Conservation, fiber digestibility, and nutritive value of corn harvested at 2 cutting heights and ensiled with fibrolytic enzymes, either alone or with a ferulic acid esterase-producing inoculant

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ABSTRACT

The aim of this study was to determine the effects of the use of a fibrolytic enzyme product, applied at ensiling either alone or in combination with a ferulic acid esterase-producing bacterial additive, on the chemical composition, conservation characteristics, and in vitro degradability of corn silage harvested at either conventional or high cutting height. Triplicate samples of corn were harvested to leave stubble of either a conventional (15 cm; NC) or high (45 cm; HC) height above ground. Sub-samples of chopped herbage were ensiled untreated or with a fibrolytic enzyme product containing xylanases and cellulases applied either alone (ENZ) or in combination with a ferulic acid esterase-producing silage inoculant (ENZ+FAEI). The fibrolytic enzyme treatment was applied at 2 mL of enzyme product/kg of herbage dry matter (DM), and the inoculant was applied at 1.3×10^5 cfu/g of fresh herbage. Samples were packed into laboratory-scale silos, stored for 7, 28, or 70 d, and analyzed for fermentation characteristics, and samples ensiled for 70 d were also analyzed for DM losses, chemical composition, and in vitro ruminal degradability. After 70 d of ensiling, the fermentation characteristics of corn silages were generally unaffected by cutting height, whereas the neutral detergent fiber, acid detergent fiber, and ash concentrations were lower and the starch concentration greater for silages made with crops harvested at HC compared with NC. After 70 d of ensiling, the acetic acid, ethanol concentrations, and the number of yeasts were greater, and the pH and neutral detergent fiber concentrations were lower, in silages produced using ENZ or ENZ+FAEI than the untreated silages, whereas ENZ+FAEI silages also incurred higher DM losses. No effect of additive treatment was observed on in vitro degradability indices after 48 h ruminal incubation. The use of a fibrolytic enzyme product, either alone or in combination with a ferulic acid esterase-producing inoculant, at ensiling

did not improve corn silage fermentation or its nutritive value and resulted in some negative effects on these parameters. The effects of using a fibrolytic enzyme product at ensiling, either alone or in combination with a ferulic acid esterase-producing inoculant, did not differ between corn harvested at either NC or HC. Silage made from HC had a greater starch content and lower fiber content than NC silage, whereas cutting height did not affect the in vitro digestibility indices.

Key words: corn, fibrolytic enzyme, ferulic acid esterase, silage, additive

INTRODUCTION

Feed contributes a major input cost in animal production systems (Archer et al., 1999), and because grain prices are expected to remain high in coming years (Guyomard et al., 2013), the use of the forage components of ruminant diets needs to be maximized to improve the efficiency of livestock production systems. A primary limiting factor of forage utilization is fiber digestibility, with potentially under 600 g/kg of fiber available for digestion by the ruminant animal (Van Soest, 1994).

Previous studies have observed the potential benefits of the use of feed additives containing fibrolytic enzymes, primarily consisting of xylanases and cellulases, to improve the fiber digestibility of corn silage (Colombatto et al., 2004; Phakachod et al., 2013) and other forages (Pinos-Rodríguez et al., 2002; Eun and Beauchemin, 2008; Elghandour et al., 2013) when applied to the feed just before feeding. However, the application of fibrolytic enzymes before the ensilage of corn may facilitate an increased duration of interaction between the forage and the applied enzymes, potentially allowing for a greater impact on the nutritive value of the subsequent silage. Furthermore, silage inoculants that contain ferulic acid esterase-producing bacteria have the potential to improve the nutritive value of corn silage and other preserved feeds through the degradation of the linkages between lignin and cell wall carbohydrates (Kang et al., 2009; Addah et al., 2012). Therefore, the application of a silage inoculant containing ferulic acid esterase-

Received August 21, 2014.

Accepted October 8, 2014.

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producing bacteria to corn silage in combination with exogenous fibrolytic enzymes may increase the opportunity for the hydrolysis of cell wall carbohydrates and enable a greater improvement in forage nutritive value than silages produced using fibrolytic enzyme products alone.

Previous studies indicate that the effects of fibrolytic enzymes on the nutritive value of forages can be highly variable, depending on a range of factors including plant variety, harvest maturity, and forage chemical composition (Sheperd and Kung, 1996; Pinos-Rodríguez et al., 2002; Elghandour et al., 2013). Corn silage consists of 2 contrasting components, the high-starch ear and the high-fiber stover. Harvesting the crop at a higher cutting height can improve the nutritive value, and alter the chemical composition, of the resultant silage through increasing the proportion of cob and decreasing the proportion of stover in the harvested herbage (Neylon and Kung, 2003; Caetano et al., 2011). Therefore, the magnitude of the effects of fibrolytic enzyme additives on the nutritive value of corn, and its subsequent preservation, harvested at contrasting cutting heights may differ, due to the contrasting chemical composition of the ensiled forage. Furthermore, the degree of potential enzyme additive effects on these herbage may be enhanced by the use of a ferulic acid esterase inoculant, as the degradation of linkages between lignin and cell wall carbohydrates can increase the potential substrate for fibrolytic enzyme hydrolysis.

The objective of this study was to determine the effect of the use of a fibrolytic enzyme product produced for ruminants, applied at ensiling either alone or in combination with a ferulic acid esterase-producing bacterial additive, on the chemical composition, conservation characteristics, and degradability of corn silage harvested at either a conventional or high cutting height.

MATERIALS AND METHODS

Triplicate samples of corn harvested at either a conventional or high cutting height were allocated to 1 of 3 additive treatments and ensiled in laboratory-scale silos for 1 of 3 durations of ensiling ($n = 54$ samples). The silage fermentation profile of samples was investigated after each ensiling duration, whereas the estimated nutritive value, *in vitro* digestibility, and aerobic stability were assessed after only 70 d of ensiling.

Forage Preparation

Forage corn (*Zea mays* L.; hybrid: 39T67; Pioneer Hi-Bred Ltd., Chatham, ON, Canada) was grown on a commercial farm in Iron Springs, Alberta, Canada

(49°56' N, 6°39' W) in 2012. On September 27, for each of 2 plots (6 rows wide, 10 m long) allocated to either a normal cutting height (NC) or high cutting height (HC) in 3 replicate blocks, plots of corn were harvested at a target DM content of 300 g of fresh forage/kg using a CLAAS Jaguar 960 corn harvester (CLAAS of America, Omaha, NE) fitted with a corn processor and chopped to approximately 0.9 cm theoretical chop length. Plots allocated to NC were harvested to leave 15 cm of stubble above ground, whereas plots allocated to HC were harvested to leave 45 cm of stubble. For each plot, chopped forage was transported to Lethbridge Research Centre, Alberta, within 30 min of harvest, placed on a shaded polyethylene sheet, and mixed thoroughly. Subsequently, three 20-kg sub-samples were randomly taken, placed into pails, allocated to 1 of 3 additive treatments, and stored in a cool shaded area until ensiling.

Ensilage

Each 20-kg sub-sample was placed onto a polyethylene sheet, sprayed with half of the allocated treatment, mixed thoroughly, sprayed with the remaining treatment, and mixed a second time. Three treatments were used for this study consisting of either an untreated control of distilled water or a commercial enzyme product (ENZ; 75:25 mix of Cellulase PLUS and Xylanase PLUS, respectively; Dyadic International, Jupiter, FL), applied alone or in combination with a ferulic acid esterase-producing inoculant (ENZ+FAEI; Pioneer 11GFT, Pioneer Hi-Bred Ltd.). The xylanase activity of ENZ was 7,527 xylose equivalents·min⁻¹·mL⁻¹ of enzyme, whereas the endoglucanase activity of ENZ was 420 μmol of glucose equivalents·min⁻¹·mL⁻¹ of enzyme. The ENZ was applied at a rate of 2 mL/kg of forage DM, whereas the bacterial inoculant was applied at 1 g/1,000 kg of fresh forage to achieve 1.3×10^5 cfu/g of fresh forage for the ENZ+FAEI treatment. The inoculant contained a mixed bacterial culture of 1.0×10^{11} cfu/g of *Lactobacillus buchneri* LN4017 (ATCC no. PTA-6138) that produced ferulic acid esterase, 2.0×10^{10} cfu/g of *Lactobacillus plantarum* LP7109 (ATCC no. PTA-6139) and 1.0×10^{10} cfu/g of *Lactobacillus casei* LC3200 (ATCC no. PTA-6135). All treatments were diluted in distilled water and applied using a total volume of 3 mL/kg of fresh forage. Aseptic techniques were observed throughout ensiling to prevent contamination of samples. A 500-g sample of herbage was taken from each pile following treatment for microbial and chemical composition analyses.

Approximately 2.3 kg of herbage was packed into laboratory-scale polyvinyl chloride silos (10.4 cm diameter $\times$ 35.6 cm height) using a hydraulic press to

achieve a density of approximately 760 kg of fresh weight/m³. Four laboratory silos were filled per 20-kg pile, to evaluate samples after 7, 28, and 70 d of ensiling. Two laboratory silos were produced for 70 d of ensiling, which were pooled upon silo opening to create a larger sample for subsequent analyses. Therefore, 72 silos were produced in total. Silos were sealed airtight with rubber caps fastened by metal ties, whereas gas was released manually with a valve daily for the first 21 d of ensiling, and then weekly until gas pressure was no longer evident. Silos were weighed empty and filled, and stored at 20°C for the ensilage period. Following each silo's allocated ensiling duration, gas was released and silos were weighed before opening. Silage was thoroughly mixed and subsampled for microbial and chemical analyses.

Chemical Analysis

The DM content of samples was determined by drying at 55°C in a forced-air oven for 48 h. Fresh-cut samples and samples ensiled for 70 d were lyophilized and ground through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) for CP, starch, NDF, ADF, ash, and *in vitro* digestibility analyses. To determine CP ($N \times 6.25$) and starch, ground feed samples were further ground to a fine powder using a ball grinder (Mixer Mill MM200, Retsch Inc., Newtown, PA). Nitrogen was quantified by flash combustion with gas chromatography and thermal conductivity detection (Nitrogen Analyzer 1500 series, Carlo Erba Instruments, Milan, Italy). Starch was determined after hydrolysis to α -glucose polymers using amyloglucosidase (Megazyme Int. Ltd., Wicklow, Ireland) and 1,4 α -D-glucan glucano-hydrolase (Brennfag Canada Inc., Toronto, ON, Canada) as described by Herrera-Saldana et al. (1990). Samples were read on a micro-plate reader (Appliskan, Thermo Electron Corporation, Vantaa, Finland) at a wavelength of 490 nm. The NDF analysis was conducted according to the AOAC Official First Action method (Mertens, 2002) with the inclusion of heat-tolerant α -amylase and sodium sulfite, and ADF was determined according to the AOAC International (2005; method 973.18). Both procedures were modified for use with the Ankom filter bag technique (Ankom Technology, Macedon, NY). The procedures were conducted sequentially and values are expressed on an ash-inclusive basis. Ash concentration was determined by complete combustion in a muffle furnace (Thermo Fisher Scientific, Rochford, UK) at 550°C for 5 h. Buffering capacity was analyzed using the method of Playne and McDonald (1966).

Aqueous extracts were obtained from all ensiled samples by blending 15 g of fresh forage or silage with

135 mL of distilled water in a Waring blender (Waring Commercial, Torrington, CT) for 30 s at full speed, and subsequently filtering through 2 layers of cheesecloth. The pH of the filtrate was determined using a Symphony pH meter (VWR, Mississauga, ON, Canada). For determination of VFA and ethanol, 1.5 mL of the supernatant was deproteinized with 0.3 mL of 25% (wt/vol) metaphosphoric acid, combined with 0.2 mL of 0.1 M crotonic acid as internal standard, and analyzed on a Hewlett Packard model 6890N gas-liquid chromatograph (Agilent Technologies Inc., Santa Clara, CA) with a ZB-FFAP fused silica capillary column (30 m $\times$ 0.32 mm i.d. and 1.0 m film thickness; Phenomenex, Torrance, CA) and a flame ionization detector. For lactic acid determination, 400 μ L of the deproteinized sample was combined with 50 μ L of malonic acid (5 mg/mL) as an internal standard. Lactic acid was methylated with BF₃-MeOH then quantified using the method of Kudo et al. (1987) on the same column and chromatograph as were used for VFA analysis. Total fermentation product was calculated as the sum of the VFA, lactic acid, and ethanol concentrations. To determine ammonia nitrogen, 1.6 mL of the supernatant was combined with 0.15 mL of 65% (wt/vol) trichloroacetic acid and analyzed by the salicylate method (Kempers and Zweers, 1986). The water-soluble carbohydrate (WSC; glucose equivalent) concentration was determined by the Nelson-Somogyi (Nelson, 1944) method using a Dynatech MRX micro-plate reader (Dynatech Laboratories Inc., Chantilly, VA) at 630 nm.

In Vitro Digestibility Analysis

The *in vitro* digestibility procedure used was based on that described by Colombatto et al. (2003) and Eun et al. (2007). The procedure was conducted on 2 separate occasions (i.e., 2 replicate runs). Approximately 0.7 g of DM was weighed into filter bags (F57, Ankom Technology, Macedon, NY) that were previously washed with acetone, dried at 55°C for 24 h and weighed. Four replications of each sample were prepared for both incubation durations (24 and 48 h) to reduce analytical error. Bags were heat-sealed, and a single bag was placed in a 125-mL capacity serum vial (Wheaton Scientific, Millville, NJ). Rumenal fluid was obtained 2 h after feeding from 2 ruminally cannulated, nonlactating Angus cows fed a diet that consisted of barley silage (600 g/kg of DM), barley grain (350 g/kg of DM) and a vitamin-mineral supplement (50 g/kg of DM). Rumen fluid was obtained from various locations within the rumen, composited, and strained through 2 layers of cheesecloth under CO₂ and was immediately transported to the laboratory, and kept at 39°C in a water bath while continually flushed with CO₂.

Anaerobic buffer medium (pH 6.8) was prepared as described by Eun et al. (2007). Vials were flushed with CO₂ before the addition of 48 mL of anaerobic buffer medium and 12 mL of rumen fluid. Vials were sealed and crimped with aluminum caps. Negative controls that contained buffer medium, rumen fluid, and an empty filter bag were also inoculated in 4 replications for each incubation duration. Inoculation of vials was complete within 1 h of rumen fluid collection and vials were incubated at 39°C for either 24 or 48 h. Headspace gas production (**GP**) was measured after 3, 7, 19, 24, and 48 h of incubation. Measurements were made using a 23-gauge (0.6 mm) needle attached to a pressure transducer (model PX4200-015GI, Omega Engineering Inc., Laval, QC, Canada) connected to a visual display (Data Track, Christchurch, UK). Following measurement, the needle was left in place until gas pressure was completely released and then removed. Pressure values were corrected for values from negative controls and the amount of substrate DM incubated and subsequently used to generate volume estimates using the quadratic equation described by Mauricio et al. (1999). After each incubation duration, the appropriate vials were removed, placed in a water bath at 4°C to stop fermentation, and the filter bag was removed. Filter bags were hand washed under cold water until excess water ran clear, dried at 55°C for 24 h, and weighed to determine DM degradability (**DMD**). The **NDF** and **ADF** digestibility (**NDFD** and **ADFD**, respectively) were determined by sequential analysis of the digested sample and filter bag using the methods described previously.

Microbial Analysis

Forage and silage samples were initially stored at 4°C, and the isolation of microbes from the herbage commenced within 2 h of silo opening. Samples (10 g) were added to 90 mL of sterile 70 mM potassium phosphate buffer and homogenized for 60 s at 260 rpm in a Stomacher 400 Laboratory Blender (Seward Medical Limited, London, UK), with a subsequent 1-mL aliquot serially diluted in potassium phosphate buffer. Media consisting of de Man, Rogosa, and Sharpe (Oxoid, Basingstoke, UK) and cycloheximide (200 µg/mL; Sigma-Aldrich, St. Louis, MO) were used for the enumeration of lactic acid bacteria (**LAB**), and nutrient agar (Difco, Detroit, MI) amended with cycloheximide (200 µg/mL; Sigma-Aldrich) was used for enumeration of total aerobic culturable bacteria. The de Man, Rogosa, and Sharpe and nutrient agar plates were incubated at 37°C for 48 h. Yeasts and molds were enumerated with Sabouraud's dextrose agar (Difco) amended with each of tetracycline and chloramphenicol (100 µg/mL; Sigma-

Aldrich) and were incubated at 20°C for 72 h. Colonies were counted from plates containing a minimum of 30 and a maximum of 300 colonies.

Experimental Design and Statistical Analysis

Data to assess the nutritive value of samples taken before ensilage (i.e., d 0 samples) were subjected to a 1-way ANOVA, whereas all other data were subjected to an analysis as a split-plot design using a model that accounted for cutting height ($n = 2$) as the main plot, and additive treatment ($n = 3$) as the sub-plot, their interactions, and replication. The ANOVA was performed separately for different ensiling durations (i.e., 7, 28, and 70 d). All procedures were performed with the PROC MIXED procedure of the SAS statistical program (SAS Institute, 2002). Data from the in vitro digestibility assays were similarly analyzed with the inclusion of in vitro digestibility run as a random factor. Data for microbial population numbers were transformed by $\log_{10}$. The significance of specific treatment differences was calculated using the Fisher's least significant differences test. Treatment differences and trends were declared significant at $P \leq 0.05$ and $P \leq 0.10$, respectively.

RESULTS

Pre-ensiling

Crops harvested at HC had lower ($P < 0.05$; Table 1) **ADF** and ash concentrations and total bacteria population numbers than crops harvested at NC. In addition, crops harvested at HC tended to have greater **DM** ($P = 0.073$) concentration and buffering capacity ($P = 0.060$), and lower **NDF** concentration ($P = 0.062$) compared with NC, whereas the starch, **WSC**, and **CP** concentrations and **LAB**, yeasts, and mold population numbers were unaffected ($P > 0.05$) by crop cutting height.

Fermentation—Cutting Height

After 7 and 28 d of ensiling, the lactic acid, acetic acid, ethanol, and total fermentation product concentrations, proportion of lactic acid in the total fermentation products, and **LAB** and yeast (only measured after 28 d of ensiling) population numbers were unaffected ($P > 0.05$) by corn cutting height (Tables 2 and 3), whereas silages made from crops harvested at HC tended to have a greater ($P < 0.063$) pH value than crops harvested at NC. Propionic acid and butyric acid were below detectable values in all samples.

Table 1. Chemical composition of corn, harvested at a normal (NC) or high (HC) cutting height at ensiling (n = 3)

Item	NC	HC	SEM ¹	P-value
DM (g/kg of fresh forage)	318	361	12.4	0.073
Buffering capacity (mEq/kg of DM)	115	135	5.4	0.060
NDF (g/kg of DM)	484	469	4.1	0.062
ADF (g/kg of DM)	257	233	4.2	0.017
Starch (g/kg of DM)	280	313	21.9	0.200
Water-soluble carbohydrates (g/kg of DM)	25	20	2.1	0.167
CP (g/kg of DM)	85	88	1.8	0.389
Ash (g/kg of DM)	49	46	0.7	0.024
Lactic acid bacteria (log ₁₀ cfu of fresh forage/g)	6.3	6.1	0.28	0.403
Total culturable bacteria (log ₁₀ cfu of fresh forage/g)	7.65	7.39	0.065	0.049
Yeasts (log ₁₀ cfu of fresh forage/g)	6.49	6.49	0.140	0.983
Molds (log ₁₀ cfu of fresh forage/g)	5.25	5.36	0.219	0.624

Similarly after 70 d of ensiling the fermentation characteristics of corn silages were generally unaffected ($P > 0.05$) by cutting height (Table 4); however, the pH ($P = 0.071$) and yeast population numbers ($P = 0.055$) tended to be greater in HC silages than NC.

Fermentation—Additive Treatment

After 7 d of ensiling, the fermentation characteristics of corn silages were generally unaffected ($P > 0.05$) by additive treatment; however, the LAB population numbers for HC silages treated with ENZ+FAEI were lower ($P < 0.01$) than other HC silages (Table 2). When averaged across cutting height, the lactic acid and total fermentation product concentration of corn silages

produced using ENZ+FAEI were greater ($P < 0.05$) than untreated silages after 28 d of ensiling, whereas the acetic acid and ethanol concentrations and yeast population numbers were greater ($P < 0.05$) for silage made using either ENZ alone or ENZ+FAEI when compared with untreated silages (Table 3). In addition, the proportion of lactic acid in the total fermentation products was lower ($P < 0.05$) in silages produced using ENZ alone compared with the other silages after 28 d of ensiling, when averaged across cutting heights. The pH of corn silages produced with ENZ or ENZ+FAEI was greater ($P = 0.001$) than untreated silages, whereas the yeast population numbers of corn silages produced with ENZ or ENZ+FAEI were higher ($P > 0.001$) than untreated silages, after 28 d of ensiling.

Table 2. Fermentation characteristics after 7 d of ensilage for corn silage harvested at a normal (NC) or high (HC) cutting height and treated with a fibrolytic enzyme (ENZ) and ferulic acid esterase-producing inoculant (FAEI)

Item	pH	Lactic ¹	Acetic ¹	Ethanol ¹	TFP ¹	LA/TFP ²	LAB ³
Treatment							
Cut							
NC	3.84	44	10	4.6	58	744	9.18
HC	3.95	32	10	3.7	45	705	9.14
Additive							
None	3.92	34	9	3.6	46	729	9.16
ENZ	3.91	39	10	4.7	53	715	9.18
ENZ+FAEI	3.87	41	11	4.2	56	731	9.14
Cut Additive							
NC None	3.86	38	9	3.3	50	755	9.15 ^a
NC ENZ	3.84	48	10	5.8	63	747	9.17 ^a
NC ENZ+FAEI	3.83	45	12	4.7	62	729	9.22 ^a
HC None	3.98	29	9	3.8	42	702	9.16 ^a
HC ENZ	3.97	30	10	3.6	43	682	9.19 ^a
HC ENZ+FAEI	3.90	36	10	3.7	49	732	9.06 ^b
SEM	0.033	4.9	0.752	1.59	5.99	21.0	0.025
Significance							
Cut	0.061	0.094	0.275	0.533	0.097	0.177	0.141
Additive	0.263	0.414	0.056	0.734	0.279	0.698	0.215
Cut × additive	0.670	0.563	0.158	0.665	0.598	0.245	0.005

^{a,b}Means (n = 3) within a column and category with different superscripts differ ($P < 0.05$).

¹g/kg of DM; TFP = total fermentation products (lactic acid + volatile fatty acids + ethanol).

²g of lactic acid/g of total fermentation products.

³LAB = lactic acid bacteria, log₁₀ (cfu/g of wet silage).

Table 3. Fermentation characteristics after 28 d of ensilage for corn silage harvested at a normal (NC) or high (HC) cutting height and treated with a fibrolytic enzyme (ENZ) and ferulic acid esterase-producing inoculant (FAEI)

Item		pH	Lactic ¹	Acetic ¹	Ethanol ¹	TFP ¹	LA/TFP ²	LAB ³	Yeasts ³
Treatment									
Cut									
	NC	3.75	50	15	5.9	70	701	8.87	4.77
	HC	3.85	41	13	4.3	58	702	8.85	5.11
Additive									
	None	3.85 ^a	39 ^b	12 ^b	3.6 ^b	54 ^b	722 ^a	8.84	4.18 ^b
	ENZ	3.78 ^b	45 ^{ab}	15 ^a	6.4 ^a	65 ^{ab}	673 ^b	8.81	5.19 ^a
	ENZ+FAEI	3.77 ^b	53 ^a	15 ^a	5.4 ^a	73 ^a	711 ^a	8.93	5.46 ^a
Cut Additive									
	NC	3.80	42	12	4.5	58	717	8.76	3.90
	NC	3.74	50	16	7.6	73	673	8.85	5.19
	NC	3.72	57	16	5.5	78	714	8.99	5.21
	HC	3.90	36	11	2.6	50	727	8.92	4.45
	HC	3.82	39	14	5.1	57	672	8.77	5.19
	HC	3.82	48	14	5.3	67	707	8.87	5.70
SEM		0.021	6.4	1.1	1.53	7.5	21.0	0.080	0.202
Significance									
	Cut	0.063	0.300	0.169	0.517	0.238	0.961	0.806	0.285
	Additive	0.001	0.040	0.020	0.002	0.015	0.037	0.170	<0.001
	Cut × additive	0.670	0.817	0.786	0.120	0.758	0.880	0.103	0.136

^{a,b}Means (n = 3) within a column with different superscripts differ ($P < 0.05$).

¹g/kg of DM; TFP = total fermentation products (lactic acid + volatile fatty acids + ethanol).

²g of lactic acid/g of total fermentation products.

³LAB = lactic acid bacteria, log₁₀ (cfu/g of wet silage).

After 70 d of ensiling, the acetic acid and ethanol concentrations and yeast population numbers were greater ($P < 0.05$) and the pH was lower ($P < 0.01$) in silages produced using either ENZ or ENZ+FAEI,

when averaged across cutting height (Table 4). In addition, the total fermentation product concentration and DM losses of silages produced using ENZ+FAEI was greater ($P < 0.05$) than untreated silages, when

Table 4. Fermentation characteristics after 70 d of ensilage for corn silage harvested at a normal (NC) or high (HC) cutting height and treated with a fibrolytic enzyme (ENZ) and ferulic acid esterase-producing inoculant (FAEI)

Item		pH	Lactic ¹	Acetic ¹	Ethanol ¹	TFP ¹	LA/TFP ²	LAB ³	Total bacteria ³	Yeast ³
Treatment										
Cut										
	NC	3.56	52	22	5.7	80	654	8.59	8.63	3.32
	HC	3.67	46	18	7.3	72	632	8.74	8.84	4.07
Additive										
	None	3.69 ^a	45	17 ^b	3.7 ^b	65 ^b	682	8.68 ^a	8.73	2.75 ^b
	ENZ	3.58 ^b	44	22 ^a	7.0 ^{ab}	73 ^{ab}	600	8.58 ^b	8.68	4.13 ^a
	ENZ+FAEI	3.59 ^b	60	22 ^a	8.9 ^a	90 ^a	648	8.73 ^a	8.80	4.21 ^a
Cut Additive										
	NC	3.62	50	17	3.1	70	712	8.58	8.50	2.55
	NC	3.53	51	25	6.5	83	619	8.55	8.63	3.62
	NC	3.54	55	24	7.5	86	632	8.63	8.76	3.79
	HC	3.75	39	16	4.2	59	652	8.78	8.96	2.94
	HC	3.62	36	19	7.5	62	580	8.60	8.72	4.63
	HC	3.64	64	20	10.2	94	664	8.83	8.83	4.63
SEM		0.026	7.6	2.6	1.109	6.7	43.6	0.072	0.272	0.225
Significance										
	Cut	0.071	0.433	0.272	0.224	0.283	0.544	0.656	0.636	0.055
	Additive	0.001	0.080	0.032	0.005	0.012	0.149	0.042	0.444	<0.001
	Cut × additive	0.717	0.232	0.309	0.725	0.151	0.471	0.315	0.106	0.404

^{a,b}Means (n = 3) within a column with different superscripts differ ($P < 0.05$).

¹g/kg of DM; TFP = total fermentation products (lactic acid + volatile fatty acids + ethanol).

²g of lactic acid/g of total fermentation products.

³LAB = lactic acid bacteria, log₁₀ (cfu/g of wet silage).

Table 5. Chemical composition, and DM losses after 70 d of ensilage for corn silage harvested at a normal (NC) or high (HC) cutting height and treated with a fibrolytic enzyme (ENZ) and ferulic acid esterase-producing inoculant (FAEI)

Item		DM ¹	NDF ²	ADF ²	WSC ²	Starch ²	CP ²	Ash ²	Ammonia-N ³	DM loss ⁴
Treatment										
Cut										
NC		311 ^a	437	238 ^a	9	259 ^b	87	52 ^a	63	61
HC		355 ^b	403	205 ^b	12	296 ^a	83	46 ^b	63	62
Additive										
None		331	456 ^a	244 ^a	8 ^b	269	85	50	63	31 ^a
ENZ		334	405 ^b	214 ^b	11 ^a	279	84	49	65	55 ^a
ENZ+FAEI		334	400 ^b	208 ^b	12 ^a	285	87	48	62	99 ^b
Cut Additive										
NC	None	307	477	260	7.7	252	86	52	63	37
NC	ENZ	310	414	227	9.2	267	88	54	62	42
NC	ENZ+FAEI	315	420	227	9.2	259	86	50	63	105
HC	None	354	435	227	7.8	286	83	48	62	25
HC	ENZ	359	395	200	12.7	291	80	44	67	68
HC	ENZ+FAEI	352	380	189	14.8	311	87	45	60	92
SEM		6.93	10.3	5.5	0.99	15.8	2.9	1.3	2.7	21.0
Significance										
Cut		0.034	0.056	0.024	0.061	0.002	0.227	0.024	0.923	0.991
Additive		0.676	0.001	<0.001	0.006	0.390	0.796	0.228	0.543	0.020
Cut × additive		0.441	0.498	0.619	0.066	0.483	0.313	0.092	0.330	0.514

^{a,b}Means (n = 3) within a column with different superscripts differ ($P < 0.05$).

¹g/kg of fresh forage.

²g/kg of DM.

³g/kg of CP.

⁴g of DM disappearance/kg of DM ensiled.

averaged across cutting heights. The LAB population numbers of silages produced using ENZ+FAEI were greater ($P < 0.05$) than silages made using ENZ alone, when averaged across cutting heights. The lactic acid concentration, proportion of lactic acid in total fermentation products and total bacteria population numbers were unaffected ($P > 0.05$) by additive treatment.

Chemical Composition and In Vitro Digestibility—Cutting Height (70 d of Ensiling)

The ADF and ash concentrations were lower ($P < 0.05$) and the DM and starch concentration greater ($P < 0.05$) for silages made with crops harvested at HC compared with NC (Table 5). Furthermore, the NDF concentration tended to be greater ($P = 0.056$) in silages made with crops harvested at NC than at HC. The WSC concentration and ammonia-N proportion of corn silages were unaffected ($P > 0.05$) by cutting height. The in vitro DMD and gas production of corn silages were unaffected ($P > 0.05$; Table 6) by cutting height after all incubation periods. After 24 h of in vitro ruminal incubation, the ADFD was greater ($P < 0.05$) and NDFD tended to be greater ($P = 0.071$) for silages made from crops harvested at NC, compared with silages made from HC. However, after 48 h of in vitro ruminal incubation, NDFD and ADFD of corn silages were not affected ($P > 0.05$) by cutting height.

Chemical Composition and In Vitro Digestibility—Additive (70 d of Ensiling)

The NDF and ADF concentrations of corn silages made using either ENZ alone or ENZ+FAEI were lower ($P < 0.001$) than untreated silages, whereas the WSC concentration was greater ($P < 0.01$) for both ENZ-treated silages than untreated silages, when averaged across cutting heights. The starch, CP, and ash concentration and ammonia-N proportion were unaffected ($P > 0.05$) by additive treatment.

The DMD of corn silage made using crops harvested at HC and ensiled with ENZ alone was lower ($P < 0.001$) than other HC silages, after 24 h in vitro incubation, whereas no effect of additive treatment was observed on silages after 48 h of in vitro incubation. When averaged across cutting heights, corn silages made using ENZ alone had a lower ($P < 0.05$) in vitro NDFD and ADFD than other silages after 24 h digestion, whereas no effect of additive treatment ($P > 0.05$) was observed on silages after 48 h of in vitro incubation.

DISCUSSION

Cutting Height

The DM, NDF, and CP concentrations of corn before ensiling was indicative of a crop harvested at a normal silage maturity (NRC, 2001) and was within the

Table 6. In vitro digestibility indices after 70 d of ensilage for corn silage harvested at a normal (NC) or high (HC) cutting height and treated with a fibrolytic enzyme (ENZ) and ferulic acid esterase-producing inoculant (FAEI)

Item	DMD ¹		NDFD ²		ADFD ³		Gas production ⁴				
	24 h	48 h	24 h	48 h	24 h	48 h	3 h	7 h	19 h	24 h	48 h
Treatment											
Cut											
NC	595	691	193	343	155	275	13	40	111	139	178
HC	590	688	171	327	127	262	14	43	114	144	184
Additive											
None	600 ^a	689	201 ^a	340	160 ^a	276	13 ^b	40 ^b	112	141	183
ENZ	582 ^b	678	159 ^b	319	118 ^b	255	13 ^b	40 ^b	110	139	176
ENZ+FAEI	596 ^{ab}	701	187 ^a	347	145 ^a	275	15 ^a	47 ^a	115	145	185
Cut Additive											
NC None	596 ^a	686	204	346	174	284	12	38	111	139	177
NC ENZ	602 ^a	693	180	335	134	268	12	39	110	139	179
NC ENZ+FAEI	586 ^a	694	196	347	156	273	15	44	111	140	179
HC None	604 ^a	692	197	333	145	267	13	41	113	143	189
HC ENZ	561 ^b	663	138	303	102	242	13	40	110	138	173
HC ENZ+FAEI	605 ^a	708	177	346	133	276	15	49	119	150	190
SEM	15.5	11.2	14.6	23.7	12.8	25.4	0.9	3.0	4.5	4.7	5.5
Significance											
Cut	0.587	0.735	0.071	0.399	0.033	0.433	0.339	0.197	0.484	0.311	0.231
Additive	0.020	0.147	0.002	0.328	<0.001	0.386	0.004	0.009	0.343	0.322	0.253
Cut × additive	<0.001	0.128	0.233	0.698	0.899	0.693	0.869	0.689	0.564	0.502	0.179

^{a,b}Means (n = 3) within a column with different superscripts differ ($P < 0.05$).¹g/kg of DM; DMD = in vitro DM digestibility.²g/kg of NDF; NDFD = NDF digestibility.³g/kg of ADF; ADFD = ADF digestibility.⁴mL/g of DM.

range of NDF concentrations (426 to 510 g/kg of DM) reported by previous studies that investigated the use of fibrolytic enzymes or ferulic acid esterase-producing inoculants (Sheperd and Kung, 1996; Colombatto et al., 2004; Kang et al., 2009).

The higher pre-ensilage concentrations of NDF and ADF and the lower DM concentration for corn samples harvested at NC compared with herbage harvested at HC reflected the higher proportion of the more fibrous crop component, corn stover, in the harvested herbage. This is in accord with similar studies by Lewis et al. (2004) and Caetano et al. (2011) who reported increased concentrations of cell wall carbohydrates for corn hybrids harvested at a range of high cutting heights (46–95 cm from ground), when compared with conventional cutting heights (5–15 cm from ground). The lack of effect of cutting height on the starch concentration of corn was in disagreement with Lewis et al. (2004), who reported greater starch concentrations in corn harvested at 46 cm compared with crops harvested at 15 cm. The greater buffering capacity of samples harvested at HC in the present study is in contrast to Caetano et al. (2011), who reported that harvesting corn at a greater cutting height reduced the herbage buffering capacity. Furthermore, Lynch et al. (2012) found that the stover component had a greater buffering capacity value when compared with the ear, and thus, the increased buffering capacity of corn samples harvested at HC in the present study was not expected.

The effects of cutting height on the chemical composition of untreated corn after 70 d of ensiling were similar to the pre-ensiled herbage. This is a reflection on the general lack of effect of cutting height on the silage fermentation of corn samples, in accordance with Neylon and Kung (2003), who reported no difference in the silage fermentations of corn harvested at either 12.7 or 45.7 cm. The tendency for lower pH of silages harvested at NC compared with HC was likely due to the lower buffering capacity of these herbages, as forages with low buffering capacity require lesser volumes of lactic acid to achieve a unit reduction in pH (Greenhill, 1964). The effects of cutting height on the indices of *in vitro* degradability were also minor, with the exception of increased NDFD and ADFD for NC silages after 24 h incubation. Despite this, no effects of cutting height were observed for any degradability indices after 48 h of incubation. These results are in disagreement with previous studies by Lewis et al. (2004) and Caetano et al. (2011) who found greater *in vitro* DMD for samples harvested at higher cutting heights than conventional, whereas Neylon and Kung (2003) and Caetano et al. (2011) also reported greater NDFD for samples harvested at increased cutting heights.

Additive Treatments

Despite a lack of effect of fibrolytic enzyme treatment after 7 d of ensilage, samples ensiled for 28 or 70 d with ENZ or ENZ+FAEI had a lower pH and greater acetic acid concentrations, whereas samples ensiled using ENZ+FAEI underwent a more extensive fermentation and had a greater ethanol concentration, compared with untreated silages. However, the magnitudes of differences were minor, with no differences in ammonia-N proportions observed between fibrolytic enzyme treatments. Similarly, Stokes and Chen (1994) and Sheperd and Kung (1996) found only minor effects of fibrolytic enzyme use on corn silage fermentation. However, silages produced using either ENZ or ENZ+FAEI and stored for 70 d had greater yeast population numbers, which may increase the inefficient production of ethanol and the susceptibility of the silage to aerobic deterioration upon exposure to air (Woolford, 1990). Similarly, previous studies on the production of corn silage using fibrolytic enzyme mixtures have reported increases in yeast numbers in enzyme-treated silages (Spoelstra et al., 1992; Sheperd and Kung, 1996). Despite increased DM losses, the silage fermentation of samples produced using a ferulic acid esterase-producing inoculant in addition to the fibrolytic enzyme product did not generally differ from silages produced using the enzyme product alone, with the exceptions of greater concentrations of ethanol and total fermentation products after 70 d, indicating that the inoculated lactic acid bacteria did not dominate the epiphytic microbial populations during the silage fermentation. The greater ethanol concentrations may have been a product of yeast fermentation, which also produces CO₂ and therefore partially contributed to the increased DM losses (Buxton et al., 2003). It should be noted that the use of the FAEI inoculant together with enzymes for preservation of corn was beyond the recommended use of the product by the manufacturer.

The reduced NDF and ADF concentrations of silages produced using ENZ or ENZ+FAEI compared with untreated silages likely reflects increased hydrolysis of cell-wall carbohydrates by the added fibrolytic enzymes. These observations are in accord with similar studies by Chen et al. (1994) and Sheperd and Kung (1996) that ensiled corn with a fibrolytic enzyme mixture and reported reductions in the concentrations of cell wall carbohydrates. Despite this, the lack of an effect of additive treatment on the *in vitro* DM or fiber degradability after 48 h incubation indicates that the use of fibrolytic enzymes did not improve the nutritive value of the corn silage. Similarly, previous studies that have ensiled corn with fibrolytic enzyme additives

have reported inconsistent effects on silage digestibility (Chen et al., 1994; Sheperd and Kung, 1996; Kung et al., 2000). However, it should be noted that improvements in fiber digestibility of corn silages treated with fibrolytic enzymes before in vitro digestion have also been reported (Colombatto et al., 2004; Phakachoe et al., 2013). The reduced in vitro NDFD and ADFD after 24 h of incubation of silages produced using ENZ, compared with untreated silages, indicates that the more easily digestible components of NDF were likely hydrolyzed during the ensiling of these samples. Despite a lack of difference in the in vitro NDFD and ADFD of untreated corn silages and silages produced using ENZ+FAEI, the greater NDFD and ADFD after 24 h of incubation for silages produced using ENZ+FAEI compared with silages produced using ENZ alone indicates that the cell wall carbohydrates were more easily digestible in silages produced with both the fibrolytic enzyme and the ferulic acid esterase-producing inoculant. A previous study by Kang et al. (2009) that used a similar inoculant reported the ability of ferulic acid esterase-producing bacteria to increase the digestibility of cell wall carbohydrates of corn silage. Therefore, despite a minimal effect on the fermentation dynamics of corn silages indicating that the inoculated organisms in silages made with ENZ+FAEI did not dominate the epiphytic microbial populations, the use of the FAEI had a significant impact on fiber digestibility and DM losses, likely through increasing the availability of substrate for microbial degradation.

CONCLUSIONS

The use of a fibrolytic enzyme product at ensiling did not improve corn silage fermentation or its nutritive value and resulted in some negative effects on these parameters. The use of a ferulic acid esterase-producing inoculant in combination with a fibrolytic enzyme product did not improve the silage fermentation or nutritive value of corn silage and increased DM losses during preservation, compared with untreated silages, but did reduce the negative effects of fibrolytic enzyme use on fiber digestibility. The effects of using a fibrolytic enzyme product at ensiling, either alone or in combination with a ferulic acid esterase-producing inoculant, did not differ between corn harvested at either a conventional (15 cm) or high (45 cm) cutting height. Silage produced from corn harvested at a high cutting height had a greater starch content and lower fiber content than conventional cut silage, whereas cutting height did not affect the in vitro digestibility indices of corn silages.

ACKNOWLEDGMENTS

Financial support for this study was provided by the Alberta Livestock and Meat Agency Ltd. (Project LOI 2012CO13R). The authors thank the staff of Agriculture and Agri-Food Canada, Lethbridge, especially L. Jin, Z. Matic, C. Barkley, B. Farr, F. Van Herk, B. Baker, A. David, L. Duniere, and W. Addah, for their invaluable assistance on this project and Dyadic International Ltd. (Jupiter, FL) for providing samples of their commercial products for use in the study. The inoculant was purchased commercially, with its use approved by Pioneer Hi-Bred Ltd. (Johnston, IA).

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