Effect of reduced ferulate-mediated lignin/arabinoxylan cross-linking in corn silage on feed intake, digestibility, and milk production¹

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ABSTRACT

Cross-linking of lignin to arabinoxylan by ferulates limits in vitro rumen digestibility of grass cell walls. The effect of ferulate cross-linking on dry matter intake (DMI), milk production, and in vivo digestibility was investigated in ad libitum and restricted-intake digestion trials with lambs, and in a dairy cow performance trial using the low-ferulate *sfe* corn mutant. Silages of 5 inbred corn lines were fed: W23, 2 W23*sfe* lines (M04-4 and M04-21), B73, and B73*bm3*. As expected, the W23*sfe* silages contained fewer ferulate ether cross-links and B73*bm3* silage had a lower lignin concentration than the respective genetic controls. Silages were fed as the sole ingredient to 4 lambs per silage treatment. Lambs were confined to metabolism crates and fed ad libitum for a 12-d adaptation period followed by a 5-d collection period of feed refusals and feces. Immediately following the ad libitum feeding trial, silage offered was limited to 2% of body weight. After a 2-d adaptation to restricted feeding, feed refusals and feces were collected for 5 d. Seventy Holstein cows were blocked by lactation, days in milk, body weight, and milk production and assigned to total mixed ration diets based on the 5 corn silages. Diets were fed for 28 d and data were collected on weekly DMI and milk production and composition. Fecal grab samples were collected during the last week of the lactation trial for estimation of feed digestibility using acid-insoluble ash as a marker. Silage, total mixed ration, feed refusals, and fecal samples were analyzed for crude protein, starch, neutral detergent fiber (NDF), cell wall polysaccharides, and lignin. The W23*sfe* silages resulted in lower DMI in the ad libitum trial than the W23 silage, but DMI did not differ in the

restricted trial. No differences were observed for NDF or cell wall polysaccharide digestibility by lambs with restricted feeding, but the amount of NDF digested daily increased for lambs fed the M04-21 W23*sfe* silage ad libitum. Lambs were less selective against NDF and lignin when offered W23*sfe* silages. The B73*bm3* silage did not affect DMI or digestibility of cell walls at the restricted feeding level, but total daily NDF digested was greater at ad libitum intake. Intake, milk production, and cell wall digestibility were greater for cows fed diets containing W23*sfe* silages than for those fed W23 silage. Although milk production was greater for the B73*bm3* diet, DMI and cell wall digestibility were not altered. Cows were less selective against cell wall material when fed both W23*sfe* and B73*bm3* silages. Reduced ferulate cross-linking in *sfe* corn silage is a new genetic mechanism for improving milk production. **Key words:** corn silage, ferulate, digestibility, milk production

INTRODUCTION

Digestibility of forage fiber (cell walls) has long been known to be negatively associated with lignin concentration (Jung and Deetz, 1993). However, this relationship between lignin and fiber digestibility is strongest when examined across forage maturity stages and is less clear when forages are harvested at a similar maturity stage, such as corn silage or bud-stage alfalfa (Cone and Engels, 1993; Wolf et al., 1993; Jung et al., 1994). To explain the observed variation in fiber digestibility of forages with similar lignin concentrations, composition of lignin and chemical cross-linking of lignin to cell wall polysaccharides have been suggested as additional mechanisms limiting fiber digestibility (Jung and Deetz, 1993). The more digestible brown midrib (*bm*) mutants in corn, sorghum, and pearl millet have been shown to have both decreased lignin concentration and a shift in lignin composition (Cherney et al., 1991). Nevertheless, results from model systems and mutant *Arabidopsis* lines strongly suggest that alterations in lignin composition, as measured by syringyl:guaiacyl ratio, do not account for the improved fiber digestibility of *bm* mutants (Grabber et al., 1997; Jung et al., 1999).

Received April 28, 2011.

Accepted June 27, 2011.

¹Mention of a proprietary product does not constitute a recommendation or warranty of the product by USDA or the University of Minnesota and does not imply approval to the exclusion of other suitable products.

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In grasses, lignin is cross-linked to arabinoxylans by ferulate molecules (Iiyama et al., 1990). These ferulates are esterified to arabinose units of arabinoxylans, and some ferulate esters subsequently form a second linkage to lignin via ether and other covalent bonds, thereby cross-linking these polymers (Ralph et al., 1992). Some ferulate esters combine to form diferulates to cross-link arabinoxylan chains, with a portion of these diferulates also becoming linked to lignin, as occurs with ferulates (Ralph et al., 1998). It has been proposed that cross-linking of lignin and arabinoxylans will impede cell wall digestibility by placing lignin in very close proximity to the polysaccharides and preventing physical access by hydrolytic microbial enzymes (Jung and Ralph, 1990; Jung and Deetz, 1993). Model studies using corn cell cultures that manipulated ferulate cross-linking and correlation analyses with grass genotypes of divergent (low vs. high ferulate ether concentration) have demonstrated that in vitro rumen cell wall degradability is negatively affected by increasing ferulate ether cross-link concentration (Grabber et al., 1998a,b; Casler and Jung, 1999; Casler et al., 2008).

A putative mutant in corn has been identified that has lower concentrations of ferulate esters in seedling leaves (Jung and Phillips, 2010). It was hypothesized that reduced deposition of ferulate esters in immature tissues would result in fewer opportunities for formation of ferulate ether cross-links in mature lignified tissues, with an associated increase in potential cell wall digestibility. Jung and Phillips (2010) showed that the seedling leaf ferulate ester (*sfe*) mutant had reduced concentration of ferulate ether cross-links and higher in vitro rumen cell wall digestibility for corn stover at the silage maturity stage as predicted. Small reductions in stover cell wall and lignin concentrations were also associated with the *sfe* mutation. Although several candidate genes have been suggested as modifiers of ferulate biosynthesis in other plant species (Nair et al., 2004; Mitchell et al., 2007; Piston et al., 2010), the gene responsible for the *sfe* corn mutation has not yet been identified. Plants with the *sfe* mutation do not exhibit the reddish-brown coloration present in *bm* mutants; therefore, the *sfe* mutation is probably not caused by the same genes as those mutated in *bm* corn mutants.

Of the 4 characterized *bm* mutations in corn, *bm3* has been shown to cause the largest reduction in lignin concentration and improvement in digestibility (Barriere and Argillier, 1993). It has been demonstrated that corn silage from *bm3* mutants resulted in greater fiber digestibility and increased weight gain by ruminants with low levels of feed intake (Muller et al., 1972; Tjardes et al., 2000) and increased feed intake and milk production of high-producing dairy cows (Oba and Allen, 1999, 2000; Tine et al., 2001). Similar animal

performance data are not available for the *sfe* mutant. The objectives of the current project were to evaluate the effect of reduced ferulate ether cross-linking of lignin to arabinoxylan in *sfe* corn silage on (1) feed intake and selectivity, (2) in vivo cell wall digestibility, and (3) milk production. Two studies were conducted to address these objectives, a lamb intake and digestion trial including ad libitum and restricted intake phases with *sfe* corn silage as the sole dietary ingredient and a lactating dairy cow trial where *sfe* corn silage was included as part of a TMR. A *bm3* corn silage was included in the trial as a positive control.

MATERIALS AND METHODS

Corn Silages

Five inbred corn lines were planted at the US Dairy Forage Research Center near Prairie du Sac, Wisconsin, in 2008. The experimental lines included the parental control line W23, 2 near-isogenic W23*sfe* mutant lines (M04-4 and M04-21), B73, and B73*bm3*. Both W23*sfe* lines were derived from the same original mutant plant and had shown the greatest reduction in ferulate ether cross-linking and increased in vitro rumen cell wall digestibility in an earlier plot trial involving 4 W23*sfe* lines (Jung and Phillips, 2010). Because inbred lines yield less biomass than commercial hybrids and seed supplies were limited, the corn lines were seeded at a low density (~59,000 plants/ha) to maximize per-plant and total silage yield rather than yield per hectare. The 5 corn lines were grown on adjacent plots and managed according to University of Wisconsin recommendations for corn silage production. The W23 and W23*sfe* corn lines were chopped for silage on September 19, 2008, and the B73 and B73*bm3* lines were chopped on September 21 and 22, 2008. The silage chopper was equipped with a kernel processor. Corn silages were ensiled in 2.4-m-diameter plastic bags. One compressed, plastic-wrapped silage bale (~265 kg) was made from each corn line using a custom-built stationary press (Digman et al., 2010).

Lamb Trial

Assignment and Covariate Period. The lamb trial was conducted from October 29 to December 10, 2008. Lambs were housed in an environmentally controlled animal room on the University of Wisconsin-Madison campus. The plastic-wrapped corn silage bales were fed in the lamb trial. Twenty crossbred wether lambs (44.1 ± 1.6 kg) were fed a reference alfalfa hay for 6 d in a group pen. Body weights were taken on 3 consecutive days and lambs were assigned to 5 blocks

based on BW. Lambs within each block were randomly assigned to silage treatment and moved to individual crates (d 1 of trial). Lambs had free access to water and a trace mineral block throughout the trial. The lambs were fed the reference alfalfa hay ad libitum during a 4-d adaptation period, followed by a 5-d feed intake and fecal collection covariate period. Feed refusal weights were collected on d 6 through 10, and total fecal collections occurred on d 7 through 11.

Ad Libitum Phase. Beginning on d 10, lambs were fed their respective treatment corn silage ad libitum as the sole dietary ingredient. The amount of corn silage offered to each lamb was adjusted daily to achieve an approximately 10% refusal rate. After a 12-d adaptation period to the corn silage diets, a 5-d feed intake and digestibility trial was conducted starting on d 22. Daily grab samples of feed offered were collected and composited by silage treatment from d 22 through 26. Individual lamb feed refusal grab samples and total feces were collected daily and composited over the 5-d collection period. Feed refusal collections began on d 23 of the trial and continued through d 27. Fecal collections began on d 24 of the trial and continued through d 28. On d 19 during the adaptation period, visual observations of lamb activity (eating, rumination, drinking, and inactivity) were recorded every 5 min for 24 h.

Restricted Phase. During the restricted-intake phase of the trial, corn silage DM offered was restricted for all treatments to about 2% of BW to represent a maintenance level of DMI. Feed restriction began on d 27. After a 2-d adjustment period to restricted intake, data collection commenced for 5 d. Feed, refusal, and fecal collections were done as in the ad libitum phase of the trial. Feed grab samples were collected from d 29 through 33, refusals were collected from d 30 through 34, and fecal samples were collected from d 31 through 35. The short adjustment period to restricted intake was judged sufficient because although fecal weights declined during d 28 to 30 after the ad libitum feeding was ended, fecal output was consistent during d 31 through 35 of fecal collection during the restricted phase.

Lactation Trial

The lactation study was conducted at the US Dairy Forage Research Center near Prairie du Sac, Wisconsin, from December 30, 2008, to March 3, 2009. Seventy Holstein cows were blocked into 14 groups based on lactation number (2.0 ± 0.1), DIM (93 ± 7 d), BW (619 ± 9 kg), and milk production (45.8 ± 0.6 kg/d) during a 14-d covariate period. During the covariate period cows were fed a TMR containing approximately 17% CP,

28.5% NDF, and 26% starch (DM basis) from 26% alfalfa silage, 31% corn silage, and 43% concentrate (DM basis). The 5 cows in each block were subsequently randomly assigned to the corn silage treatments. Ingredient and nutrient composition of the experimental diets is shown in Table 1. Diets were formulated to be similar in CP and NDF concentrations, provide 70% of dietary NDF from corn silage, and exceed CP, mineral, and vitamin requirements for 39 kg/d of milk production (NRC, 2001). Proportions of high-moisture corn and treatment corn silages were adjusted to obtain similar NDF concentrations in the TMR across treatments. To ensure fiber utilization, diets were formulated to provide <26% starch and CP that was 30% A-fraction and 36% RUP as defined by NRC (2001). Cows were housed in tie stalls, fed once daily, and milked twice daily.

Cows were switched to their respective treatment diets on January 13, 2009. A rapid decline in feed intake and milk production occurred across all treatments. Because of the severity of the reduced performance, alfalfa silage in the diets was increased from 10 to 20% of diet DM (in place of concentrate ingredients) in all diets on January 20. Feed intake and milk production returned to covariate period levels over the next 7 d. Alfalfa silage was reduced to 15% of diet DM on January 27, and the experimental period began on February 3, when alfalfa silage was reduced to 10% of diet DM and cows returned to the experimental diets shown in Table 1. Cows were fed diets containing their respective treatment corn silage during the transition period. The cause of the decrease in performance upon the initial switch of cows to the experimental diets is unknown.

The length of the lactation trial was 28 d because of limited supplies of the experimental corn silages. Diets were adjusted on d 14 to account for changes in DM and NDF of the corn and alfalfa silages. Cows were fed for ad libitum intake, allowing approximately 10% daily refusals. Grab samples of the corn silages, TMR diets, and feed refusals were collected daily and composited weekly by treatment. Milk production was measured daily, and weekly means were calculated for each cow. Milk samples were collected for compositional analysis weekly at consecutive p.m. and a.m. milkings. Fecal grab samples were collected during the last week of the experiment. On 2 consecutive days, fecal grab samples were obtained from every cow during feeding after the a.m. milking, in early afternoon before the p.m. milking, and in the evening after the p.m. milking. The 6 fecal samples from each cow were composited. Lambs and cows were cared for according to separate protocols approved by the Research Animal Resource Center of the University of Wisconsin.

Table 1. Ingredients and nutrient composition of diets fed to lactating Holstein cows

Composition	Diet ¹				
	W23	M04-4	M04-21	B73	B73bm3
Ingredient (% of DM)					
Corn silage	39.7	38.2	39.1	41.4	42.2
Alfalfa silage	10.0	10.0	10.0	10.0	10.0
High-moisture corn	12.0	13.5	12.6	10.4	9.5
Roasted soybeans	6.0	6.0	6.0	6.0	6.0
Molasses	5.0	5.0	5.0	5.0	5.0
Premix ²	27.3	27.3	27.3	27.3	27.3
Nutrient					
DM (%)	53.8	52.6	51.1	46.1	46.7
Ash (% of DM)	8.5	8.6	8.6	8.3	8.3
CP (% of DM)	16.5	15.6	16.8	16.7	16.3
Ether extract (% of DM)	7.0	6.9	7.4	7.3	6.7
Starch (% of DM)	24.5	26.0	22.3	22.8	23.1
NDF (% of DM)	31.0	30.5	30.9	31.0	32.1
Acid detergent lignin (% of NDF)	5.7	5.7	4.9	6.6	4.3
Cell wall ³ (% of DM)	33.6	31.9	34.3	34.3	35.2
Klason lignin (% of cell wall)	14.5	14.6	14.7	14.0	13.8
48-h in vitro NDF digestibility	55.0	57.1	56.0	61.8	74.5
48-h in vitro cell wall polysaccharide digestibility	67.6	69.0	70.0	68.1	78.6

¹Diets contained 1 of 5 inbred corn lines: the parental control line W23, 2 near-isogenic W23*sfe* mutant lines (M04-4 and M04-21), B73, and B73bm3.

²Premix consisted (on DM basis) of 48% CP soybean meal (47.87%), ground dry corn (36.69%), Energy Booster (3.67%; Milk Specialties Co., Dundee, IL), blood meal (1.28%), urea (0.66%), CaCO₃ (4.40%), CaHPO₄ (0.22%), Dynamate (0.95%; Mosaic Co., Plymouth, MN), NaHCO₃ (2.75%), MgO (0.37%), NaCl (0.73%), and vitamins, trace minerals, and Rumensin (0.40%; Elanco Animal Health, Greenfield, IN).

³Sum of all cell wall monosaccharide residues (glucose, xylose, arabinose, galactose, mannose, rhamnose, fucose, and uronic acids), Klason lignin, ferulates, and *p*-coumarates.

Analytical Methods

Composition. Feed, refusal, and fecal samples from both lamb and cow trials were dried at 60°C and ground to pass a 1-mm screen in a Wiley mill. Crude protein concentration was determined by the Kjeldahl method and lipids by exhaustive extraction with diethyl ether (AOAC, 1995). Samples were combusted at 450°C overnight to determine ash content. Starch (plus any free glucose) was measured by hydrolysis with heat-stable α -amylase and amyloglucosidase, and resulting glucose was measured by HPLC (Dien et al., 2006). Samples were analyzed for NDF and sulfuric acid detergent lignin (**ADL**) in the sequential mode (Van Soest et al., 1991) using the Ankom filter bag (Ankom Technology Corp., Fairport, NY) technique. Heat-stable amylase was included in the determination of NDF.

Cell wall polysaccharide components (glucose, xylose, arabinose, galactose, mannose, rhamnose, fucose, and uronic acids) and Klason lignin were determined by a 2-stage sulfuric acid hydrolysis of starch-free, alcohol-insoluble residues prepared from feed, refusal, and fecal samples using the Uppsala Dietary Fiber method (Theander et al., 1995). Neutral sugars were derivatized and analyzed by GC. Total uronic acids in cell wall polysaccharides were determined by colorimetry on aliquots

from the first-stage acid hydrolysis using glucuronic acid as the standard (Ahmed and Labavitch, 1977).

Corn silage, TMR diets, and refusal samples were analyzed for *p*-coumarates and ferulates. Starch-free, alcohol-insoluble residues were extracted with 2 *M* NaOH at 39°C to release esterified *p*-coumarates and ferulates (Jung and Shalita-Jones, 1990). Duplicate samples were extracted with 4 *M* NaOH at 170°C to release both ester and ether-linked ferulates (Iiyama et al., 1990). *p*-Coumaric and ferulic acids released by these alkaline extractions were quantified by HPLC. Ferulate ether cross-links were calculated as the difference between total and esterified ferulates (Iiyama et al., 1990).

Total cell wall concentration was calculated as the sum of cell wall glucose, xylose, arabinose, galactose, mannose, rhamnose, fucose, uronic acids, Klason lignin, esterified *p*-coumarates and ferulates, and etherified ferulates.

Samples of TMR diet, refusals, and feces from the lactation trial were analyzed for acid-insoluble ash (Van Keulen and Young, 1977). Digestibility was calculated using acid-insoluble ash as an internal marker (Van Soest, 1994). Data for feed and refusal composition, and amounts from wk 4 of the lactation trial were used in the digestibility calculations.

Milk samples were analyzed for fat, protein, lactose, SCC, and MUN by AgSource Cooperative Services (Menomonie, WI).

In Vitro Digestibility. Corn silage samples collected during both phases of the lamb trial, and silage and TMR samples from the lactation trial were analyzed for *in vitro* rumen digestibility of NDF (IVNDFD) and cell wall polysaccharides (IVCWPD). Dried and ground silage samples were incubated with rumen fluid for 24, 48, and 96 h, whereas TMR samples were incubated for 48 h. Rumen fluid was collected from a fistulated, lactating Holstein cow housed on the University of Minnesota-St. Paul campus and fed a TMR including corn silage, alfalfa hay, and concentrates. Rumen fluid was collected 4 h postfeeding, strained through 4 layers of cheesecloth, and mixed (1:4 vol/vol) with anaerobic McDougall's buffer (McDougall, 1948) under CO₂ sparging. Ankom filter bags and the Daisy rotating-jar incubator were used for measurement of IVNDFD. Incubations were conducted in 50-mL disposable, capped centrifuge tubes in a water bath for IVCWPD. To avoid problems related to starch fermentation creating a slimy layer on Ankom bag surfaces, which experience has shown to increase variability of IVNDFD measurements for high-starch samples, corn silage samples were incubated with porcine amylase overnight and the supernatant removed by centrifugation before inoculation with the rumen fluid:buffer mixture. The IVCWPD procedure did not require removal of starch. All incubations were done at 39°C. Blank bags with glass wool were included in the IVNDFD determination, and empty tubes were used for IVCWPD to correct for NDF and cell wall polysaccharides contributed by the rumen inoculum. Residues from the *in vitro* rumen incubations were analyzed for NDF and cell wall polysaccharide components as described above. Care of the fistulated cow was approved by the University of Minnesota Institutional Animal Care Committee.

Calculations and Statistical Analysis

All laboratory analysis data are the mean of duplicate determinations, and data were corrected to a 100°C DM basis. A selectivity index was calculated for both trials, where the composition of the feed consumed was calculated from appropriate feed and refusal composition and quantity data. The composition of the feed actually consumed was divided by the composition of the feed offered to obtain the selectivity index for individual nutrients. A selectivity index value >1.0 indicated selection for the nutrient, an index of 1.0 indicated no selection, and an index <1.0 indicated selection against the nutrient. Daily chewing activity (total, eating, and rumination) per gram of NDF consumed was calculated

as the number of activity events recorded during the 24-h observation period and assuming each activity event lasted the full 5 min between observations.

Data for the lamb trial were analyzed as a randomized complete block design with 4 replicates and 5 corn silage treatments using PROC GLM of SAS ver. 9.1 (SAS Institute Inc., Cary, NC). Analysis of data from the covariate period indicated a significant ($P < 0.05$) difference among lambs assigned to treatment groups for DMI, but not DM digestibility of the reference alfalfa hay. Based on this result, DMI of the alfalfa hay was used as a covariate for analysis of corn silage intake and amount of NDF digested per day by lambs. Analysis of other traits did not include a covariate. Data for feed intake, milk production, and milk composition were analyzed as a randomized complete block design with weekly means as repeated measures. The PROC MIXED routine in SAS was used with a heterogeneous autoregressive covariance structure and the Satterthwaite degrees of freedom method. Cow was treated as a random effect and block and treatment were considered fixed effects. Covariate period data were not included as part of the statistical model for the lactation study because of the 3-wk interval between the end of the covariate period and actual initiation of data collection for the trial and the intervening transition diets fed during those 3 wk. Data for nutrient digestibility and selection indices from the fecal collection period for the lactation trial were analyzed as a randomized complete block design with 14 replicates and 5 treatments using PROC GLM.

Orthogonal contrasts were used to compare treatments in both lamb and lactation trial data sets. The contrasts included W23 versus W23*sfe* (combination of lines M04-4 and M04-21), W23 versus M04-21, B73 versus B73*bm3*, and W23 versus B73. The contrast including only W23 versus W23*sfe* line M04-21 was included because the earlier plot trial indicated that M04-21 had a greater reduction in ferulate ether cross-linking and a greater improvement in IVNDFD and IVCWPD than observed for M04-4 (Jung and Phillips, 2010).

RESULTS

Corn Silages

The overall composition of the corn silages used for the lamb and cow trials is shown in Table 2. These mean data were based on analysis of 1 composite silage sample from the *ad libitum* phase and 1 composite silage sample from the restricted phase of the lamb trial, and 4 weekly composite silage samples from the lactation trial. Unfortunately, the W23 and W23*sfe* corn lines dried faster than anticipated and were drier than the

Table 2. Mean composition of corn silages of inbreds W23, W23*sfe* lines (M04-4 and M04-21), B73, and B73*bm3* fed to lambs during ad libitum and restricted intake phase digestibility studies, and included in lactating Holstein cow trial

Component	Corn line				
	W23	M04-4	M04-21	B73	B73 <i>bm3</i>
DM (%)	48.9	42.4	40.4	31.5	32.8
Ash (% of DM)	5.1	5.6	5.5	4.3	4.5
CP (% of DM)	8.3	8.1	8.2	7.2	7.5
Ether extract (% of DM)	3.3	3.9	4.6	5.1	4.5
Starch (% of DM)	25.7	20.7	21.9	21.5	21.8
NDF (% of DM)	45.0	48.1	47.3	47.0	47.4
Acid detergent lignin (% of NDF)	4.9	6.7	4.9	5.3	4.0
Cell wall ¹ (% of DM)	45.1	50.4	50.0	45.9	46.1
Klason lignin (% of cell wall)	16.1	16.6	15.9	15.3	12.9
Glucose (% of cell wall)	38.6	39.6	39.1	40.2	43.2
Xylose (% of cell wall)	27.5	26.5	27.7	29.2	29.0
Arabinose (% of cell wall)	6.4	6.1	6.3	5.2	5.2
Galactose (% of cell wall)	2.2	2.3	2.3	2.0	2.0
Mannose (% of cell wall)	1.4	1.6	1.6	1.4	1.5
Uronic acids (% of cell wall)	4.2	4.0	3.9	3.1	3.5
Ferulate esters (% of cell wall)	0.83	0.69	0.72	0.88	0.84
Ferulate ethers (% of cell wall)	0.92	0.70	0.67	0.95	0.90
<i>p</i> -Coumarate esters (% of cell wall)	1.76	1.79	1.72	1.70	1.01

¹Sum of all cell wall polysaccharide component sugars (glucose, xylose, arabinose, galactose, mannose, rhamnose, fucose, and uronic acids), Klason lignin, ferulates, and *p*-coumarates.

B73 and B73*bm3* lines at harvest. Although the B73 and B73*bm3* silages were similar in DM content, W23 was drier than the 2 W23*sfe* silages. Due to an error in communication, nondried silages samples were not retained for analysis of pH or fermentation acids. Our subjective assessment was that all silages were well preserved and probably had normal fermentation profiles.

As expected, the W23*sfe* silages had lower numerical concentrations of ferulate ether cross-links in the cell wall than W23, with M04-21 being marginally lower than M04-4 (27 and 24% reductions, respectively). A similar difference in ferulate ether concentration was not observed between the B73 and B73*bm3* silages. The W23 silage contained more starch, and lower concentrations of NDF and total cell wall than the W23*sfe* silages. The B73 and B73*bm3* silages were similar to the W23*sfe* silages in starch and NDF concentrations, but more similar to W23 for cell wall concentration. Silages for the W23*sfe* lines were similar in ADL and Klason lignin concentration to W23. Cell wall polysaccharide composition was similar for W23 and W23*sfe* silages. The B73 silage was similar to the W23 and W23*sfe* silages in lignin concentration; however, the B73*bm3* silage contained less ADL (24% lower) and Klason lignin (16% lower) than B73, as was expected. Composition of the B73 and B73*bm3* silages did not differ for other major cell wall components, but *p*-coumarate ester concentration was substantially lower for the B73*bm3* silage relative to B73.

In vitro digestibility of total cell wall polysaccharides and individual polysaccharide components (with the exception of uronic acids) were uniformly greater for the W23*sfe* silages than for W23 after the 24-h incubation (Table 3). However, in vitro digestibility of the M04-21 silage was similar to W23 silage after the 48-h incubation and only slightly higher after 96 h. In contrast, in vitro digestibility of M04-4 silage tended to be lower than that of W23 silage after the 48-h incubation and similar after 96 h. In vitro digestibility of NDF did not follow the same pattern as IVCWPD. The W23 and M04-21 silages were similar in IVNDFD, and M04-4 silage was lower, at all incubation times. Greater in vitro digestibility of NDF, total cell wall polysaccharides, and all polysaccharide components was observed for the B73*bm3* silage compared with the B73 silage at all 3 incubation times.

Although the silage composition data in Table 2 were averaged across both phases of the lamb trial and the lactation trial, digestibility and selection index calculations were done using composition data from the appropriate silage composites collected for each individual trial phase.

Lamb Trial

Ad libitum DMI was lower ($P < 0.05$) for lambs fed the W23*sfe* silages than for W23 silage; however, this effect was due to low DMI of M04-4 silage because intake

Table 3. Mean in vitro digestibility of NDF, total cell wall polysaccharides, and major cell wall polysaccharide component sugars of silages from corn inbred W23, W23*sfe* lines (M04-4 and M04-21), B73, and B73*bm3* fed to lambs during ad libitum and restricted-intake phase digestibility studies and included in lactating Holstein cow trial

Component	Time (h)	Digestibility of corn lines (%)				
		W23	M04-4	M04-21	B73	B73 <i>bm3</i>
NDF	24	34.4	28.1	35.0	29.3	40.5
	48	46.5	41.4	46.2	46.3	61.2
	96	60.2	57.2	60.7	59.4	75.0
CWP ¹	24	36.7	39.7	51.4	37.4	61.3
	48	63.0	61.6	62.7	56.4	72.2
	96	68.3	68.1	70.4	67.3	81.8
Glucose	24	36.9	39.7	51.5	38.1	63.5
	48	66.1	63.7	65.8	60.8	77.0
	96	71.7	71.1	73.6	71.2	85.5
Xylose	24	31.7	35.1	48.5	34.2	56.2
	48	57.0	56.2	58.0	50.5	67.8
	96	63.6	63.5	65.8	63.1	79.3
Arabinose	24	48.8	51.7	61.1	49.2	67.1
	48	63.4	62.9	63.7	57.6	71.1
	96	70.3	71.0	72.1	68.3	78.6
Uronics	24	59.2	55.9	55.5	43.1	60.6
	48	70.8	69.7	69.4	62.9	76.3
	96	75.1	74.9	81.2	68.9	83.4

¹CWP = total cell wall polysaccharides.

of M04-21 silage did not differ ($P > 0.10$) from that of W23 (Table 4). Although ad libitum DMI of B73 and B73*bm3* silages did not differ ($P > 0.10$), lambs consumed more ($P < 0.01$) of the W23 silage than the B73 silage. Intake of DM by the lambs did not differ ($P > 0.10$) among the silages when feed offered was restricted, as was the intent of this phase of the trial.

Digestibility of total DM by lambs was lower ($P < 0.01$) in the ad libitum phase and tended to be lower ($P < 0.10$) in the restricted phase for W23*sfe* silages than for W23 (Table 4). Unlike for DMI, lamb digestibility response to the M04-21 silage was similar to that for M04-4. Crude protein digestibility was lower for lambs

fed W23*sfe* silages in the ad libitum phase of the trial, but similar to that of lambs fed W23 silage in the restricted phase. Starch digestibility did not differ ($P > 0.10$) for the W23*sfe* silages relative to W23 in either ad libitum or restricted intake phases. Digestibility of CP was lower ($P < 0.05$) for B73*bm3*-fed lambs compared with the B73-fed lambs during both intake phases of the trial. A trend was observed toward lower ($P < 0.10$) starch digestibility of B73*bm3* compared with B73 silage in the restricted phase. During both the ad libitum and restricted phases of the lamb trial, starch digestibility was greater ($P < 0.01$ and 0.05 , respectively) for the B73 silage than for the W23 silage; however, total DM

Table 4. Silage intake and digestibility of DM, CP, and starch for corn inbreds W23, W23*sfe* lines (M04-4 and M04-21), B73, and B73*bm3* fed to lambs during ad libitum (Ad lib) and restricted intake (Res) phase digestibility studies

Corn line	Intake (g of DM/d)		Digestibility (%)					
			DM		CP		Starch	
	Ad lib	Res	Ad lib	Res	Ad lib	Res	Ad lib	Res
W23	984	750	67.0	64.1	56.9	46.9	97.6	98.5
M04-4	839	751	59.5	61.5	40.1	46.1	97.0	99.3
M04-21	943	754	61.9	60.7	45.6	45.5	97.6	98.7
B73	883	743	66.1	64.8	52.9	49.1	99.2	99.4
B73 <i>bm3</i>	918	753	62.8	65.6	34.2	42.7	98.5	98.7
SEM	22	27	1.4	1.2	2.4	1.9	0.3	0.2
Contrasts								
W23 vs. W23 <i>sfe</i>	*	NS	**	†	***	NS	NS	NS
W23 vs. M04-21	NS	NS	*	†	**	NS	NS	NS
B73 vs. B73 <i>bm3</i>	NS	NS	NS	NS	***	*	NS	†
W23 vs. B73	**	NS	NS	NS	NS	NS	**	*

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS: $P > 0.10$.

and CP digestibility did not differ ($P > 0.10$) between the 2 corn genetic backgrounds.

Digestibility of NDF by lambs did not differ ($P > 0.10$) among any corn silages in the ad libitum phase (Table 5). Total cell wall polysaccharide digestibility of W23*sfe* silages was actually lower ($P < 0.05$) than that of W23; however, this significant effect was due to the low digestibility of the M04-4 silage. Although digestibility of cell wall glucose did not differ ($P > 0.10$) among silages fed ad libitum to lambs, digestibility of the other major polysaccharide components (xylose, arabinose, and uronic acids) was lower ($P < 0.10$ to 0.01) for W23*sfe* silages than for W23. The digestibility of NDF and cell wall polysaccharides did not differ ($P > 0.10$) for the B73 and B73*bm3* silages in the ad libitum phase. When the corn silages were fed at the restricted level of intake, no differences ($P > 0.10$) in digestibility of NDF or cell wall polysaccharide components were observed among any of the silage contrasts (data not shown).

Because the composition of the refusals for lambs fed the W23*sfe* and B73*bm3* silages were different than their respective control silage refusals (data not shown), we examined the possibility that lambs fed these mutant silages were more or less selective than their counterparts fed the control silages. During the ad libitum intake phase, the percentage of offered feed that was refused by the lambs did not differ ($11.8 \pm 1.0\%$; $P > 0.10$) among the silages. Although the selectivity indices for starch, total cell wall material, ADL, and Klason lignin did not differ ($P > 0.10$) among the silages (data not shown), lambs fed the W23*sfe* silages were less selective for CP (1.012 and 1.035 for W23*sfe* and W23, respectively; $P < 0.01$) and against NDF (0.958 and 0.924 for W23*sfe* and W23, respectively; $P < 0.05$) in the silage consumed compared with lambs fed the W23 silage. Although lambs were fed to minimize refusals in

the restricted phase, lambs fed the W23*sfe* and B73*bm3* silages had lower ($P < 0.05$) refusal percentages than lambs fed their respective control silages (Table 6). Selectivity indices for the W23*sfe*-fed lambs indicated less selection for starch ($P < 0.05$) and less selection against total cell wall material ($P < 0.01$) and Klason lignin ($P < 0.01$), and a tendency to less selection against NDF ($P < 0.10$) than for W23-fed lambs. Only a trend was observed for less selectivity against NDF ($P < 0.10$) for B73*bm3*-fed lambs in the restricted phase of the trial compared with B73-fed lambs. Refusal percentage did not differ ($P > 0.10$) for lambs fed W23 and B73 silages at either intake level, and the only significant selectivity difference was a greater selectivity for CP by W23-fed lambs.

When feed intake, composition of silage consumed, and NDF digestibility were combined to calculate amount of NDF digested per day, lambs fed M04-21 digested more NDF than lambs fed W23 silage in the ad libitum phase but not in the restricted phase (Figure 1). Similarly, B73*bm3*-fed lambs digested more NDF daily in the ad libitum, but not restricted, phase than B73-fed lambs. Total time spent chewing (eating plus rumination) was lower for lambs fed W23*sfe* than for those fed W23 silage (2.29 and 2.76 min/g of NDF consumed/d, respectively; $P < 0.05$) during the ad libitum phase, but not for the restricted phase. However, time spent in eating and rumination activities individually did not differ ($P > 0.10$) among silages. The B73- and B73*bm3*-fed lambs did not differ ($P > 0.10$) in chewing activity per gram of NDF consumed.

Lactation Trial

Cows fed diets containing the W23*sfe* silages had greater ($P < 0.01$) DMI than cows fed the TMR containing W23 silage (Table 7). Total milk production

Table 5. Digestibility of NDF, total cell wall polysaccharides, and the major cell wall polysaccharide component sugars for silages of corn inbreds W23, W23*sfe* lines (M04-4 and M04-21), B73, and B73*bm3* fed to lambs during the ad libitum intake phase digestibility study

Corn line	Digestibility (%)					
	NDF	CWP ¹	Glucose	Xylose	Arabinose	Uronics
W23	49.4	57.9	58.6	54.8	69.8	64.0
M04-4	46.5	48.8	51.4	44.8	54.8	57.8
M04-21	52.0	52.2	55.4	47.0	59.7	60.1
B73	48.7	55.9	57.6	52.6	63.8	61.0
B73 <i>bm3</i>	53.3	57.8	60.3	55.3	65.4	63.1
SEM	2.5	2.7	2.6	3.1	2.8	1.8
Contrasts						
W23 vs. W23 <i>sfe</i>	NS	*	NS	*	**	*
W23 vs. M04-21	NS	NS	NS	†	*	NS
B73 vs. B73 <i>bm3</i>	NS	NS	NS	NS	NS	NS
W23 vs. B73	NS	NS	NS	NS	NS	NS

¹CWP = total cell wall polysaccharides.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; NS: $P > 0.10$.

Table 6. Feed refusal and nutrient selectivity by lambs fed corn inbreds W23, W23*sfe* lines (M04-4 and M04-21), B73, and B73*bm3* silages during the restricted intake phase digestibility study

Corn line	Refusal (%)	Selectivity index ¹					
		CP	Starch	NDF	Cell wall	Acid detergent lignin	Klason lignin
W23	3.7	1.007	1.024	0.980	0.978	0.962	0.955
M04-4	1.3	1.002	1.008	0.996	0.996	0.990	0.995
M04-21	0.7	1.003	1.004	0.992	1.001	0.986	1.002
B73	3.9	1.007	1.018	0.989	0.986	0.958	0.971
B73 <i>bm3</i>	1.0	1.006	1.008	1.000	0.999	0.987	0.989
SEM	0.9	0.003	0.006	0.006	0.004	0.015	0.010
Contrasts							
W23 vs. W23 <i>sfe</i>	*	NS	*	†	**	NS	**
W23 vs. M04-21	†	NS	†	NS	**	NS	**
B73 vs. B73 <i>bm3</i>	*	NS	NS	NS	†	NS	NS
W23 vs. B73	NS	NS	NS	NS	NS	NS	NS

¹Selectivity index was calculated as the composition of the feed actually consumed (offered minus refusal) divided by the composition of the feed offered. Values >1.00 indicate selection for a component; values <1.00 indicate selection against a component.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; NS: $P > 0.10$.

and 3.5% FCM were also increased ($P < 0.001$ and 0.05, respectively) for cows on the W23*sfe*-containing diets. Milk fat percentage and fat yield were not different ($P > 0.10$) between the W23 and W23*sfe* diets, but a decrease ($P < 0.001$) was found in protein percentage for W23*sfe*-fed cows. Although milk protein yield did not differ ($P > 0.10$) for the W23 versus W23*sfe* contrast, the greater milk yield of the M04-21-fed cows resulted in greater protein yield ($P < 0.001$) than that in W23-fed cows. Lactose milk content did not differ ($P > 0.10$) between W23 and W23*sfe* diets, although

lactose concentration was lower ($P < 0.05$) for M04-21 diets, and lactose yield was greater ($P < 0.001$) for cows fed W23*sfe* silages. Milk urea nitrogen concentration was lower ($P < 0.001$) for cows fed the M04-21 silage (14.6 mg/dL) compared with the W23 diet (16.0 mg/dL). Cows fed B73 and B73*bm3* diets had similar ($P > 0.10$) DMI; however, we observed increased ($P < 0.01$) total milk production and 3.5% FCM for B73*bm3*-fed cows. Milk fat percentage did not differ ($P > 0.10$) for the B73 and B73*bm3* diets, but both protein and lactose concentrations were greater ($P < 0.001$) on B73*bm3* diets. Yield of all components was increased ($P < 0.05$) for B73*bm3*-fed cows. Cows fed the B73*bm3* diet had a lower ($P < 0.001$) MUN concentration compared with those fed the B73 diet (14.0 and 15.3 mg/dL, respectively). Dry matter intake and milk production were greater ($P < 0.001$) for cows fed the B73 silage compared with W23, and protein percentage was lower ($P < 0.001$) for B73-fed cows. Milk urea nitrogen concentration was higher ($P < 0.05$) for cows receiving the W23 silage diet than for those receiving B73.

Lactating cows had greater digestibility of DM ($P < 0.05$), starch ($P < 0.001$), and total cell wall polysaccharides ($P < 0.01$) on the W23*sfe* compared with W23 silage diets (Table 8). Digestibility of CP also trended higher ($P < 0.10$) for the W23*sfe* diets, although NDF digestibility did not differ ($P > 0.10$). However, digestibility of all nutrients was greater ($P < 0.001$) for cows fed the M04-21 silage diet compared with the W23-fed cows. Feeding B73*bm3* silage diets did not ($P > 0.10$) improve digestibility compared with B73 and a trend was observed for reduced ($P < 0.10$) CP digestibility by B73*bm3*-fed cows. The W23 genetic background resulted in lower digestibility of all nutrients than observed for cows fed the B73 silage.

Figure 1. Amount of NDF digested per day in the ad libitum and restricted phases of the lamb trial and by cows in the lactation trial. Significant differences among treatments are discussed in text. The experimental lines included the parental control line W23, 2 near-isogenic W23*sfe* mutant lines (M04-4 and M04-21), B73, and B73*bm3*.

Table 7. Feed intake and milk production and composition of Holstein cows fed diets containing silage from corn inbreds W23, W23*sfe* lines (M04-4 and M04-21), B73, and B73*bm3*

Corn line	DMI (kg/d)	Milk (kg/d)	3.5%FCM (kg/d)	Milk composition (%)			Component yield (kg/d)		
				Fat	Protein	Lactose	Fat	Protein	Lactose
W23	21.8	38.9	41.8	3.94	3.03	4.92	1.54	1.17	1.92
M04-4	23.4	39.3	43.0	4.08	2.86	4.99	1.60	1.12	1.96
M04-21	23.3	41.6	44.1	3.88	2.92	4.88	1.61	1.21	2.03
B73	31.4	43.1	45.3	3.81	2.82	4.90	1.64	1.21	2.11
B73 <i>bm3</i>	31.5	44.3	48.0	4.00	2.97	5.01	1.77	1.31	2.22
SEM	0.3	0.3	0.6	0.08	0.01	0.01	0.04	0.01	0.01
Contrasts									
W23 vs. W23 <i>sfe</i>	**	***	*	NS	***	NS	NS	NS	***
W23 vs. M04-21	*	***	*	NS	***	*	NS	***	***
B73 vs. B73 <i>bm3</i>	NS	**	**	NS	***	***	*	***	***
W23 vs. B73	***	***	***	NS	***	NS	*	***	***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS: $P > 0.10$.

Cows fed the TMR diets containing W23*sfe* silage were less ($P < 0.001$) selective for starch and less selective against NDF ($P < 0.05$) and Klason lignin ($P < 0.001$) in feed consumed than were cows fed the W23 silage TMR (Table 9). A trend was found for W23*sfe*-fed cows to be less ($P < 0.10$) selective against total cell wall material. These selectivity patterns for cows fed mixed diets were similar to those exhibited by the lambs fed only W23 and W23*sfe* silages. Although lambs fed the B73*bm3* silage were not more selective than those fed B73 silage, lactating cows were more selective for starch ($P < 0.001$) and less ($P < 0.001$) selective against total cell wall material, ADL, and Klason lignin when fed B73*bm3*. Cows were uniformly more ($P < 0.001$) selective when fed the W23 silage containing TMR than when fed the B73 diet.

Amount of NDF digested per day by the cows did not differ ($P > 0.10$) for the W23 versus W23*sfe* contrast; however, cows fed the M04-21 silage-containing diet digested more NDF per day than those fed the W23 diet

(Figure 1). Cows fed the B73 and B73*bm3* silages did not differ ($P > 0.10$) for amount of NDF digested daily.

DISCUSSION

Silage of the 2 near-isogenic W23*sfe* corn inbreds fed to lambs and cows had lower concentrations of ferulate ether cross-links than W23 silage. The reduction in ferulate ethers was of similar magnitude and in agreement with results for stover of these same lines evaluated in an agronomic plot trial (Jung and Phillips, 2010). The previous trial found higher IVNDFD and IVCWPD of the W23*sfe* stover, but the current in vitro rumen digestibility data for the silages only partly agree with these earlier results. Of the 2 W23*sfe* lines included in the current report, only silage from the line with the lower concentration of ferulate ethers (M04-21) consistently had higher in vitro cell wall polysaccharide digestibility than the W23 silage. Casler and coworkers (Casler and Jung, 1999; Casler et al., 2008)

Table 8. Digestibility of dietary nutrients by lactating Holstein cows fed diets containing silage from corn inbreds W23, W23*sfe* lines (M04-4 and M04-21), B73, and B73*bm3*

Corn line	Digestibility (%)				
	DM	CP	Starch	NDF	CWP ¹
W23	71.1	68.7	93.3	57.7	61.9
M04-4	69.1	65.1	93.7	56.3	64.3
M04-21	78.1	76.9	96.9	65.2	69.5
B73	79.4	78.7	97.4	62.0	73.4
B73 <i>bm3</i>	78.2	75.9	98.1	62.5	72.4
SEM	0.9	1.1	0.5	1.7	1.4
Contrasts					
W23 vs. W23 <i>sfe</i>	*	†	**	NS	**
W23 vs. M04-21	***	***	***	**	***
B73 vs. B73 <i>bm3</i>	NS	†	NS	NS	NS
W23 vs. B73	***	***	***	†	***

¹CWP = total cell wall polysaccharides.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS: $P > 0.10$.

Table 9. Nutrient selectivity by lactating Holstein cows fed diets containing silage from corn inbreds W23, W23*sfe* lines (M04-4 and M04-21), B73, and B73*bm3* during the week when the digestibility trial was conducted

Corn line	Selectivity index ¹					
	CP	Starch	NDF	Cell wall	Acid detergent lignin	Klason lignin
W23	1.014	1.030	0.977	0.979	0.943	0.918
M04-4	1.014	1.026	0.984	0.985	0.942	0.953
M04-21	1.014	1.015	0.976	0.968	0.942	0.928
B73	1.005	0.995	0.983	0.986	0.981	0.979
B73 <i>bm3</i>	1.006	1.004	0.985	1.000	0.971	0.995
SEM	0.001	0.001	0.001	0.001	0.003	0.003
Contrasts						
W23 vs. W23 <i>sfe</i>	NS	***	*	†	NS	***
W23 vs. M04-21	NS	***	NS	***	NS	*
B73 vs. B73 <i>bm3</i>	NS	***	NS	***	*	***
W23 vs. B73	***	***	***	***	***	***

¹Selectivity index was calculated as the composition of the feed actually consumed (offered minus refusal) divided by the composition of the feed offered. Values >1.00 indicate selection for a component; values <1.00 indicate selection against a component.

† $P < 0.10$; * $P < 0.05$; *** $P < 0.001$; NS: $P > 0.10$.

found negative correlations between the concentration of ferulate ether cross-links and IVNDFD for several perennial grasses; therefore, it would be expected that the corn inbred line (M04-21) with the lower ferulate ether cross-link concentration would exhibit the greater increase in digestibility. Given the relatively small increase in digestibility observed by Jung and Phillips (2010) for W23*sfe* lines in a highly replicated plot trial, it is not unexpected that similar small increases in digestibility were not evident for the nonreplicated silages. Jung and Phillips (2010) concluded that all of the improvement in W23*sfe* stover in vitro digestibility occurred during the first 24 h of incubation with rumen fluid, indicating that the effect of the *sfe* mutation was on the more rapidly digestible cell wall fraction. In vitro digestibility data for whole-plant corn silage generally supported this conclusion. The improvement for in vitro digestibility associated with the *bm3* mutation (Barriere and Argillier, 1993; Oba and Allen, 1999, 2000) was evident in the B73*bm3* silage.

One concern in interpreting the animal response data is that the silages differed in DM content. The higher DM of the W23 silage probably reflected a greater grain content of this line than for the W23*sfe* silages. Jung and Phillips (2010) reported that W23*sfe* lines produced more total biomass than W23 but that all of the extra biomass was in the stover fraction. Because corn grain typically dries faster than stover (Lauer, 2010), a lower grain:stover ratio in the W23*sfe* silages should slow preharvest whole-plant moisture loss. The greater starch and lower NDF and cell wall concentrations of the W23 silage than the W23*sfe* silages would be expected with a higher grain:stover ratio for the

W23 silage. Although silage DM and genetic line (W23 vs. W23*sfe*) were confounded, we have no reason to believe that the wetter W23*sfe* silages directly affected animal response.

As expected, the B73*bm3* silage had less lignin than did B73. Although Jung and Phillips (2010) found slightly reduced lignification of W23*sfe* stover compared with its control, a similar small reduction in lignin was not apparent for the silages in the current study. The lower *p*-coumarate ester concentration of B73*bm3* silage reflects the shift observed in lignin composition for the *bm3* mutation toward a lower syringyl:guaiacyl ratio because *p*-coumarates are specifically esterified to syringyl monolignol units (Ralph et al., 1994). No similar change in *p*-coumarate esters was observed or expected for the *sfe* mutant. Stems from *bm3* corn stover have a lower concentration of ferulate ether cross-links compared with isogenic controls (Goto et al., 1994; Lam et al., 1996), but very little difference was observed between the B73*bm3* and B73 silages in the current study.

Because this is the first report on the in vivo effect of reduced ferulate cross-linking in corn silage on animal performance, no literature is available for comparison. Direct comparisons between the W23*sfe* and B73*bm3* silages are difficult because the 2 mutations have different genetic backgrounds (W23 and B73, respectively). Extensive literature has documented that corn genetic lines differ markedly in composition and digestibility even in the absence of major mutations such as *bm3* (Wolf et al., 1993; Argillier and Barriere, 1996). Composition and in vitro digestibility data for the silages and animal response data from the 2 genetic backgrounds differed for numerous traits; therefore,

any differences between the *sfe* and *bm3* mutations cannot be separated from genetic background effects. By necessity, our discussion of the results for the animal trials will limit comparisons between the *sfe* and *bm3* mutations to subjective comments rather than statistical differences.

Cows fed the W23*sfe* silages as part of a TMR exhibited greater DMI and milk production. Contrary to previous results (Oba and Allen, 1999; Tine et al., 2001), we did not observe an increase in DMI for cows fed the B73*bm3* mutant silage compared with B73. However, 3.5% FCM production was improved 6.0% for the B73*bm3* silage diet relative to the B73 control and this increase was similar (6.8%) to that reported earlier (Oba and Allen, 1999). The increases in 3.5% FCM production for the *sfe* mutant were 2.9 and 5.5% for the M04-4 and M04-21 silages, respectively. The improvement in FCM for the M04-21 silage diet was similar in magnitude to the increase observed for the B73*bm3* silage in the trial. Although cows fed the B73*bm3* diet produced 8.8% more FCM than cows fed the M04-21 silage, this difference can be attributed to differences between the 2 corn genetic backgrounds, not the 2 mutations per se, because the B73 diet supported 8.4% greater milk production compared with the W23 silage. Neither mutation altered milk fat concentration; however, the W23*sfe* silages reduced protein percentage, whereas B73*bm3* increased protein percentage. The W23*sfe* silages did not have a consistent effect on lactose concentration, whereas B73*bm3* increased lactose percentage. These milk composition shifts for the B73*bm3* silage match the pattern reported by Oba and Allen (1999) for *bm3* corn silage.

In vitro digestibility of cell wall material has consistently been shown to increase when the *bm3* mutation is incorporated into different corn genetic backgrounds (Mechin et al., 1998). Although increased in vivo cell wall digestibility is often observed for *bm3* corn silage (Muller et al., 1972; Tjardes et al., 2000; Tine et al., 2001), such a positive digestibility response is not always the case (Rook et al., 1977; Oba and Allen, 2000). In the current trial we did not detect changes in the digestion coefficients for cell wall material or amount of NDF digested daily by cows fed the B73*bm3* diet; however, digestibility of cell wall polysaccharides was greater for the W23*sfe* diets. Again, the greatest improvement in cell wall digestibility was seen for the M04-21 silage, which also resulted in a greater amount of NDF digested per day. The lack of a response for the B73*bm3* silage was puzzling, particularly because DMI was also not different for this silage and its control. Oba and Allen (1999) theorized that cows fed the *bm3* silage-containing diet in their study did not exhibit greater in vivo NDF digestibility because DMI was

increased on the *bm3* diet and a negative relationship existed between DMI and NDF digestibility.

Lambs fed the W23*sfe* silages did not exhibit greater cell wall digestibility relative to their respective control silages, although the amount of NDF digested per day was greater for the M04-21 silage in the ad libitum phase only. This result was surprising given the improvements in IVNDFD and IVCWPD found for the W23*sfe* silages and the previous report on W23*sfe* stover (Jung and Phillips, 2010). Of particular concern was the lack of an increase in digestibility for the B73*bm3* silage when the *bm3* mutation has consistently been shown to improve NDF digestibility by lambs and cattle at low levels of feed intake (Muller et al., 1972; Weller and Phipps, 1986; Tjardes et al., 2000; Tine et al., 2001). Clearly, some factor(s) in this digestion trial was preventing the lambs from achieving the higher NDF digestibility reasonably expected for the B73*bm3* silage and, possibly, the W23*sfe* silages. One possible explanation for the lack of a positive response by lambs to the *sfe* mutant may relate to selectivity of the silage consumed.

Although selectivity differed little between lambs fed B73 and B73*bm3* silages, the W23*sfe*-fed lambs clearly consumed a diet richer in cell wall material compared with the lambs fed the W23 silage. Consumption of forages with higher concentrations of cell wall material generally results in lower total DM digestibility because non-cell-wall nutrients are more digestible than cell walls (Van Soest, 1994), and cell wall digestibility declines because the degree of cell wall lignification is usually greater as forages accumulate higher concentrations of cell wall (Jung and Deetz, 1993). Therefore, the lower DM and cell wall digestibility observed for lambs fed W23*sfe* silages ad libitum compared with W23 was perhaps expected given the small degree of improvement in IVNDFD and IVCWPD for the *sfe* mutant (Jung and Phillips, 2010) and the consumption of W23*sfe* silage with more cell wall material than was consumed by lambs fed W23 silage. Reduced in vivo DM and cell wall digestibility was not observed for W23*sfe* in the restricted intake phase of the lamb trial. An increase in digestibility would be expected at restricted intake due to the longer rumen retention time (Oba and Allen, 1999); however, lambs were even less selective against cell wall material in W23*sfe* silage consumed in the restricted phase of the trial. It remains unclear why cell wall digestibility was not increased for the B73*bm3* silage in the lamb trial because we observed little difference in selectivity of lambs fed the B73 and B73*bm3* silages. Similar to lambs fed the W23*sfe* silages, lactating cows fed W23*sfe* diets were also less selective against cell wall material than those fed the W23 diet. Unlike lambs fed the B73*bm3* silage, which showed no change in selectivity, cows fed a B73*bm3* TMR were

less selective against cell wall material than cows on the B73 silage. The cause for the reduced selectivity of cows fed the 2 corn silage mutants is unknown.

It has been suggested that diferulate cross-linking of Chinese water chestnut cell wall polymers contributes to the maintenance of crispness even when cooked (Parker et al., 2003). The W23*sfe* lines have recently been found to contain lower concentrations of both ester-only and ester/ether linked diferulates (R. D. Hatfield, USDA-ARS, Madison, WI, and H. G. Jung, unpublished data). Perhaps the lower concentration of diferulate cross-links and ferulate ether cross-links cause the *sfe* mutant to be more fragile, resulting in greater particle size breakdown during silage chopping or mastication. Rupture of lignified tissues is essential for exposure of potentially digestible interior cell wall surfaces to microbial attack (Jung et al., 2000; Jung and Engels, 2001). Unfortunately, data were not collected in the current trials on silage particle size after chopping or particle size breakdown during and after ingestion. However, the observation that lambs fed the W23*sfe* silages spent less total time chewing per gram of NDF consumed may indicate greater fragility due to the *sfe* mutation.

CONCLUSIONS

Silage of the *sfe* corn mutant had reduced ferulate ether cross-linking and higher in vitro cell wall digestibility, and resulted in increased DMI and milk production by lactating cows. In vivo digestibility of cell walls by cows was greater on *sfe* corn silage-containing diets, but not by lambs fed *sfe* silages at a restricted level of feed intake. Both cows and lambs were less selective against cell wall material when fed *sfe* silages, which may have contributed to the lack of an in vivo digestibility response by the lambs. Surprisingly, the low-lignin *bm3* corn silage was not more digestible by lambs or cows, and did not increase DMI by cows. Milk production was greater on *bm3* diets, but the source of the extra nutrients to support the additional milk is unknown. The relative magnitude of the improved milk production for the *sfe* and *bm3* mutants was similar. Combining *sfe* and *bm3* mutations in the same corn line may provide even greater improvements in animal performance because these mutations involve different genes and appear to have different modes of action (ferulate cross-linking and lignin concentration).

ACKNOWLEDGMENTS

The authors thank Wilfred Vermerris (University of Florida, Gainesville) for providing the seed of the B73 and B73*bm3* inbred corn lines, Richard Jeo (USDA-ARS, Madison, WI) and many undergraduates for

thousands of hand pollinations to increase seed supplies of the 5 inbred lines and laboratory analysis of samples at St. Paul, Minnesota, and staff at the US Dairy Forage Research Center (Madison, WI) for producing the corn silages and for animal care. This research was funded by the USDA-Agricultural Research Service.

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