 corn silage, respectively. The BM3 corn silage had a 13.5%-unit greater NDFD at 24 h and a 3.4%-unit lower uNDF240om compared to the CON corn silage (62.1 vs. 48.6% of aNDFom; 7.6 vs. 11.0 % of DM, respectively).

The chemical composition, in vitro NDFD, and particle size distribution of the treatment diets (based on analysis of individual ingredients collected over the course of the study) are shown in Tables 4, 5, and 6. As expected, the lower NDF diets had a lower NDF and peNDF concentration than the higher NDF diets. Likewise, the BM3 diets had greater NDFD at 24 h than the CON diets (61.1 vs. 55.2, % of aNDF). The BM3-LNDF diet had the lowest uNDF240om content (7.1% of DM) compared to the CON-HNDF diet (10.2% of DM) with the BM3-HNDF and CON-LNDF diets being intermediate (8.0 and 8.3% of DM, respectively). As a result of differences in NDF and uNDF240, the BM3 diets had higher pdNDF (i.e., aNDFom – uNDF240om) compared to the CON diets (77.5 vs. 73.0% of aNDFom). As expected, the starch content of the lower NDF diets was greater than the higher NDF diets (28.0 vs. 22.5% of DM).

DMI and Lactational Performance

Dry matter intake, nutrient intake, and milk production and composition measures are presented in Table 7. Cows fed the BM3 corn silage had higher DMI than cows fed the CON corn silage (*P* = 0.005) whereas cows fed the HNDF diet consumed less DM than cows fed the LNDF diet (*P* = 0.009). A significant interaction (*P* = 0.02) between NDFD and dietary NDF content resulted in the HNDF diet reducing DMI with CON corn silage but not with BM3 silage. Oba and Allen (2000a) had previously reported that cows fed brown midrib-3 corn silage had greater DMI than cows fed conventional corn silage, whether in low (28.9% of DM) or high (38.0% of DM) NDF diets. Interestingly, in our study, the cows fed the BM3-LNDF diet did not have greater DMI than cows fed the CON-LNDF diet. The similar DMI for the two LNDF diets is most likely related to the relatively low uNDF240om content in the CON-LNDF and the BM3-LNDF diets (8.3 and 7.1% of DM, respectively). Undegradable NDF240om is an in vitro measure of undegradable NDF and has been related to digestion and passage kinetics, gut fill, and physical effectiveness (Nousiainen et al., 2003; Cotanch et al., 2014). In contrast, the BM3 corn silage with greater NDFD and lower uNDF240 did allow for greater DMI than CON corn silage at the higher dietary NDF concentration. The CON-HNDF diet contained 10.2% uNDF240 (% of DM) and this higher concentration in the diet clearly limited DMI. Similarly, with corn silage-based diets, Smith et al. (2018) observed a reduction in DMI for lactating dairy cows fed a ration containing 11.5 versus 8.9% uNDF240 (% of DM). With alfalfa-based diets, Fustini et al. (2017) observed that lower NDFD forage reduced DMI by 20% compared with a higher NDFD forage in both lower (9.5% of DM) and higher (10.9% of DM) uNDF240 diets. Legumes and grasses differ markedly in their content of uNDF240 and uNDF240/lignin (Palmonari et al., 2016; Raffrenato et al. (2018), and it would be expected that differences in DMI and other lactational responses would be observed when cows are fed diets comprised primarily of corn silage or alfalfa hay.

A new metric that combines dietary particle size (pef) and uNDF240 content has been related to DMI for corn-silage based diets fed to lactating dairy cows (Miller et al., 2020). This combination measure, termed peuNDF240, is calculated as the simple product of pef x uNDF240 (Miller et al., 2020). In the present study, DMI tracked reasonably well with peuNDF240, increasing as peuNDF240 decreased. The peuNDF240 values for the diets were: CON-LNDF, 4.6% of DM; CON-HNDF, 6.5% of DM; BM3-LNDF, 4.2% of DM; and BM3-HNDF, 5.0% of DM.

Intake of aNDFom was not affected by source of corn silage ($P = 0.07$), but it was increased as expected for cows fed the higher NDF diets ($P = 0.001$). However, a significant interaction ($P = 0.02$) between NDFD and dietary NDF content was observed whereby cows fed BM3 corn silage consumed more aNDFom on the HNDF versus LNDF diet compared with those cows fed the CON corn silage. The greater aNDFom intake was due most likely to the higher NDFD of the BM3 corn silage that allowed for greater DMI despite the higher ration aNDFom content (Oba and Allen, 2000a). Cows fed the BM3 corn silage consumed less uNDF240om than cows fed CON corn silage ($P < 0.001$) at both dietary NDF concentrations. Lower dietary NDF concentration resulted in less uNDF240om intake than the higher dietary NDF content ($P = 0.004$). This response in uNDF240om intake reflected the greater uNDF240om and lower pdNDF content of the CON diets compared to the BM3 diets. Several recent studies suggest that, when uNDF240om intake exceeds approximately 2.35 kg/d (approximately 10% of ration DM or 0.36% of BW), DMI may become restricted due to gut fill in corn silage-based diets fed to high producing Holstein cattle (Smith et al., 2018; Coons et al., 2019; Miller et al., 2020). With high NDFD alfalfa hay-based diets, Fustini et al. (2017) observed greater uNDF240 intake for cows fed higher uNDF240 (0.48% of BW) than lower uNDF240 diets (0.40% of BW). This relationship between uNDF240 and DMI warrants further investigation with a greater variety of diets, forage sources, and stages of lactation.

Cows fed the BM3 corn silage had greater milk and ECM production than cows fed the CON corn silage (Table 7; $P = 0.005$, $P = 0.02$). For either degradability of corn silage, the

HNDF diet decreased milk and ECM production ($P = 0.02$, $P = 0.05$). This pattern of milk yield response generally followed the responses observed in DMI (Table 7). Milk fat percentage was greater for cows fed the HNDF than LNDF diet ($P = 0.008$), although there was no effect ($P = 0.27$) of corn silage NDFD. Output of milk fat was unaffected by either the NDFD ($P = 0.08$) of the corn silage or the dietary NDF content ($P = 0.17$). The lower milk fat content for cows fed the LNDF diets was most likely due to more fermentable carbohydrates supplied by both starch and degradable NDF compared with the other HNDF diets. Our results agree with Oba and Allen (2000a) who reported that cows fed brown midrib-3 corn silage in a lower NDF diet produced milk with lower fat content compared with cows fed a conventional corn silage in the same lower NDF diet. In contrast, milk fat content was unaffected by NDFD for high NDF diets (Oba and Allen, 2000a).

A significant interaction between NDFD of corn silage and dietary NDF content was observed for milk true protein percentage and yield ($P = 0.05$; $P = 0.04$). Cows fed BM3 corn silage had greater milk protein percentage ($P < 0.001$) and output ($P < 0.001$) than cows fed CON corn silage, and cows fed the HNDF diets produced less milk protein than cows fed the LNDF diets ($P = 0.04$). Our results agree with Coons et al. (2019) who reported that cows fed brown midrib-3 corn silage had greater true protein content and yield compared to cows fed conventional corn silage. Cows fed the BM3 corn silage had greater milk lactose content ($P = 0.001$) and yield ($P < 0.001$) versus cows fed CON corn silage. Likewise, cows fed the LNDF diets had greater lactose yield than cows fed the HNDF diets ($P < 0.001$). A significant interaction occurred between NDFD of corn silage and dietary NDF content with the HDF diet reducing milk lactose more with the CON than BMR corn silage. This response in milk composition aligns with both Oba and Allen (2000a) and Coons et al. (2019) who reported that cows fed brown midrib-3 corn silage had greater lactose content and yield compared to cows fed conventional corn silage. A previous study (Miller et al., 2020) with corn silage-based rations found no effect of uNDF240 on milk fat, true protein, or lactose yield at similar dietary uNDF240 concentrations (7.8 and 10.6% of DM).

Cows fed BM3 corn silage had lower MUN than cows fed CON corn silage (*P* = 0.008). Similarly, cows fed the LNDF diets had lower MUN than cows fed HNDF diets (*P* = 0.03). Our results agree with Hassanat et al. (2017) who reported that, when cows were fed brown midrib-3 corn silage, they had lower MUN than cows fed conventional corn silage. The lower MUN and greater milk protein yield were thought to be related to an increase in AA supply as a result of enhanced rumen OM fermentability (Hassanat et al., 2017). Feed efficiency, whether expressed as milk yield per unit of DMI or ECM yield per unit of DMI, was not affected by type of corn silage or dietary NDF content (*P* > 0.10). Similarly, Hassanat et al. (2017) and Coons et al. (2019) reported no difference in feed efficiency for cows fed brown midrib-3 or conventional corn silage. In contrast, Miller et al. (2020) observed greater milk/DMI when cows were fed corn silage-based rations containing 6.9% versus 8.6% uNDF240. As with DMI and milk yield, the relationship between uNDF240, NDFD, and feed efficiency requires further research with multiple sources of NDF.

Rumen pH and VFA

Rumen pH and VFA data are presented in Table 8. Cows fed the CON-HNDF diet had a greater (*P*[RG3] = 0.02) mean ruminal pH and maximum pH (*P* = 0.03) compared with cows fed the BM3-LNDF diet, with CON-LNDF and BM3-HNDF being intermediate (6.17 vs. 6.00 vs. 6.08; 6.68 vs. 6.52 vs. 6.59, respectively). The lower ruminal pH observed for cows fed the BM3 diets was likely related to more fermentable corn silage for the BM3 versus CON. Similarly, Oba and Allen (2000a) reported that cows fed brown midrib corn silage had a lower mean ruminal pH than cows fed a conventional corn silage diet even when starch content was similar between the two diets. However, Hassanat et al. (2017) reported no difference in mean ruminal pH and maximum pH for cows fed brown midrib-3 or conventional corn silage although both diets had similar starch content. The reason for these differences among the studies is not clear, although it may be due to differences in dietary starch and NDF content. For example, the dietary starch content for Oba and Allen (2000a) averaged approximately 37.5% of ration DM whereas the starch content was only approximately 17% for Hassanat et al. (2017). The dietary starch content for our diets was intermediate relative to these two previous studies and ranged between 21.2 and 28.0% of DM. Differences among the diets in ruminal pH were manifested primarily in mean and maximum pH since the standard deviation of pH, minimum pH, range, and measures of sub-acute rumen acidosis (SARA; AUC < 5.8, pH < 5.5, min/d, pH < 5.8, min/d) were not affected by type of corn silage or dietary NDF content (*P* > 0.05).

Rumen VFA data are presented in Table 9. The interactions of time of rumen fluid collection relative to feeding among treatments were not statistically significant (*P* > 0.10) for all measures. However, time was significant (*P* < 0.05) and this response was expected because substrate availability and digestion fluctuate throughout the day and are influenced by meal bouts (Dijkstra et al., 2012[RG4]).

Cows fed the BM3 corn silage had greater total VFA concentration than cows fed CON corn silage (*P* = 0.008). The greater VFA concentration reflected greater DMI and more fermentable corn silage for the cows fed the BM3 diets compared to the cows fed the CON diets at either dietary NDF content. Likewise, Oba and Allen (2000a) reported that cows fed a higher NDF diet (i.e., 37.5% of dietary DM) containing brown midrib-3 corn silage had greater total ruminal VFA concentration than cows fed a higher NDF diet with conventional corn silage.

Acetate, butyrate, valerate, and isovalerate molar percentages were not affected by NDFD of corn silage or dietary NDF content (*P* > 0.05). In contrast to molar percentage of acetate, cows fed the BM3 and LNDF diets had greater propionate percentage (*P* = 0.004, *P* = 0.001) than cows fed the CON corn silage or the LNDF diets. This response was most likely because cows fed the BM3 and LNDF diets had greater DMI and these diets contained more starch and fermentable OM than the CON or HNDF diets. Our results are in accordance with Oba and Allen (2000a) who reported greater propionate concentration for cows fed a low NDF diet with greater starch content compared to cows fed a higher NDF diet with lower starch content. As a result of the changes in propionate, cows fed the BM3 corn silage had lower acetate-to-propionate ratios than cows fed CON corn silage (*P* = 0.005). Additionally, cows fed the HNDF diets had greater acetate-to-propionate ratios than cows fed the LNDF diets (*P* < 0.001). The change in acetate-to-propionate ratio reflects the increased propionate percentage due to cows consuming more DM and fermentable carbohydrates, particularly starch. Oba and Allen (2000a) also reported lower acetate-to-propionate ratio for cows fed a lower NDF diet containing more starch.

Chewing Behavior and Total Tract Nutrient Digestibility

Chewing behavior data are presented in Table 10. There was no interaction between NDFD of corn silage and dietary NDF content for eating time or eating time per kilogram of DM or aNDFom intake (*P* = 0.73, *P* = 0.10, *P* = 0.18). Cows fed the HNDF diet spent more time eating than cows fed the LNDF diet and cows fed the BM3 corn silage spent less time eating than cows fed the CON corn silage. The increased eating time reflected the greater aNDFom, uNDF240om, and peNDF content for the HNDF diets versus the LNDF diets and the CON versus the BM3 corn silages. In fact, it took cows fed the CON-HNDF diet approximately 50 min more per day to consume 2.5 kg/d less DM than cows fed the BM3-LNDF diet. Within a level of dietary NDF, cows fed BM3 versus CON corn silage spent approximately 20 to 30 min/d less time eating. Overall, it seems clear that dietary NDF content, and specifically the relative proportion of uNDF240 and degradable NDF, influences eating time for corn silage-based diets. Oba and Allen (2000b) reported that high NDF diets elicited longer eating times compared with low NDF diets, and the source of corn silage did not have an effect. Grant and Ferraretto (2018) published a review that concluded that diets with greater NDF, uNDF240, and peNDF can all result in cows spending more time to consume feed versus diets with lower and more degradable peNDF.

Similar to eating time, there was no interaction between NDFD of corn silage and dietary NDF content on rumination time or rumination time per kilogram of DM or aNDFom intake (P = 0.17, P = 0.89, P = 0.66). Cows fed the HNDF diets ruminated more than cows fed LNDF diets, although NDFD of corn silage did not have a significant effect on rumination time (P = 0.08). Similarly, Oba and Allen (2000b) reported that cows fed high NDF diets spent more time ruminating than cows fed low NDF diets. Cows fed the HNDF diets had greater ($P < 0.001$) rumination time, expressed as minutes per kilogram of DM, compared to cows fed the LNDF diets. The greater rumination time was due to greater forage NDF in the high-NDF compared to the LNDF diet. Meal length and bouts were not different among treatments ($P > 0.10$). Similarly, Miller et al. (2020) reported that meal length and bouts were unaffected by feeding diets containing either brown midrib-3 corn or conventional corn silage.

Total-tract digestibility of DM, OM, ADF, aNDFom, and pdNDF is presented in Table 11. There was a significant interaction (*P* = 0.02, *P* = 0.03) between NDFD of corn silage and dietary NDF content for DM and OM total-tract digestibility. The reduction in total-tract DM and OM digestibility when HNDF diets were fed was greater for cows fed the CON corn silage than BM3 corn silage. This digestibility response may be attributed to the uNDF240om content of the diets because the CON-HNDF diet had 2.4%-unit greater uNDF240om content than the BM3 and CON-LNDF diets. Our results agree with Miller et al. (2020) who reported that cows fed diets containing brown midrib-3 corn silage had greater total tract degradability of DM and OM compared to cows fed diets containing conventional corn silage. They also reported a 1.7%-unit difference in uNDF240om between brown midrib-3 and conventional corn silage diets (Miller et al., 2020).

A significant interaction between type of corn silage and dietary NDF content was observed for ADF and aNDFom total-tract degradability (*P* = 0.03, *P* = 0.02). The reduction in total-tract degradability of ADF and aNDFom for cows fed HNDF diets was greater for CON than for BM3 corn silage. The increased total tract degradability of ADF and aNDFom was likely due to greater fiber degradability and lower uNDF240om content of the BM3 corn silage in high NDF diets. Similarly, Ebling and Kung (2004) reported that cows fed brown midrib-3 corn silage-based diets had greater total tract degradability of ADF and NDF compared with cows fed conventional corn silage diets.

Oba and Allen (2000c) reported that cows fed high NDF diets had greater total tract degradability of NDF compared to cows fed low NDF concentration diets. The discrepancy between Oba and Allen (2000c) and our study could be due to differences in ruminal pH as our lower NDF diets supported a mean ruminal pH of 6.05 and only 6.6 h/d with pH less than 5.8, whereas their low NDF diets elicited a mean ruminal pH of 5.70 and 13.6 h/d with pH less than 5.8[RG5]. Grant and Mertens (1992) and Krajcarski-Hunt et al. (2002) reported that low ruminal pH increased the time for fiber degradation to initiate, slowed the rate of fiber degradation, and decreased the extent of fiber degradation. Total tract degradability of pdNDF was not affected (*P* > 0.05) by NDFD of corn silage or dietary NDF content and averaged approximately 73%. In contrast to our study with corn silage-based diets, Fustini et al. (2017) observed a reduction in pdNDF degradability for cows fed high versus low uNDF240 diets based on alfalfa hay. It is possible that the difference in total-tract pdNDF degradability is related to the intrinsic differences between corn silage and alfalfa in uNDF240 and uNDF240/lignin content (Palmonari et al., 2016; Raffrenato et al., 2018).

Ruminal Digesta Pools and Turnover

Ruminal digesta characteristics, pools, and turnover are presented in Table 12. There was no interaction (*P* > 0.05) of NDFD of corn silage with dietary NDF content for any measure of rumen digesta characteristics, pool size, or turnover. Cows fed BM3 diets had lower ruminal digesta volume (*P* = 0.003) and mass (*P* = 0.009) than cows fed CON corn silage. Cows fed the HNDF diet had greater ruminal digesta mass than cows fed the LNDF diets (*P* = 0.04). The cows fed the BM3 and LNDF diets were able to obtain the required nutrient supply from a smaller ruminal mass and this response was supported by greater total-tract digestibility of DM, OM, and NDF compared to cows fed the CON diet. Despite differences in digesta mass and volume, ruminal particulate digesta density was not different among treatments (*P* > 0.05).

The rumen OM pool was not different among treatments (*P* > 0.05). However, cows fed the higher NDFD corn silage had greater aNDFom pool size than cows fed lower NDFD corn silage (*P* = 0.05). Additionally, HNDF diets resulted in greater pool size of aNDFom than LNDF diet (*P* = 0.03). Cows fed the BM3 diets had faster turnover rate (*P* = 0.002) and shorter (*P* =0.003) turnover time of OM than cows fed the CON diets. The faster turnover rate observed for the BM3 diets was likely due to increased NDFD compared to CON corn silage. Likewise, Oba and Allen (2000b) reported that cows fed brown midrib-3 corn silage diets had faster OM turnover rate compared to cows fed conventional corn silage diets.

The cows fed the CON diet had a greater (*P* < 0.001) ruminal aNDFom pool than cows fed the BM3 diet. Similarly, cows fed the BM3 diet had faster turnover rate (*P* < 0.001) and shorter (*P* = 0.004) turnover time of aNDFom than the cows fed the CON diets. Interestingly, the ruminal aNDFom pool size was similar between CON and BM3 corn silage in the high NDF diets, but the turnover rate and time were different. The BM3 corn silage in the higher NDF diet allowed for greater DMI, faster NDF turnover rate, and shorter turnover time relative to the CON corn silage in the other high NDF diet. Greater fiber degradability and lower undegradable fiber in the BM3 corn silage likely drove these results. Similarly, Oba and Allen (2000b) reported that cows fed brown midrib-3 corn silage diet had faster NDF turnover rate compared to cows fed a conventional corn silage diet. Despite having lower total tract digestibility of OM and NDF, cows fed the CON-HNDF diet had slower ruminal turnover rate and longer retention times.

CONCLUSIONS

The objective of the study was to determine the effects of NDFD of corn silage fed at lower or higher dietary inclusion. Higher forage diets (i.e., >60% of DM) require higher NDFD

(>60% of NDF) and lower uNDF240 (<10% of ration DM) to avoid reductions in DMI and milk yield. Higher NDF diet with lower NDFD resulted in depressed DMI and milk yield associated with greater levels of ruminal fill, longer ruminal OM and NDF retention time, and nearly an hour more eating time than a higher forage diet with greater NDFD. Higher forage NDFD and lower uNDF240 allowed for greater NDF intake. Because ECM yield was reduced with the higher NDF diets, but much less so for the BM3 corn silage, we concluded that forage NDF in higher forage diets may support similar milk production when forage NDFD is high (i.e., >60% of NDF). Gut fill limitations may occur for corn silage-based diets when dietary uNDF240 content exceeds approximately 10% of ration DM.

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Table 1. Ingredient composition of diets (% of DM) containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

	CON		BM3	
Item	Low NDF	High NDF	Low NDF	High NDF
Conventional corn silage (CON)	39.25	54.95	-	-
Brown midrib corn silage (BM3)	-	-	36.11	50.24
Haycrop silage	13.35	13.35	13.34	13.34
Corn meal	17.27	1.57	20.41	6.28
Grain mix	30.13	30.13	30.14	30.14
Canola meal	6.21	6.21	6.32	6.32
Soybean meal	5.43	5.43	4.09	4.09
Soybean hulls	3.31	3.31	5.07	5.07
Corn germ meal	3.31	3.31	3.17	3.17
Distillers dried grains with solubles	1.55	1.55	0.00	0.00
Wheat red dog	0.00	0.00	1.43	1.43
Wheat middlings	0.83	0.83	0.63	0.63
Dextrose	1.15	1.15	0.99	0.99
Cane molasses	1.00	1.00	0.63	0.63
AminoPlus[1]	1.66	1.66	1.59	1.59
Blood meal	0.83	0.83	0.79	0.79

Urea	0.23	0.23	0.40	0.40
Optigen[2]	0.23	0.23	0.24	0.24
Smartamine M[3]	0.05	0.05	0.05	0.05
Rumen inert fat[4]	0.83	0.83	1.26	1.26
Fat mixer[4]	0.41	0.41	0.40	0.40
Calcium carbonate	1.27	1.27	1.21	1.21
Sodium sesquicarbonate	0.75	0.75	0.72	0.72
Sodium chloride	0.39	0.39	0.37	0.37
Magnesium oxide	0.18	0.18	0.14	0.14
Potassium CH50[4]	0.17	0.17	0.34	0.34
Dicalcium phosphate	0.14	0.14	0.15	0.15
Potassium bicarbonate	0.04	0.04	0.00	0.00
Trace minerals[5]	0.03	0.03	0.03	0.03
Organic trace minerals[6]	0.03	0.03	0.03	0.03
Organic selenium[7]	0.02	0.02	0.02	0.02
Vitamins A, D, and E[8]	0.03	0.03	0.03	0.03
Vitamin E[9]	<0.01	<0.01	<0.01	<0.01
Biotin, 1%	0.01	0.01	0.01	0.01
Rumensin[10]	<0.01	<0.01	<0.01	<0.01

[1] Ag Processing Inc., Omaha, NE.

[2] Alltech, Nicholasville, KY.

[3] Addisseo USA, Inc., Alpharetta, GA.

[4] Cargill, Inc., Minneapolis, MN. Rumen inert fat is a blend of C16:0 and C18:0 fatty acids; mixer fat is tallow-based and used to aid grain mixing process.

[5] Contained 0.02% Ca, 18.82% S, 153,737 mg Zn/kg, 30,303 mg Cu/kg, 136,364 mg Mn/kg, 3,384 mg Co/kg, and 3,030 mg I/kg.

[6] Contained 51,633 mg Zn/kg, 17,959 mg Cu/kg, 28,571 mg Mn/kg, and 3,673 mg Co/kg.

[7] Contained 2,041 mg Se/kg.

[8] Contained 30,492 kIU vitamin A/kg, 5,867 kIU vitamin D/kg, and 93,980 IU vitamin E/kg.

[9] Contained 44,639 IU vitamin E/kg.

[10] Elanco, Greenfield, IN.

Table 2. Data (mean ± standard error[RG6]) characterizing the analyzed chemical composition of ingredients used in diets containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

Item	CON corn silage	BM3 corn silage	Haycrop silage	Corn meal	CON grain mix[1]	BM3 grain mix[2]
Composite samples, n	4	4	4	4	4	4
DM, %	37.1 ± 0.4	36.4 ± 1.0	36.5 ± 1.2	90.9 ± 0.7	91.6 ± 0.4	92.6 ± 1.0
CP, % of DM	7.2 ± 0.1	7.9 ± 0.2	18.2 ± 0.7	8.4 ± 0.1	36.4 ± 0.8	34.1 ± 0.7
Soluble protein, % of CP	59.9 ± 1.0	59.4 ± 0.9	65.0 ± 1.8	17.0 ± 1.6	24.6 ± 2.2	29.1 ± 1.9
Ammonia, % of CP	16.9 ± 0.5	17.7 ± 0.3	15.8 ± 0.7	-	-	-
ADF, % of DM	23.5 ± 1.1	24.4 ± 0.3	33.8 ± 0.5	4.2 ± 0.4	12.8 ± 0.4	14.6 ± 0.3
aNDFom[3], % of DM	36.1 ± 1.2	34.8 ± 1.6	46.2 ± 0.6	-	-	-
24-h aNDFom degradability, % of aNDFom	48.6 ± 0.8	62.1 ± 1.0	57.7 ± 0.8	-	-	-
Undegradable NDFom at 240 h, % of DM	11.0 ± 0.3	7.6 ± 0.2	14.0 ± 0.5	-	-	-
Potentially degradable NDF, % of aNDFom	69.5 ± 0.9	78.1 ± 0.6	69.7 ± 1.1	-	-	-
ADL, % of DM	3.0 ± <0.1	2.4 ± 0.2	5.1 ± 0.3	1.3 ± <0.1	3.4 ± 0.4	3.6 ± 0.3
Starch, % of DM	36.0 ± 0.8	34.5 ± 1.0	1.3 ± 0.2	72.7 ± 0.5	8.1 ± 0.4	10.1 ± 0.7
Sugar (ESC[4]), % of DM	1.0 ± 0.1	1.0 ± 0.1	2.2 ± 0.5	3.0 ± <0.1	11.4 ± 0.2	9.7 ± 0.5
Crude fat, % of DM	3.1 ± 0.1	3.3 ± 0.2	3.8 ± 0.2	3.3 ± 0.2	6.1 ± 0.3	6.7 ± 0.4
Ash, % of DM	4.4 ± 0.1	5.0 ± 0.1	10.1 ± 0.3	1.4 ± 0.1	14.5 ± 0.4	13.9 ± 0.6
Ca, % of DM	0.25 ± 0.02	0.29 ± 0.01	1.07 ± 0.04	0.03 ± 0.00	2.53 ± 0.11	2.46 ± 0.22
P, % of DM	0.21 ± 0.00	0.24 ± 0.00	0.36 ± 0.03	0.30 ± 0.02	0.76 ± 0.02	0.71 ± 0.01
Mg, % of DM	0.22 ± 0.00	0.17 ± 0.00	0.29 ± 0.01	0.12 ± 0.00	0.82 ± 0.03	0.81 ± 0.04
K, % of DM	0.74 ± 0.03	1.02 ± 0.05	2.92 ± 0.23	0.35 ± 0.01	1.67 ± 0.04	1.62 ± 0.05
S, % of DM	0.11 ± 0.01	0.15 ± 0.02	0.26 ± 0.02	0.10 ± 0.01	0.62 ± 0.02	0.76 ± 0.08
Na, % of DM	0.01 ± 0.00	0.01 ± 0.00	0.05 ± 0.01	0.02 ± 0.00	1.45 ± 0.05	1.37 ± 0.07
Cl ion, % of DM	0.23 ± 0.00	0.2 ± 0.01	0.6 ± 0.09	0.06 ± 0.00	1.06 ± 0.03	0.91 ± 0.01
Fe, mg/kg	256 ± 10	492 ± 45	195 ± 9	29 ± 2	493 ± 13	460 ± 5
Cu, mg/kg	8 ± 1	12 ± 1	12 ± 1	3 ± 1	50 ± 5	62 ± 4

Mn, mg/kg	43 ± 1	28 ± 1	41 ± 2	9 ± 1	162 ± 10	222 ± 10
Zn, mg/kg	34 ± 1	29 ± 3	31 ± 2	26 ± 3	241 ± 18	243 ± 17
Lactic acid, % of DM	4.10 ± 0.32	6.03 ± 0.23	9.00 ± 1.30	-	-	-
Acetic acid, % of DM	3.20 ± 0.29	3.37 ± 0.12	2.90 ± 0.10	-	-	-
Propionic acid, % of DM	0.16 ± 0.04	0.28 ± 0.01	0.20 ± 0.00	-	-	-
Butyric acid, % of DM	-	-	0.3 ± 0.0	-	-	-
Total VFA, % of DM	7.5 ± 0.1	9.7 ± 0.2	12.3 ± 1.3	-	-	-
pH	3.9 ± <0.1	3.8 ± <0.1	4.4 ± 0.1	-	-	-

[1] Grain mix utilized in diets containing conventional corn silage (CON).

[2] Grain mix utilized in diets containing BM3 corn silage (BM3).

[3] Amylase-modified NDF on an OM basis.

[4] Ethanol-soluble carbohydrates.

Table 3. Particle size distribution, physical effectiveness factor (pef), and physically effective NDF (peNDF) of ingredients (mean ± standard deviation) used in diets containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

Item	CON corn silage	BM3 corn silage	Haycrop silage	Corn meal	CON grain mix[1]	BM3 grain mix[2]
Composite samples, n	4	4	4	4	4	4
Particle size distribution, % of DM						
>19.00 mm	0.3 ± 0.2	0.0 ± 0.0	0.7 ± 0.1	-	-	-
13.20 to 19.00 mm	1.1 ± 0.2	0.8 ± 0.1	1.4 ± 0.5	-	-	-
9.50 to 13.20 mm	4.6 ± 0.4	3.9 ± 0.5	2.1 ± 0.7	-	-	-
6.70 to 9.50 mm	17.6 ± 0.2	14.1 ± 1.3	4.7 ± 0.5	0.1 ± <0.1	-	0.1 ± <0.1
4.75 to 6.70 mm	22.1 ± 0.7	22.3 ± 0.3	5.9 ± 0.5	0.1 ± <0.1	0.1 ± 0.1	0.1 ± 0.1
3.35 to 4.75 mm	22.4 ± 0.6	23.0 ± 0.6	9.9 ± 0.7	0.1 ± <0.1	0.2 ± <0.1	0.2 ± 0.1
2.36 to 3.35 mm	13.0 ± 0.5	13.9 ± 1.0	15.7 ± 0.9	0.7 ± 0.4	0.8 ± 0.1	0.9 ± 0.2
1.18 to 2.36 mm	10.6 ± 0.3	12.7 ± 0.6	38.3 ± 3.2	15.1 ± 6.3	15.6 ± 0.6	15.1 ± 1.6
0.60 to 1.18 mm	4.8 ± 0.2	5.4 ± <0.1	15.3 ± 0.6	31.2 ± 1.4	34.2 ± 0.2	32.1 ± 0.3
0.30 to 0.60 mm	2.3 ± 0.1	2.6 ± <0.1	4.2 ± 0.3	31.7 ± 2.9	33.2 ± 0.2	32.3 ± 0.9
<0.30 mm	1.5 ± 0.2	1.4 ± 0.1	1.8 ± 0.1	20.9 ± 3.9	16.0 ± 0.6	19.1 ± 1.6
pef[3]	0.92 ± <0.01	0.91 ± <0.01	0.79 ± 0.01	0.16 ± 0.07	0.17 ± <0.01	0.16 ± 0.02
peNDF[4], % of DM	35.8 ± 0.8	36.4 ± 0.4	36.6 ± 0.1	1.8 ± 0.8	3.8 ± 0.0	3.9 ± 0.4

[1] Grain mix utilized in diets containing conventional corn silage (CON).

[2] Grain mix utilized in diets containing BM3 corn silage (BM3).

[3] physical effectiveness factor, % of DM retained on ≥1.18 mm with dry sieving; based on methods and assumptions in Mertens (1997).

[4] pef x NDF (Mertens, 1997).

Table 4. Calculated composition (mean ± standard deviation) based on chemical analysis of individual ingredients of diets containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

	CON		BM3	
Item	Low NDF	High NDF	Low NDF	High NDF
Composite samples, n	4	4	4	4
DM, %	52.2 ± 0.8	45.8 ± 0.5	52.8 ± 1.3	47.0 ± 0.8
CP, % of DM	17.0 ± 0.1	17.0 ± 0.1	16.7 ± 0.3	16.7 ± 0.1
Soluble protein, % of CP	34.5 ± 1.0	37.6 ± 1.8	37.6 ± 2.1	40.0 ± 1.1
ADF, % of DM	19.4 ± 0.4	23.0 ± 0.6	19.3 ± 0.8	22.0 ± 0.6
aNDFom[1], % of DM	32.4 ± 1.2	35.9 ± 0.6	31.5 ± 0.5	35.6 ± 0.3
ADL, % of DM	3.1 ± 0.1	3.6 ± 0.2	2.7 ± 0.1	2.9 ± 0.1
Starch, % of DM	28.0 ± 0.9	21.2 ± 0.5	27.8 ± 0.9	23.8 ± 0.7
Sugar (ESC[2]), % of DM	4.4 ± 0.2	3.9 ± 0.3	4.3 ± 0.2	4.3 ± 0.4
Crude fat, % of DM	4.0 ± 0.1	3.9 ± 0.1	4.4 ± 0.2	4.5 ± 0.2
Ash, % of DM	7.2 ± 0.1	7.6 ± 0.1	8.2 ± 0.5	7.8 ± 0.1
Ca, % of DM	1.02 ± 0.05	1.04 ± 0.04	1.03 ± 0.04	1.05 ± 0.04
P, % of DM	0.41 ± 0.01	0.40 ± 0.01	0.41 ± 0.02	0.40 ± 0.01
Mg, % of DM	0.39 ± 0.01	0.42 ± 0.01	0.36 ± 0.01	0.37 ± 0.01
K, % of DM	1.31 ± 0.07	1.43 ± 0.08	1.41 ± 0.07	1.49 ± 0.09
S, % of DM	0.31 ± 0.04	0.28 ± 0.01	0.33 ± 0.02	0.31 ± 0.01
Na, % of DM	0.48 ± 0.01	0.48 ± 0.01	0.43 ± 0.03	0.44 ± 0.01
Cl ion, % of DM	0.53 ± 0.02	0.56 ± 0.03	0.45 ± 0.02	0.48 ± 0.02
Fe, mg/kg	331 ± 25	324 ± 8	476 ± 34	396 ± 17
Cu, mg/kg	22 ± 2	24 ± 2	28 ± 3	28 ± 1
Mn, mg/kg	82 ± 4	90 ± 3	91 ± 5	92 ± 4
Zn, mg/kg	91 ± 5	96 ± 4	99 ± 8	103 ± 6

[1] Amylase-modified NDF on an OM basis.

[2] Ethanol-soluble carbohydrates.

Table 5. Data (mean ± standard deviation) characterizing the fiber fractions of diets containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

	CON		BM3	
Item	Low NDF	High NDF	Low NDF	High NDF
Composite samples, n	4	4	4	4
aNDFom[1], % of DM	32.4 ± 1.2	35.9 ± 0.6	31.5 ± 0.5	35.6 ± 0.3
24-h aNDF degradability, % of aNDF	56.3 ± 1.5	54.0 ± 1.4	62.0 ± 1.8	60.3 ± 1.4
Undegradable NDFom at 240 h, % of DM	8.3 ± 0.4	10.2 ± 0.2	7.1 ± 0.4	8.0 ± 0.0
Potentially degradable NDF, % of aNDFom	74.4 ± 0.7	71.6 ± 0.3	77.5 ± 0.2	77.5 ± 0.9

[1] Amylase-modified NDF on an OM basis.

Table 6. Particle size distribution, physical effectiveness factor (pef), and physically effective NDF (peNDF) of diets (mean ± standard deviation) containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

	CON		BM3	
Item	Low NDF	High NDF	Low NDF	High NDF
Composite samples, n	4	4	4	4
Particle size distribution, % of DM				
>19.00 mm	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2
13.20 to 19.00 mm	0.6 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
9.50 to 13.20 mm	1.5 ± 0.1	2.4 ± 0.9	1.5 ± 0.5	1.9 ± 0.2
6.70 to 9.50 mm	6.6 ± 0.7	8.4 ± 1.5	6.9 ± 1.1	7.3 ± 1.0
4.75 to 6.70 mm	8.9 ± 0.6	11.1 ± 1.6	10.3 ± 1.7	11.7 ± 1.1
3.35 to 4.75 mm	10.3 ± 0.5	12.5 ± 1.3	11.8 ± 1.5	12.9 ± 0.0
2.36 to 3.35 mm	9.1 ± 0.3	10.2 ± 0.4	9.4 ± 0.7	10.3 ± 0.2
1.18 to 2.36 mm	18.7 ± 1.4	18.0 ± 0.5	19.1 ± 1.8	18.5 ± 0.9
0.60 to 1.18 mm	20.7 ± 0.5	18.8 ± 1.5	19.8 ± 1.7	19.5 ± 0.1
0.30 to 0.60 mm	15.5 ± 1.1	12.4 ± 2.2	13.8 ± 1.8	12.4 ± 0.8
<0.30 mm	7.9 ± 0.7	5.3 ± 1.6	6.9 ± 1.4	4.9 ± 0.6
pef[1]	0.56 ± 0.02	0.64 ± 0.05	0.59 ± 0.05	0.63 ± 0.01
peNDF[2], % of DM	17.3 ± 0.8	23.1 ± 2.0	18.5 ± 2.0	21.5 ± 1.2

[1] physical effectiveness factor, % of DM retained on ≥1.18 mm with dry sieving; based on methods and assumptions in Mertens (1997).

[2] pef x NDF (Mertens, 1997).

Table 7. Least squares means of intake, BW, BCS, and lactational performance for lactating Holstein cows fed a diet containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

Item	CON		BM3		SE	*P*-value		
	Low NDF	High NDF	Low NDF	High NDF		NDFD[1]	NDF[2]	NDFD x NDF[3]
DMI, kg/d	29.0	26.5	29.3	29.2	0.7	0.005	0.009	0.02
DMI, % of BW/d	4.31	3.96	4.37	4.36	0.12	0.001	0.005	0.008
aNDFom[4] intake, kg/d	8.80	8.95	8.69	9.54	0.20	0.07	0.001	0.02
aNDFom intake, % of BW/d	1.31	1.33	1.30	1.43	0.04	0.03	<0.001	0.009
uNDF240om[5] intake, kg/d	2.33	2.53	1.93	2.14	0.07	<0.001	0.004	0.95
uNDF240om intake, % of BW/d	0.35	0.38	0.29	0.32	0.01	<0.001	0.003	0.97
BW, kg	676	673	673	673	19	0.61	0.53	0.70
BCS	2.92	2.88	2.89	2.89	0.13	0.83	0.67	0.75
Milk, kg/d	47.0	43.1	48.6	47.2	1.6	0.005	0.007	0.15
ECM, kg/d	49.5	45.9	50.8	50.1	1.2	0.02	0.05	0.16
Fat, %	3.82	4.02	3.76	3.94	0.14	0.27	0.008	0.84
Fat, kg/d	1.83	1.71	1.87	1.85	0.05	0.08	0.17	0.28
True protein, %	3.06	2.92	3.10	3.02	0.05	<0.001	<0.001	0.05
True protein, kg/d	1.48	1.25	1.55	1.43	0.04	<0.001	0.04	0.04
Lactose, %	4.73	4.65	4.75	4.73	0.05	0.001	0.002	0.04
Lactose, kg/d	2.28	2.00	2.38	2.25	0.09	<0.001	<0.001	0.05
SNF, %	8.67	8.47	8.73	8.62	0.07	<0.001	<0.001	0.09
SNF, kg/d	4.18	3.63	4.36	4.09	0.13	<0.001	<0.001	0.05
Urea nitrogen, mg/dL	14.3	15.5	13.7	14.1	0.6	0.008	0.03	0.25
SCS	0.52	0.75	0.53	0.57	0.34	0.43	0.19	0.37
Milk/DMI	1.62	1.62	1.66	1.61	0.04	0.49	0.33	0.29
ECM/DMI	1.71	1.73	1.73	1.72	0.03	0.87	0.84	0.25

[1] NDFD: Effect of NDF degradability of corn silage.

[2] NDF: Effect of dietary NDF content.

[3] NDFD x NDF: Interaction of NDF degradability of corn silage and dietary NDF content.

[4] Amylase-modified NDF on an OM basis.

[5] uNDF240om = undegradable NDFom at 240 h of in vitro fermentation.

Table 8. Least squares means of ruminal pH data for lactating Holstein cows fed a diet containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

	CON		BM3			*P*-value				
Item	Low NDF	High NDF	Low NDF	High NDF	SE	NDFD[1]	NDF[2]	NDFD x NDF[3]	Time	Treatment Interactions with Time
24-h mean pH	6.09	6.17	6.00	6.07	0.08					
24-h standard deviation	0.26	0.24	0.25	0.25	0.02	0.99	0.45	0.71	-	-
24-h minimum pH	5.51	5.63	5.42	5.50	0.11	0.13	0.17	0.78	-	-
24-h maximum pH	6.62	6.68	6.52	6.56	0.06	0.008	0.16	0.78	-	-
24-h range (max pH –	1.11	1.05	1.10	1.07	0.07	0.95	0.40	0.82	-	-
AUC < 5.8, pH units x	1.07	0.71	1.96	1.13	0.44	0.11	0.15	0.56	-	-
pH < 5.5, min/d	81	52	168	93	43	0.12	0.20	0.57	-	-
pH < 5.8, min/d	312	205	475	319	89	0.08	0.10	0.74	-	-

[1] NDFD: Effect of NDF degradability of corn silage.

[2] NDF: Effect of dietary NDF content.

[3] NDFD x NDF: Interaction of NDF degradability of corn silage and dietary NDF content.

Table 9. Least squares means of VFA profiles for lactating Holstein cows fed a diet containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

	CON		BM3			*P*-value				
Item	Low NDF	High NDF	Low NDF	High NDF	SE	NDFD^1	NDF^2	NDFD x NDF^3	Time	Treatment Interactions with Time
Total VFA, m*M*	105	102	110	111	8	0.008	0.59	0.40	<0.001	-
VFA, molar %										-
Acetate (A)	65.2	64.8	65.3	67.0	5.3	0.45	0.67	0.49	<0.001	-
Propionate (P)	26.2	21.5	29.0	25.7	2.3	0.004	0.001	0.50	<0.001	NDFamtxtime = 0.04
Butyrate (B)	13.0	12.2	13.4	13.1	1.2	0.32	0.37	0.64	<0.001	-
Isobutyrate	0.77	0.83	0.72	0.68	0.05	<0.001	0.86	0.03	0.004	-
Valerate	2.45	2.45	2.67	2.28	0.28	0.92	0.45	0.45	0.04	-
Isovalerate	1.24	1.49	1.16	1.03	0.17	0.03	0.61	0.10	<0.001	-
A:P	2.56	3.08	2.31	2.69	0.15	0.005	<0.001	0.49	<0.001	NDFamt x time = 0.09
(A+B):P	3.08	3.64	2.79	3.22	0.19	0.01	0.001	0.60	<0.001	NDFamt x time = 0.04

Ammonia-N, mg/dL	10.49	11.60	9.88	10.42	0.79	0.24	0.27	0.70	<0.001	NDFamt x time = 0.09

[1] NDFD: Effect of NDF degradability of corn silage.

[2] NDF: Effect of dietary NDF content.

[3] NDFD x NDF: Interaction of NDF degradability of corn silage and dietary NDF content.

Table 10. Least squares means of chewing data for lactating Holstein cows fed a diet containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

	CON		BM3			*P*-value		
Item	Low NDF	High NDF	Low NDF	High NDF	SE	NDFD[1]	NDF[2]	NDFD x NDF[3]
Eating time, min/d	273	301	250	273	14	0.003	0.003	0.73
Eating time per kg of DMI, min/kg DM	9.4	11.3	8.7	9.6	0.6	<0.001	<0.001	0.10
Eating time per kg of aNDFom[4] intake, min/kg aNDFom	31.2	33.6	29.2	29.2	1.8	0.002	0.20	0.18
Rumination time, min/d	514	543	463	536	17	0.08	0.004	0.17
Rumination time per kg of DMI, min/kg DM	17.8	20.4	16.1	18.8	0.8	0.004	<0.001	0.89
Rumination time per kg of aNDFom intake, min/kg aNDFom	58.9	60.4	54.2	57.4	2.6	0.05	0.22	0.66
Meal length, min/meal	29.2	31.3	27.5	28.4	2.5	0.11	0.28	0.64
Meal bout, bouts/d	11.8	12.1	11.5	11.9	0.7	0.45	0.33	0.95

[1] NDFD: Effect of NDF degradability of corn silage.

[2] NDF: Effect of dietary NDF content.

[3] NDFD x NDF: Interaction of NDF degradability of corn silage and dietary NDF content.

[4] Amylase-modified NDF on an OM basis.

Table 11. Least squares means of total-tract digestibility data for lactating Holstein cows fed a diet containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

	CON		BM3			*P*-value		
Item[1]	Low NDF	High NDF	Low NDF	High NDF	SE	NDFD2	NDF3	NDFD x NDF4
DM, %	73.5	68.0	73.4	72.3	0.9	0.03	0.001	0.02
OM, %	75.0	70.0	75.3	74.3	0.9	0.01	0.002	0.03
ADF, %	52.6	44.3	53.4	52.0	1.6	0.009	0.004	0.03
aNDFom[5], %	55.7	48.9	57.8	56.8	1.5	<0.001	0.004	0.02
pdNDF[6], %	74.1	70.9	72.2	72.8	1.6	0.94	0.26	0.11

[1] All values are ash-corrected.

[2] NDFD: Effect of NDF degradability of corn silage.

[3] NDF: Effect of dietary NDF content.

[4] NDFD x NDF: Interaction of NDF degradability of corn silage and dietary NDF content.

[5] Amylase-modified NDF on an OM basis.

[6] Potentially degradable NDF.

Table 12. Least squares means of rumen digesta characteristics, pool sizes, and turnover for lactating Holstein cows fed a diet containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

	CON		BM3			*P*-value		
Item	Low NDF	High NDF	Low NDF	High NDF	SE	NDFD[1]	NDF[2]	NDFD x NDF[3]
Rumen digesta volume, L	123	128	113	119	3	0.003	0.06	0.73
Rumen digesta mass, kg	106	112	98	105	3	0.009	0.04	0.93
Rumen density, kg/L	0.86	0.87	0.88	0.88	0.01	0.19	0.32	0.52
Rumen pool, kg								
OM	13.0	12.5	12.1	12.6	0.6	0.32	0.99	0.17
aNDFom[1]	8.32	8.45	7.64	8.36	0.41	0.05	0.03	0.12
Rumen turnover rate, %/h								
OM	8.95	8.31	9.44	9.57	0.51	0.002	0.29	0.12
aNDFom[4]	4.84	4.76	5.12	5.52	0.30	<0.001	0.23	0.07
Rumen turnover time, h								
OM	11.4	12.2	11.0	10.9	0.5	0.003	0.13	0.09
aNDFom	21.1	21.4	20.3	19.0	1.1	0.004	0.28	0.10

[1] NDFD: Effect of NDF degradability of corn silage.

[2] NDF: Effect of dietary NDF content.

[3] NDFD x NDF: Interaction of NDF degradability of corn silage and dietary NDF content.

[4] Amylase-modified NDF on an OM basis.