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

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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

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ABSTRACT

42

43 The effect of neutral detergent fiber (NDF) **degradability** of corn silage in diets

44 containing lower and higher NDF concentrations on lactational performance, nutrient

45 digestibility, and ruminal characteristics in lactating Holstein cows was measured. Eight

46 ruminally cannulated Holstein cows averaging 91 ± 4 (standard error) days in milk were used in

47 a replicated 4 x 4 Latin square design with 21-d periods (7-d collection periods). Dietary

48 treatments were formulated to contain either conventional (CON; **48.6% 24-h NDF**

49 **degradability**) or brown midrib-3 (BM3; **61.1% 24-h NDF degradability**) corn silage and either

50 lower NDF (LNDF) or higher NDF (HNDF) concentration (32.0 and 35.8% of ration dry matter,

51 DM) by adjusting the **dietary** forage content (52 and 67% forage, DM basis). The dietary

52 treatments were: 1) CON-LNDF, 2) CON-HNDF, 3) BM3-LNDF, and 4) BM3-HNDF. **Data**

53 **were analyzed as a factorial arrangement of diets within a replicated Latin square with the**

54 **MIXED procedure of SAS with fixed effects of diet, period, and replicate. Cow within square**

55 **was the random effect^[RG1]. Cows fed the BM3 corn silage had higher dry matter intake (DMI) than**

56 **cows fed the CON corn silage whereas cows fed the HNDF diet consumed less DM than cows**

57 **fed the LNDF diet. An interaction between NDF degradability and NDF content resulted in the**

58 **HNDF diet decreasing DMI with CON corn silage but not with BM3 silage.** Cows fed the BM3

59 diets had greater energy-corrected milk yield, higher milk true protein content, and lower milk

60 urea nitrogen concentration than cows fed CON diets. Additionally, cows fed the BM3 diets had

61 greater total tract digestibility of organic matter and NDF than cows fed the CON diets.

62 Compared to CON diets, the BMR diets accelerated ruminal NDF turnover. When incorporated

63 into higher NDF diets, corn silage with greater in vitro **24-h NDF degradability** and lower

64 **undegradable NDF** at 240 h of in vitro fermentation (uNDF240) allowed for greater DMI intake

65 than CON. In contrast, for lower NDF diets, NDF **degradability** of corn silage did not affect DMI
66 which suggests that a threshold level of inclusion of higher **NDF degradability** corn silage is
67 necessary to observe enhanced lactational performance. Results suggest that there is a maximum
68 gut fill of dietary uNDF240 and that higher NDF **degradability** corn silage can be fed at greater
69 dietary concentrations.

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

71 INTRODUCTION

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

83 Recently, a laboratory measure of **undegradable NDF**, termed **undegradable** NDF at 240
84 h of in vitro fermentation (uNDF240), has been found to be related to NDF degradation and
85 passage kinetics, gut fill and DMI, and physical effectiveness of fiber (Nousiainen et al., 2003;
86 Cotanch et al., 2014; Raffrenato et al., 2018). However, due to its novelty, few published studies

87 have used the measure in relation to dairy cow performance (Fustini et al., 2017; Miller et al.,
88 2020). Recently, Miller et al. (2020) showed that corn silage with a lower uNDF240 fraction
89 allowed for greater DMI and milk yield. The corn silage used was brown midrib (BMR) which
90 has a gene mutation that results in less lignin in the plant cell wall, lower uNDF240 content, and
91 greater NDF **degradability** (Oba and Allen, 1999a; Hassanat et al., 2017; Miller et al., 2020).
92 Generally, substituting a corn silage hybrid with higher NDFD for a hybrid with lower NDFD,
93 on a DM basis, leads to greater DMI, faster fiber turnover from the rumen, greater total tract
94 NDFD, and increased milk production (Oba and Allen, 1999; Hassanat et al., 2017). However,
95 responses have been more variable when corn silage substitution occurs on an NDF basis due in
96 part to variation in NDFD at similar NDF content (Oba and Allen, 2000abc; Hristov et al., 2020).

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

104 Therefore, the objective of this study was to evaluate the effect of lower or higher NDF
105 **degradability** of corn silage (i.e., conventional or brown midrib-3), when substituted on an NDF
106 basis, in diets containing lower and higher NDF concentration (i.e., forage percentage) **and their**
107 **interaction** on lactational performance, nutrient digestibility, and ruminal fermentation and
108 turnover characteristics in Holstein cows. Our hypothesis was that **greater** NDFD and lower

109 uNDF240 content of brown midrib-3 corn silage would increase DMI and milk yield, nutrient
110 digestibility, and ruminal fiber turnover similarly in lower and higher NDF diets compared to
111 conventional corn silage with lower NDFD and higher uNDF240.

112

113 **MATERIALS AND METHODS**

114 *Experimental Design, Diets, and Management of Cows*

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

125 *Dietary Formulation and Feeding Strategy*

126 Dietary treatments (Table 1) were formulated to contain either conventional (CON;?) or
127 brown midrib-3 corn silage (BM3?) and either lower or higher NDF concentration (32.0 and
128 35.8% of DM) by adjusting the forage percentage (52 and 67% forage, DM basis). The four
129 dietary treatments were: 1) CON and lower NDF (CON-LNDF), 2) CON and higher NDF

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

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

144 ***Data Collection, Sampling Procedures, and Analytical Methods***

145 ***Ingredient and diet sampling and analysis.*** Individual feed ingredients were collected
146 weekly and dried in a forced-air oven at 105°C for 18 to 24 h (until weight remained unchanged)
147 for DM determination to maintain consistent dietary DM content throughout the study. During
148 the collection period (d 15 to 21), feed ingredients and diets were collected daily and a portion of
149 each sample was dried in a forced-air oven at 105°C for 18 to 24 h for DM determination. The
150 remaining portion of each sample collected was frozen at -20°C until composited by collection
151 period and analyzed for chemical composition using AOAC International (2000; 2006) approved

152 methods (CPM Plus; Cumberland Valley Analytical Services, Inc., Waynesboro, PA). In vitro
153 **NDFD** of forage composite and dietary composite samples (1-mm grind; Wiley mill; Arthur H.
154 Thomas, Philadelphia, PA) at 24-h was determined using an in vitro fermentation (Daisy^{II}
155 Incubator; Ankom Technology Corp., Fairport, NY) with buffered media containing ruminal
156 fluid (Goering and Van Soest, 1970). The uNDF240 for forages and dietary composite samples
157 was assessed using a batch in vitro rumen fermentation system (Raffrenato et al., 2018).

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

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

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

171 Cows were milked 3 times daily (0430, 1230, and 2030 h). Milk yields were recorded
172 electronically (ProVantage Information Management System; Bou-Matic, Madison, WI) on d 15
173 to 21 of each period. Milk samples from 6 consecutive milkings for each cow were collected on

174 d 17 and 18 of each test period and were preserved (Bronolab-W II Liquid Preservative; D & F
175 Control Systems, Inc., Dublin, CA). Samples were sent for analyses within two to three days of
176 sampling and were analyzed for fat, true protein, lactose, solids nonfat, urea nitrogen, and
177 somatic cells by mid-infrared procedures (Dairy One, Ithaca, NY; Foss 4000; Foss Technology,
178 Eden Prairie, MN). After analysis, samples were composited mathematically by day in
179 proportion to milk yield at each sampling within the day. Somatic cell count was transformed
180 and analyzed as SCS according to Shook et al. (1993) using the equation: $SCS = \log_2(SCC/100)$
181 $+ 3$ where SCC is in units of 1,000 cells/mL. Energy-corrected milk was calculated using a
182 formula modified to account for use of true protein instead of total protein (Tyrrell and Reid,
183 1965; Mark Stephenson, University of Wisconsin;
184 <https://dairymarkets.org/PubPod/Reference/Library/Energy%20Corrected%20Milk>): $(0.327 \times \text{kg}$
185 $\text{of milk} + 12.95 \times \text{kg of fat} + 7.65 \times \text{kg of true protein})$.

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

192 Samples of ruminal fluid (approximately 300 mL) were collected from beneath the
193 ruminal digesta mat at 4-h intervals for 24 h on d 17 (1430) and d 18 (1830, 2230, 0230, 0630,
194 and 1030) of each period, and strained through 4 layers of cheesecloth; pH was determined
195 immediately. A portion of each sample of ruminal fluid (approximately 40 mL) was frozen and

196 stored at -20°C until analysis for VFA concentration (Bulletin 856B; Supelco, Inc., Bellefonte,
197 PA) by gas chromatography with use of a Varian CP-3800 gas chromatograph (Varian, Inc., Palo
198 Alto, CA) equipped with a flame-ionization detector and an 80/120 Carbopack B-DA/4%
199 Carbowax 20M column (Supelco, Inc., Bellefonte, PA). Ten mL of ruminal fluid was added to 1
200 drop (approximately 100 µL) of concentrated HCl and frozen at -20°C for analysis of ruminal
201 NH₃-N concentration according to the procedure of Chaney and Marback (1962).

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

209 ***Nutrient digestibility.*** Total tract digestibility of DM, OM, ADF, NDF, and potentially
210 digestible NDF (pdNDF) was determined on d 16 to 20 of each test period. Fecal grab samples
211 were collected on d 17 to 20 for each period so that every 3 h in a 24-h period were represented.
212 Fecal samples from each cow were composited by combining approximately 100 g of feces from
213 each time point (n = 8). Samples of diets and orts were collected on d 15 to 21. Samples of diets,
214 orts, and feces were frozen at -20°C, dried in a forced air oven at 55°C for 48 h, and ground to
215 pass through a 1-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA). Composite
216 samples of diets (by period), orts (by cow by period), and feces (by cow by period) were
217 analyzed for DM, ash, ADF, NDF, and lignin (Cumberland Valley Analytical Services, Inc.,

218 Waynesboro, PA). Undegraded NDF at 120 h (uNDF120) was used as an internal marker and
219 was quantified as NDF content of diets, orts, and feces samples following an in vitro
220 fermentation (Ankom Technology Corp., Fairport, NY) in buffered ruminal media (Goering and
221 Van Soest, 1970) for 120 h. Total tract digestibility was calculated by the ratio technique using
222 the concentrations of the nutrients and uNDF120 in the diet and feces (Maynard et al., 1979).
223 The nutrient content of the diet used in the digestibility calculation was adjusted for each cow
224 based on the nutrient composition of the diet offered and refused.

225 *Rumen digesta measures and NDF and OM turnover.* Ruminal contents were manually
226 evacuated through the ruminal cannula after daily feeding on d 20 and prior to feeding on d 21
227 for determination of ruminal content mass and volume. To ensure that cows experienced the
228 same interval of time between rumen evacuations, cows were divided into two groups of four
229 cows, each based on square assignment. The first group was evacuated 3.5 h after feeding on d
230 20 (1830 h) and 3.5 h prior to feeding on d 21 (1130 h). The second group of cows was
231 evacuated 4.5 h after feeding on d 20 (1930 h) and 4.5 h prior to feeding on d 21 (1030 h).
232 During evacuation, approximately 10% of ruminal contents were subsampled and squeezed
233 through a nylon screen (1-mm pore size) to separate solid and liquid phases (Voelker Linton and
234 Allen, 2008). Each phase was weighed, and aliquots (300 g) from solid and liquid phases were
235 then stored at -20°C for further analysis. Solid and liquid phase samples were subsequently dried
236 at 55°C, ground (solids: 1-mm screen; Wiley mill; Arthur H. Thomas, Philadelphia, PA; liquid:
237 1-mm screen, UDY Cyclone Sample Mill; UDY Corp., Fort Collins, CO), and recombined based
238 on DM proportion of each phase for analysis of ash (modified method 942.05; AOAC, 1990; 4 h
239 at 600°C) and NDF.

240 Ruminal pool size of OM and NDF was calculated as the product of ruminal content
241 mass and nutrient content of ruminal contents. Ruminal turnover rate (%/h) of OM and NDF was
242 calculated as $[100 \times (\text{intake of nutrient}/\text{ruminal pool of nutrient})/24]$ (Voelker Linton and Allen,
243 2008). Nutrient intake was calculated using DMI from d 20 and 21 and corresponding composite
244 sample nutrient content. Ruminal turnover time (h) was calculated as $1/(\text{ruminal turnover rate}$
245 $(\%/h)/100)$.

246 **Statistical Analysis**^[RG2]

247 Ingredient and diet analyses were analyzed using the MEANS procedure of SAS (version
248 9.1, Statistical Analysis Systems Institute Inc., Cary, NC) and reported as the mean and standard
249 error. Repeated measurements on performance, chewing, ruminal digesta characteristics and
250 digestion kinetics, microbial nitrogen, and total tract nutrient digestibility were reduced to period
251 means for each cow before statistical analysis. All data, except for ruminal pH and VFA, were
252 analyzed as a replicated Latin square design with model effects for diet, period within square,
253 and square using MIXED procedure of SAS with cow within replicate as a square effect.
254 Ruminal pH and VFA were analyzed as a replicated Latin square design with repeated measures
255 using the MIXED procedure of SAS. The model included the effects of diet, period within
256 square, and square, time, and the interaction of diet and time with cow within square as a random
257 effect. Significance was declared at $P \leq 0.05$.

258 **RESULTS AND DISCUSSION**

259 *Dietary and Ingredient Nutrient Composition*

260 Chemical composition, in vitro NDFD, and particle size distribution of the ingredients are
261 presented in Tables 2 and 3. The amylase-modified NDF (OM basis; aNDFom) content averaged
262 36.1 and 34.8% (DM basis) for CON and BM3 corn silage, respectively. The BM3 corn silage
263 had a 13.5%-unit greater NDFD at 24 h and a 3.4%-unit lower uNDF240om compared to the
264 CON corn silage (62.1 vs. 48.6% of aNDFom; 7.6 vs. 11.0 % of DM, respectively).

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

275 *DMI and Lactational Performance*

276 Dry matter intake, nutrient intake, and milk production and composition measures are
277 presented in Table 7. Cows fed the BM3 corn silage had higher DMI than cows fed the CON
278 corn silage ($P = 0.005$) whereas cows fed the HNDF diet consumed less DM than cows fed the
279 LNDF diet ($P = 0.009$). A significant interaction ($P = 0.02$) between NDFD and dietary NDF
280 content resulted in the HNDF diet reducing DMI with CON corn silage but not with BM3 silage.
281 Oba and Allen (2000a) had previously reported that cows fed brown midrib-3 corn silage had

282 greater DMI than cows fed conventional corn silage, whether in low (28.9% of DM) or high
283 (38.0% of DM) NDF diets. Interestingly, in our study, the cows fed the BM3-LNDF diet did not
284 have greater DMI than cows fed the CON-LNDF diet. The similar DMI for the two LNDF diets
285 is most likely related to the relatively low uNDF240om content in the CON-LNDF and the BM3-
286 LNDF diets (8.3 and 7.1% of DM, respectively). Undegradable NDF240om is an in vitro
287 measure of undegradable NDF and has been related to digestion and passage kinetics, gut fill,
288 and physical effectiveness (Nousiainen et al., 2003; Cotanch et al., 2014). In contrast, the BM3
289 corn silage with greater NDFD and lower uNDF240 did allow for greater DMI than CON corn
290 silage at the higher dietary NDF concentration. The CON-HNDF diet contained 10.2% uNDF240
291 (% of DM) and this higher concentration in the diet clearly limited DMI. Similarly, with corn
292 silage-based diets, Smith et al. (2018) observed a reduction in DMI for lactating dairy cows fed a
293 ration containing 11.5 versus 8.9% uNDF240 (% of DM). With alfalfa-based diets, Fustini et al.
294 (2017) observed that lower NDFD forage reduced DMI by 20% compared with a higher NDFD
295 forage in both lower (9.5% of DM) and higher (10.9% of DM) uNDF240 diets. Legumes and
296 grasses differ markedly in their content of uNDF240 and uNDF240/lignin (Palmonari et al.,
297 2016; Raffrenato et al. (2018), and it would be expected that differences in DMI and other
298 lactational responses would be observed when cows are fed diets comprised primarily of corn
299 silage or alfalfa hay.

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

305 4.6% of DM; CON-HNDF, 6.5% of DM; BM3-LNDF, 4.2% of DM; and BM3-HNDF, 5.0% of
306 DM.

307 Intake of aNDFom was not affected by source of corn silage ($P = 0.07$), but it was
308 increased as expected for cows fed the higher NDF diets ($P = 0.001$). However, a significant
309 interaction ($P = 0.02$) between NDFD and dietary NDF content was observed whereby cows fed
310 BM3 corn silage consumed more aNDFom on the HNDF versus LNDF diet compared with those
311 cows fed the CON corn silage. The greater aNDFom intake was due most likely to the higher
312 NDFD of the BM3 corn silage that allowed for greater DMI despite the higher ration aNDFom
313 content (Oba and Allen, 2000a). Cows fed the BM3 corn silage consumed less uNDF240om than
314 cows fed CON corn silage ($P < 0.001$) at both dietary NDF concentrations. Lower dietary NDF
315 concentration resulted in less uNDF240om intake than the higher dietary NDF content ($P =$
316 0.004). This response in uNDF240om intake reflected the greater uNDF240om and lower
317 pdNDF content of the CON diets compared to the BM3 diets. Several recent studies suggest that,
318 when uNDF240om intake exceeds approximately 2.35 kg/d (approximately 10% of ration DM or
319 0.36% of BW), DMI may become restricted due to gut fill in corn silage-based diets fed to high
320 producing Holstein cattle (Smith et al., 2018; Coons et al., 2019; Miller et al., 2020). With high
321 NDFD alfalfa hay-based diets, Fustini et al. (2017) observed greater uNDF240 intake for cows
322 fed higher uNDF240 (0.48% of BW) than lower uNDF240 diets (0.40% of BW). This
323 relationship between uNDF240 and DMI warrants further investigation with a greater variety of
324 diets, forage sources, and stages of lactation.

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

327 HNDF diet decreased milk and ECM production ($P = 0.02$, $P = 0.05$). This pattern of milk yield
328 response generally followed the responses observed in DMI (Table 7). Milk fat percentage was
329 greater for cows fed the HNDF than LNDF diet ($P = 0.008$), although there was no effect ($P =$
330 0.27) of corn silage NDFD. Output of milk fat was unaffected by either the NDFD ($P = 0.08$) of
331 the corn silage or the dietary NDF content ($P = 0.17$). The lower milk fat content for cows fed
332 the LNDF diets was most likely due to more fermentable carbohydrates supplied by both starch
333 and **degradable** NDF compared with the other HNDF diets. Our results agree with Oba and Allen
334 (2000a) who reported that cows fed brown midrib-3 corn silage in a lower NDF diet produced
335 milk with lower fat content compared with cows fed a conventional corn silage in the same lower
336 NDF diet. In contrast, milk fat content was unaffected by NDFD for high NDF diets (Oba and
337 Allen, 2000a).

338 A significant interaction between NDFD of corn silage and dietary NDF content was
339 observed for milk true protein percentage and yield ($P = 0.05$; $P = 0.04$). Cows fed BM3 corn
340 silage had greater milk protein percentage ($P < 0.001$) and output ($P < 0.001$) than cows fed
341 CON corn silage, and cows fed the HNDF diets produced less milk protein than cows fed the
342 LNDF diets ($P = 0.04$). Our results agree with Coons et al. (2019) who reported that cows fed
343 brown midrib-3 corn silage had greater true protein content and yield compared to cows fed
344 conventional corn silage. Cows fed the BM3 corn silage had greater milk lactose content ($P =$
345 0.001) and yield ($P < 0.001$) versus cows fed CON corn silage. Likewise, cows fed the LNDF
346 diets had greater lactose yield than cows fed the HNDF diets ($P < 0.001$). A significant
347 interaction occurred between NDFD of corn silage and dietary NDF content with the HDF diet
348 reducing milk lactose more with the CON than BMR corn silage. This response in milk
349 composition aligns with both Oba and Allen (2000a) and Coons et al. (2019) who reported that

350 cows fed brown midrib-3 corn silage had greater lactose content and yield compared to cows fed
351 conventional corn silage. A previous study (Miller et al., 2020) with corn silage-based rations
352 found no effect of uNDF240 on milk fat, true protein, or lactose yield at similar dietary
353 uNDF240 concentrations (7.8 and 10.6% of DM).

354 Cows fed BM3 corn silage had lower MUN than cows fed CON corn silage ($P = 0.008$).
355 Similarly, cows fed the LNDF diets had lower MUN than cows fed HNDF diets ($P = 0.03$). Our
356 results agree with Hassanat et al. (2017) who reported that, when cows were fed brown midrib-3
357 corn silage, they had lower MUN than cows fed conventional corn silage. The lower MUN and
358 greater milk protein yield were thought to be related to an increase in AA supply as a result of
359 enhanced rumen OM fermentability (Hassanat et al., 2017). Feed efficiency, whether expressed
360 as milk yield per unit of DMI or ECM yield per unit of DMI, was not affected by type of corn
361 silage or dietary NDF content ($P > 0.10$). Similarly, Hassanat et al. (2017) and Coons et al.
362 (2019) reported no difference in feed efficiency for cows fed brown midrib-3 or conventional
363 corn silage. In contrast, Miller et al. (2020) observed greater milk/DMI when cows were fed corn
364 silage-based rations containing 6.9% versus 8.6% uNDF240. As with DMI and milk yield, the
365 relationship between uNDF240, NDFD, and feed efficiency requires further research with
366 multiple sources of NDF.

367 *Rumen pH and VFA*

368 Rumen pH and VFA data are presented in Table 8. Cows fed the CON-HNDF diet had a
369 greater ($P_{\text{RG3}} = 0.02$) mean ruminal pH and maximum pH ($P = 0.03$) compared with cows fed the
370 BM3-LNDF diet, with CON-LNDF and BM3-HNDF being intermediate (6.17 vs. 6.00 vs. 6.08;
371 6.68 vs. 6.52 vs. 6.59, respectively). The lower ruminal pH observed for cows fed the BM3 diets

372 was likely related to more fermentable corn silage for the BM3 versus CON. Similarly, Oba and
373 Allen (2000a) reported that cows fed brown midrib corn silage had a lower mean ruminal pH
374 than cows fed a conventional corn silage diet even when starch content was similar between the
375 two diets. However, Hassanat et al. (2017) reported no difference in mean ruminal pH and
376 maximum pH for cows fed brown midrib-3 or conventional corn silage although both diets had
377 similar starch content. The reason for these differences among the studies is not clear, although it
378 may be due to differences in dietary starch and NDF content. For example, the dietary starch
379 content for Oba and Allen (2000a) averaged approximately 37.5% of ration DM whereas the
380 starch content was only approximately 17% for Hassanat et al. (2017). The dietary starch content
381 for our diets was intermediate relative to these two previous studies and ranged between 21.2 and
382 28.0% of DM. Differences among the diets in ruminal pH were manifested primarily in mean
383 and maximum pH since the standard deviation of pH, minimum pH, range, and measures of sub-
384 acute rumen acidosis (SARA; AUC < 5.8, pH < 5.5, min/d, pH < 5.8, min/d) were not affected
385 by type of corn silage or dietary NDF content ($P > 0.05$).

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

391 Cows fed the BM3 corn silage had greater total VFA concentration than cows fed CON
392 corn silage ($P = 0.008$). The greater VFA concentration reflected greater DMI and more
393 fermentable corn silage for the cows fed the BM3 diets compared to the cows fed the CON diets

394 at either dietary NDF content. Likewise, Oba and Allen (2000a) reported that cows fed a higher
395 NDF diet (i.e., 37.5% of dietary DM) containing brown midrib-3 corn silage had greater total
396 ruminal VFA concentration than cows fed a higher NDF diet with conventional corn silage.

397 Acetate, butyrate, valerate, and isovalerate molar percentages were not affected by NDFD
398 of corn silage or dietary NDF content ($P > 0.05$). In contrast to molar percentage of acetate, cows
399 fed the BM3 and LNDF diets had greater propionate percentage ($P = 0.004$, $P = 0.001$) than
400 cows fed the CON corn silage or the LNDF diets. This response was most likely because cows
401 fed the BM3 and LNDF diets had greater DMI and these diets contained more starch and
402 fermentable OM than the CON or HNDF diets. Our results are in accordance with Oba and Allen
403 (2000a) who reported greater propionate concentration for cows fed a low NDF diet with greater
404 starch content compared to cows fed a higher NDF diet with lower starch content. As a result of
405 the changes in propionate, cows fed the BM3 corn silage had lower acetate-to-propionate ratios
406 than cows fed CON corn silage ($P = 0.005$). Additionally, cows fed the HNDF diets had greater
407 acetate-to-propionate ratios than cows fed the LNDF diets ($P < 0.001$). The change in acetate-to-
408 propionate ratio reflects the increased propionate percentage due to cows consuming more DM
409 and fermentable carbohydrates, particularly starch. Oba and Allen (2000a) also reported lower
410 acetate-to-propionate ratio for cows fed a lower NDF diet containing more starch.

411 *Chewing Behavior and Total Tract Nutrient Digestibility*

412 Chewing behavior data are presented in Table 10. There was no interaction between
413 NDFD of corn silage and dietary NDF content for eating time or eating time per kilogram of DM
414 or aNDFom intake ($P = 0.73$, $P = 0.10$, $P = 0.18$). Cows fed the HNDF diet spent more time
415 eating than cows fed the LNDF diet and cows fed the BM3 corn silage spent less time eating

416 than cows fed the CON corn silage. The increased eating time reflected the greater aNDFom,
417 uNDF240om, and peNDF content for the HNDF diets versus the LNDF diets and the CON
418 versus the BM3 corn silages. In fact, it took cows fed the CON-HNDF diet approximately 50
419 min more per day to consume 2.5 kg/d less DM than cows fed the BM3-LNDF diet. Within a
420 level of dietary NDF, cows fed BM3 versus CON corn silage spent approximately 20 to 30 min/d
421 less time eating. Overall, it seems clear that dietary NDF content, and specifically the relative
422 proportion of uNDF240 and degradable NDF, influences eating time for corn silage-based diets.
423 Oba and Allen (2000b) reported that high NDF diets elicited longer eating times compared with
424 low NDF diets, and the source of corn silage did not have an effect. Grant and Ferraretto (2018)
425 published a review that concluded that diets with greater NDF, uNDF240, and peNDF can all
426 result in cows spending more time to consume feed versus diets with lower and more degradable
427 peNDF.

428 Similar to eating time, there was no interaction between NDFD of corn silage and dietary
429 NDF content on rumination time or rumination time per kilogram of DM or aNDFom intake ($P =$
430 0.17 , $P = 0.89$, $P = 0.66$). Cows fed the HNDF diets ruminated more than cows fed LNDF diets,
431 although NDFD of corn silage did not have a significant effect on rumination time ($P = 0.08$).
432 Similarly, Oba and Allen (2000b) reported that cows fed high NDF diets spent more time
433 ruminating than cows fed low NDF diets. Cows fed the HNDF diets had greater ($P < 0.001$)
434 rumination time, expressed as minutes per kilogram of DM, compared to cows fed the LNDF
435 diets. The greater rumination time was due to greater forage NDF in the high-NDF compared to
436 the LNDF diet. Meal length and bouts were not different among treatments ($P > 0.10$). Similarly,
437 Miller et al. (2020) reported that meal length and bouts were unaffected by feeding diets
438 containing either brown midrib-3 corn or conventional corn silage.

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

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

458 Oba and Allen (2000c) reported that cows fed high NDF diets had greater total tract
459 degradability of NDF compared to cows fed low NDF concentration diets. The discrepancy
460 between Oba and Allen (2000c) and our study could be due to differences in ruminal pH as our

461 lower NDF diets supported a mean ruminal pH of 6.05 and only 6.6 h/d with pH less than 5.8,
462 whereas their low NDF diets elicited a mean ruminal pH of 5.70 and 13.6 h/d with pH less than
463 5.8^[RG5]. Grant and Mertens (1992) and Krajcarski-Hunt et al. (2002) reported that low ruminal pH
464 increased the time for fiber degradation to initiate, slowed the rate of fiber degradation, and
465 decreased the extent of fiber degradation. Total tract degradability of pdNDF was not affected (P
466 > 0.05) by NDFD of corn silage or dietary NDF content and averaged approximately 73%. In
467 contrast to our study with corn silage-based diets, Fustini et al. (2017) observed a reduction in
468 pdNDF degradability for cows fed high versus low uNDF240 diets based on alfalfa hay. It is
469 possible that the difference in total-tract pdNDF degradability is related to the intrinsic
470 differences between corn silage and alfalfa in uNDF240 and uNDF240/lignin content (Palmonari
471 et al., 2016; Raffrenato et al., 2018).

472 *Ruminal Digesta Pools and Turnover*

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

482 The rumen OM pool was not different among treatments ($P > 0.05$). However, cows fed
483 the higher NDFD corn silage had greater aNDFom pool size than cows fed lower NDFD corn
484 silage ($P = 0.05$). Additionally, HNDF diets resulted in greater pool size of aNDFom than LNDF
485 diet ($P = 0.03$). Cows fed the BM3 diets had faster turnover rate ($P = 0.002$) and shorter (P
486 $=0.003$) turnover time of OM than cows fed the CON diets. The faster turnover rate observed for
487 the BM3 diets was likely due to increased NDFD compared to CON corn silage. Likewise, Oba
488 and Allen (2000b) reported that cows fed brown midrib-3 corn silage diets had faster OM
489 turnover rate compared to cows fed conventional corn silage diets.

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

501 CONCLUSIONS

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

504 (>60% of NDF) and lower uNDF240 (<10% of ration DM) to avoid reductions in DMI and milk
505 yield. Higher NDF diet with lower NDFD resulted in depressed DMI and milk yield associated
506 with greater levels of ruminal fill, longer ruminal OM and NDF retention time, and nearly an
507 hour more eating time than a higher forage diet with greater NDFD. Higher forage NDFD and
508 lower uNDF240 allowed for greater NDF intake. Because ECM yield was reduced with the
509 higher NDF diets, but much less so for the BM3 corn silage, we concluded that forage NDF in
510 higher forage diets may support similar milk production when forage NDFD is high (i.e., >60%
511 of NDF). Gut fill limitations may occur for corn silage-based diets when dietary uNDF240
512 content exceeds approximately 10% of ration DM.

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517

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

Item	CON		BM3	
	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

640 ¹Ag Processing Inc., Omaha, NE.

641 ²Alltech, Nicholasville, KY.

642 ³Addissee USA, Inc., Alpharetta, GA.

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

645 ⁵Contained 0.02% Ca, 18.82% S, 153,737 mg Zn/kg, 30,303 mg Cu/kg, 136,364 mg Mn/kg,
646 3,384 mg Co/kg, and 3,030 mg I/kg.

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

648 ⁷Contained 2,041 mg Se/kg.

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

650 ⁹Contained 44,639 IU vitamin E/kg.

651 ¹⁰Elanco, Greenfield, IN.

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653 **Table 2.** Data (mean \pm standard error_{REG}) characterizing the analyzed chemical composition of ingredients used in diets containing
654 either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating
655 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 \pm 0.4	36.4 \pm 1.0	36.5 \pm 1.2	90.9 \pm 0.7	91.6 \pm 0.4	92.6 \pm 1.0
CP, % of DM	7.2 \pm 0.1	7.9 \pm 0.2	18.2 \pm 0.7	8.4 \pm 0.1	36.4 \pm 0.8	34.1 \pm 0.7
Soluble protein, % of CP	59.9 \pm 1.0	59.4 \pm 0.9	65.0 \pm 1.8	17.0 \pm 1.6	24.6 \pm 2.2	29.1 \pm 1.9
Ammonia, % of CP	16.9 \pm 0.5	17.7 \pm 0.3	15.8 \pm 0.7	-	-	-
ADF, % of DM	23.5 \pm 1.1	24.4 \pm 0.3	33.8 \pm 0.5	4.2 \pm 0.4	12.8 \pm 0.4	14.6 \pm 0.3
aNDFom ³ , % of DM	36.1 \pm 1.2	34.8 \pm 1.6	46.2 \pm 0.6	-	-	-
24-h aNDFom degradability , % of aNDFom	48.6 \pm 0.8	62.1 \pm 1.0	57.7 \pm 0.8	-	-	-
Undegradable NDFom at 240 h, % of DM	11.0 \pm 0.3	7.6 \pm 0.2	14.0 \pm 0.5	-	-	-
Potentially degradable NDF, % of aNDFom	69.5 \pm 0.9	78.1 \pm 0.6	69.7 \pm 1.1	-	-	-
ADL, % of DM	3.0 \pm <0.1	2.4 \pm 0.2	5.1 \pm 0.3	1.3 \pm <0.1	3.4 \pm 0.4	3.6 \pm 0.3
Starch, % of DM	36.0 \pm 0.8	34.5 \pm 1.0	1.3 \pm 0.2	72.7 \pm 0.5	8.1 \pm 0.4	10.1 \pm 0.7
Sugar (ESC ⁴), % of DM	1.0 \pm 0.1	1.0 \pm 0.1	2.2 \pm 0.5	3.0 \pm <0.1	11.4 \pm 0.2	9.7 \pm 0.5
Crude fat, % of DM	3.1 \pm 0.1	3.3 \pm 0.2	3.8 \pm 0.2	3.3 \pm 0.2	6.1 \pm 0.3	6.7 \pm 0.4
Ash, % of DM	4.4 \pm 0.1	5.0 \pm 0.1	10.1 \pm 0.3	1.4 \pm 0.1	14.5 \pm 0.4	13.9 \pm 0.6
Ca, % of DM	0.25 \pm 0.02	0.29 \pm 0.01	1.07 \pm 0.04	0.03 \pm 0.00	2.53 \pm 0.11	2.46 \pm 0.22
P, % of DM	0.21 \pm 0.00	0.24 \pm 0.00	0.36 \pm 0.03	0.30 \pm 0.02	0.76 \pm 0.02	0.71 \pm 0.01
Mg, % of DM	0.22 \pm 0.00	0.17 \pm 0.00	0.29 \pm 0.01	0.12 \pm 0.00	0.82 \pm 0.03	0.81 \pm 0.04
K, % of DM	0.74 \pm 0.03	1.02 \pm 0.05	2.92 \pm 0.23	0.35 \pm 0.01	1.67 \pm 0.04	1.62 \pm 0.05
S, % of DM	0.11 \pm 0.01	0.15 \pm 0.02	0.26 \pm 0.02	0.10 \pm 0.01	0.62 \pm 0.02	0.76 \pm 0.08
Na, % of DM	0.01 \pm 0.00	0.01 \pm 0.00	0.05 \pm 0.01	0.02 \pm 0.00	1.45 \pm 0.05	1.37 \pm 0.07
Cl ion, % of DM	0.23 \pm 0.00	0.2 \pm 0.01	0.6 \pm 0.09	0.06 \pm 0.00	1.06 \pm 0.03	0.91 \pm 0.01
Fe, mg/kg	256 \pm 10	492 \pm 45	195 \pm 9	29 \pm 2	493 \pm 13	460 \pm 5
Cu, mg/kg	8 \pm 1	12 \pm 1	12 \pm 1	3 \pm 1	50 \pm 5	62 \pm 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	-	-	-

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

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

658 ³Amylase-modified NDF on an OM basis.

659 ⁴Ethanol-soluble carbohydrates.

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681 **Table 3.** Particle size distribution, physical effectiveness factor (pef), and physically effective NDF (peNDF) of ingredients (mean ±
 682 standard deviation) used in diets containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or
 683 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

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

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

686 ³physical effectiveness factor, % of DM retained on ≥1.18 mm with dry sieving; based on methods and assumptions in Mertens
 687 (1997).

688 ⁴pef x NDF (Mertens, 1997).

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

Item	CON		BM3	
	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

699 ¹Amylase-modified NDF on an OM basis.

700 ²Ethanol-soluble carbohydrates.

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

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

714 ¹Amylase-modified NDF on an OM basis.

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

Item	CON		BM3	
	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

729 ¹physical effectiveness factor, % of DM retained on ≥1.18 mm with dry sieving; based on methods and assumptions in Mertens
 730 (1997).

731 ²pef x NDF (Mertens, 1997).

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736 **Table 7.** Least squares means of intake, BW, BCS, and lactational performance for lactating Holstein cows fed a diet containing either
 737 conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein
 738 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

739 ¹NDFD: Effect of NDF degradability of corn silage.

740 ²NDF: Effect of dietary NDF content.
741 ³NDFD x NDF: Interaction of NDF degradability of corn silage and dietary NDF content.
742 ⁴Amylase-modified NDF on an OM basis.
743 ⁵uNDF240om = undegradable NDFom at 240 h of in vitro fermentation.
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748 **Table 8.** Least squares means of ruminal pH data for lactating Holstein cows fed a diet containing either conventional corn silage
 749 (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to lactating Holstein cows

Item	CON		BM3		SE	P-value			Time	Treatment Interactions with Time
	Low NDF	High NDF	Low NDF	High NDF		NDFD ¹	NDF ²	NDFD x NDF ³		
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 – min pH)	1.11	1.05	1.10	1.07	0.07	0.95	0.40	0.82	-	-
AUC < 5.8, pH units x min/d	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	-	-

750 ¹NDFD: Effect of NDF degradability of corn silage.

751 ²NDF: Effect of dietary NDF content.

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

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

Item	CON		BM3		SE	P-value			Time	Treatm ent Interact ions with Time
	Low NDF	High NDF	Low NDF	High NDF		NDFD ¹	NDF ²	NDFD x NDF ³		
Total VFA, mM	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	-
									<0.001	NDFa mtxtim e = 0.04
Propionate (P)	26.2	21.5	29.0	25.7	2.3	0.004	0.001	0.50		-
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	-
									<0.001	NDFa mt x time = 0.09
A:P	2.56	3.08	2.31	2.69	0.15	0.005	<0.001	0.49		-
									<0.001	NDFa mt x time = 0.04
(A+B):P	3.08	3.64	2.79	3.22	0.19	0.01	0.001	0.60		-

										<0.001	NDFa mt x time = 0.09
	Ammonia-N, mg/dL	10.49	11.60	9.88	10.42	0.79	0.24	0.27	0.70		
759	¹ NDFD: Effect of NDF degradability of corn silage.										
760	² NDF: Effect of dietary NDF content.										
761	³ NDFD x NDF: Interaction of NDF degradability of corn silage and dietary NDF content.										
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764 **Table 10.** Least squares means of chewing data for lactating Holstein cows fed a diet containing either conventional corn silage
 765 (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
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

766 ¹NDFD: Effect of NDF degradability of corn silage.

767 ²NDF: Effect of dietary NDF content.

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

769 ⁴Amylase-modified NDF on an OM basis.

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

Item ¹	CON		BM3		SE	P-value		
	Low NDF	High NDF	Low NDF	High NDF		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

780 ¹All values are ash-corrected.

781 ²NDFD: Effect of NDF degradability of corn silage.

782 ³NDF: Effect of dietary NDF content.

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

784 ⁵Amylase-modified NDF on an OM basis.

785 ⁶Potentially **degradable** NDF.

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788 **Table 12.** Least squares means of rumen digesta characteristics, pool sizes, and turnover for lactating Holstein cows fed a diet
 789 containing either conventional corn silage (CON) or brown midrib corn silage (BM3) with lower or higher NDF concentration fed to
 790 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
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

791 ¹NDFD: Effect of NDF degradability of corn silage.

792 ²NDF: Effect of dietary NDF content.

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

794 ⁴Amylase-modified NDF on an OM basis.

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