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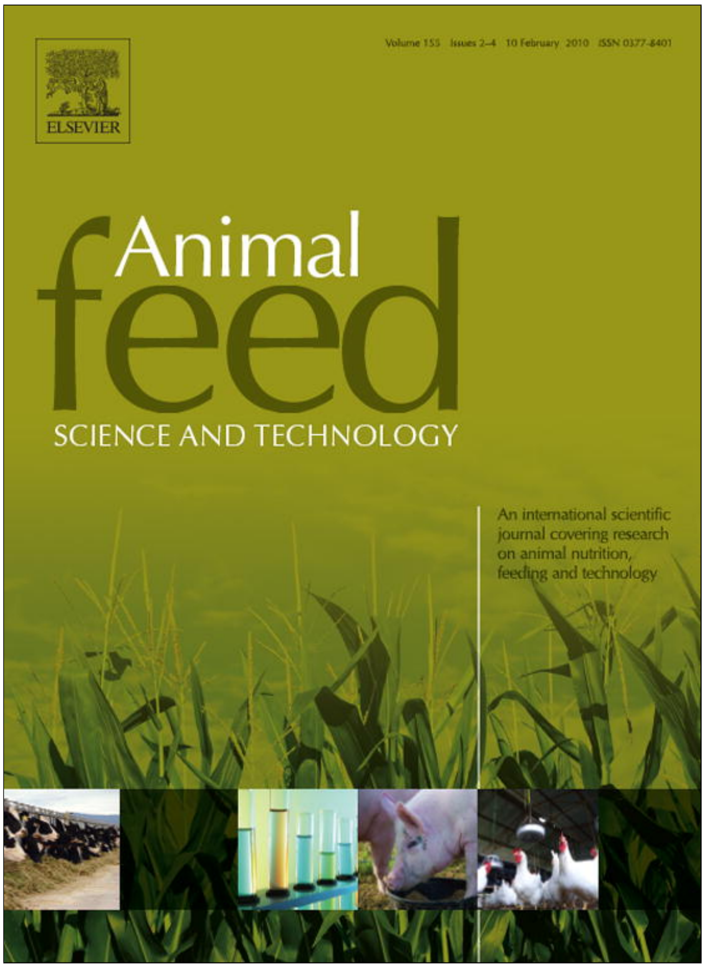

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Ammonia fiber expansion (AFEX) treatment of eleven different forages: Improvements to fiber digestibility *in vitro*

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ABSTRACT

With the increased attention to bioenergy and especially cellulosic ethanol, there are concerns regarding potential competition for available land between biofuels and feeds/foods. Ammonia fiber expansion (AFEX), a pretreatment process for cellulosic ethanol, may also be used to improve ruminant digestibility of feedstuffs not traditionally used as forages. Eleven forages – including traditional forages, agricultural residues, and dedicated energy crops – were AFEX treated and digested *in vitro* with rumen inoculum. AFEX treatment improved 48-h neutral detergent fiber (aNDFom) digestion for several moderately indigestible forages compared to untreated samples, but showed no improvement for highly digestible samples. Of particular interest are corn stover and late-harvest switchgrass, as AFEX treatment improved digestibility by 52% and 128% over untreated material, whereas the improvement was 74% and 70% over conventional ammonia treatment, respectively. The crude protein content of all treated samples increased to more than 100 g/kg dry forage. This research strongly suggests that AFEX-treated feedstuffs can be competitive with traditional forages, and thus offer expanded options for ruminant feeding.

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1. Introduction

Cellulosic ethanol is considered to be a leading alternative to fossil-fuel based liquid fuels because it is renewable, environmentally friendly, and can be produced worldwide. While research on cellulosic ethanol has been ongoing for several decades, its commercial viability has only been demonstrated recently. The Energy Independence and Security Act signed into law in 2007 mandates 16 billion gallons (60.6 billion liters) of cellulosic ethanol for the United States by 2022 (EISA, 2007). Despite this potential, there has been a great deal of resistance to the biofuels industry because of concerns over land use for biofuels *vs.* food. Although agricultural residues will account for some of this fuel, Gallagher et al. (2003) estimate that only 30 billion liters of ethanol can be produced from corn stover (assuming a standard 80 gallons per dry ton), the single largest source of agricultural residue in the United States. Thus, dedicated feedstocks ("energy crops") such as switchgrass or miscanthus will be required to further reduce petroleum consumption. These energy crops may be planted on marginal land, but there is greater potential for high yields with low inputs on nutrient-rich cropland, potentially displacing corn and other feeds.

Abbreviations: AFEX, ammonia fiber expansion; NDF, neutral detergent fiber; RBPC, Regional Biomass Processing Center; HPLC, high performance liquid chromatography; SSR, residual sum of squares; df, degrees of freedom; MSE, error mean square; S.E.M., standard error of the mean.

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With the potential for dedicated energy crops displacing traditional crops and the increasing worldwide demand for meat products (Food and Agricultural Organization, 2003), improvements in ruminant feeding will be necessary. There is great potential for feed energy from forages, but their low digestibility remains an issue. Efforts have been made for several decades to increase the digestibility of fibrous feeds by ammoniation. Ammoniation of rice straw, for example, can increase *in vitro* dry matter digestibility by 15–50% (Van Soest, 2006).

Ammonia fiber expansion or AFEX is a novel pretreatment process for cellulosic ethanol that may have similar benefits for ruminant animals. During AFEX, concentrated aqueous ammonia is contacted with biomass under moderate temperatures (80–150 °C) and pressure (200–400 psi). After a short (5–30 min) residence time, the pressure is explosively released. This process has several physical and chemical effects on the lignocellulosic material that improve its digestibility. AFEX results in cellulose depolymerization and partial solubilization of hemicellulose. Solubilized hemicellulose and lignin components appear to be moved to the exterior of the cell walls during the process, opening up the structure to facilitate access to cellulose by ruminal microbes and enzymes. These changes dramatically increase the rate and extent of both glucan and xylan release during enzymatic hydrolysis compared to untreated material (Wyman et al., 2005a; Teymouri et al., 2005). Weimer et al. (2003) included AFEX-treated rice straw at modest levels (70 g/kg dry matter) in a cattle diet and found improved milk yields and intake compared to untreated straw.

Thus, we foresee the possibility of using AFEX as a pretreatment to improve ruminant feedstuffs as well as increase the reactivity of biomass for ethanol production. Cellulosic biomass can potentially produce more biomass per acre than corn grain, thereby increasing the feed grown per acre and therefore decreasing the acreage required to feed cattle. The goal of this paper is to explore the potential of AFEX-treated animal feed within a Regional Biomass Processing Center (RBPC), which can produce both animal feed and biofuels (Carolan et al., 2007).

2. Materials and methods

2.1. Feedstuffs

Eleven different feedstuffs were used during this experiment, and are listed in Table 1. Three of these forages – corn silage, orchardgrass hay, and alfalfa hay – are defined as traditional forages in this paper, as they are commonly used for ruminant feeding without treating the forage. The remaining feedstuffs are defined as nontraditional forages. Two of these forages – corn stover harvested in October 2007 from Michigan State University (East Lansing, MI, USA) and Alamo switchgrass harvested in October 2005 from Auburn University (Auburn, AL, USA) – were chosen for further experiments. These two were chosen as a representative example of an agricultural residue and a dedicated energy crop, respectively.

2.2. Treatments

The AFEX pretreatment was performed in a 1.5-L stainless steel pressure vessel preheated to the desired treatment temperature. Approximately 150 g (dry weight) of forage was premixed with distilled water to the desired moisture concentration before adding it to the vessel. The lid was bolted shut and the air removed using a vacuum pump. A cylinder loaded with preheated anhydrous ammonia gas was connected and the ammonia charged into the vessel. After the desired residence time, the pressure was explosively released. Samples were allowed to dry under a fume hood overnight to remove residual ammonia.

The four variables in AFEX treatment are the ammonia:biomass ratio, water:biomass ratio, temperature, and residence time. As the purpose of this research is to evaluate the potential for several AFEX-treated forages rather than to compare the forages to each other, different treatment conditions were used for each forage. These conditions were chosen to be as close to optimal values as possible as reported in the literature while accounting for differences in AFEX procedures. These optimal values provide the greatest disruption of cell wall material, allowing for maximum yields of sugars after

Table 1
AFEX treatment conditions for eleven potential forages.

Name	NH_3 (g/g[a])	Water (g/g)	Time (min)	Temp. (°C)	Reference[b]
Corn silage	1.0	2.0	15	130	Unpublished data
Alfalfa hay	1.0	0.8	15	130	
Orchardgrass hay	1.0	0.8	15	130	
Rice straw	1.0	0.8	15	140	Balan et al. (2008)
Cave-in-rock switchgrass (early harvest)	1.0	0.8	15	130	Alizadeh et al. (2005)
Forage sorghum	2.0	1.2	15	140	Unpublished data
Corn stover	1.0	0.6	15	130	Teymouri et al. (2005)
Alamo switchgrass (late harvest)	1.5	1.0	30	150	
Wheat straw	1.0	0.8	15	140	
Sugarcane bagasse	1.5	1.0	30	150	
Miscanthus	2.0	1.5	30	150	Murnen et al. (2007)

[a] g/g: g ammonia or water per g dry matter.
[b] When available, optimal AFEX conditions were chosen from previous references, with time and temperature data adjusted for rapid heating of material.

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hydrolysis using commercial cellulases. If no reference was available, conditions were determined based on other forages with fiber that is expected to be similar in recalcitrance to enzymatic breakdown. In general, digestible forages required mild AFEX conditions (lower temperatures, residence times, and ammonia loadings). For these forages, harsher conditions generally do not significantly improve accessibility to enzymatic attack (Teymouri et al., 2005; Bradshaw et al., 2007). For more indigestible material, relatively harsh AFEX conditions are required to effectively break down the cell wall structure. AFEX conditions for each forage are shown in Table 1.

To compare AFEX with other treatment methods, corn stover and Alamo switchgrass were ammoniated at room temperature according to Solaiman et al. (1979). Forages were ammoniated by placing 30 g dry weight of samples into plastic bags. Concentrated (30%) ammonia was added at 40 g/kg dry matter, and the total moisture content was adjusted to 300 g/kg dry matter. Samples were thoroughly mixed and left sealed for 30 days at room temperature. After 30 days, samples were dried to remove residual ammonia. For the purpose of this paper, this treatment is defined as conventional ammoniation.

2.3. *In vitro rumen digestibility*

In vitro digestibility was performed using the method described in Tilley and Terry (1963). A rumen buffer solution was prepared including peptone, macrominerals, and microminerals, and 40 mL were added to 0.5 g forage (dry weight). The flasks were placed in a 40 °C water bath and 2 mL of a reducing solution containing cysteine, sodium hydroxide, and sodium sulfide were added. Flasks were placed under CO_2 and allowed to reduce prior to inoculating with rumen fluid. Rumen fluid was collected from a lactating fistulated dairy cow at Michigan State University's Dairy Farm approximately 2 h after feeding. The fluid and partially digested fiber from the cow was blended before filtering the fiber from the fluid, and the fluid was kept under CO_2 at all times to minimize bacterial death. After removing the fiber, 10 mL of rumen fluid was injected into each flask. Samples were kept at 40 °C for 48 h for all samples except corn stover and late-harvest switchgrass, for which 8, 12, 24, 48, and 168 h incubation times were used. After the desired residence time, 10 mL of neutral detergent solution were added to each vial to stop fiber digestion.

2.4. *Commercial enzyme hydrolysis*

Fiber was hydrolyzed using a mixture of commercial enzyme cocktails to simulate cellulosic ethanol production. The procedure was based upon the standard protocol from the National Renewable Energy Laboratory (Selig et al., 2008). Samples were hydrolyzed in scintillation vials at 30 g dry forage per liter buffered to pH 5.0 by 1 M citrate buffer. Accelerase 1000® (Genencor, Rochester, NY, USA) was used as the cellulase, and was loaded at 5 mg Accelerase per g dry matter. All samples were incubated at 50 °C with 200 rpm rotation.

2.5. *Analytical techniques*

Nitrogen content within the biomass was determined using a Skalar Primacs SN Total Nitrogen Analyzer (Breda, The Netherlands), which uses the Dumas method of combusting all nitrogen to NOx (Wiles et al., 1998).

Neutral detergent fiber, both before and after *in vitro* digestion, was determined as aNDFom as described by Mertens (2002). Heat stable amylase and sodium sulfite were both added to the samples prior to neutral detergent digestion. Samples were boiled for 1 h in neutral detergent solution and then filtered through crucibles. The remaining fiber was rinsed with water and acetone, and allowed to dry at 105 °C to determine its dry weight. Samples were then ashed at 575 °C, and the remaining ash subtracted from the weight of the sample.

For commercial enzyme hydrolysis, total sugars released was determined using high performance liquid chromatography (HPLC) analysis (Teymouri et al., 2005). Samples were collected at the desired reaction time, and glucose, xylose, and arabinose concentrations were determined using a Waters HPLC system equipped with a Bio-Rad (Richmond, CA, USA) Aminex HPX-87P carbohydrate analysis column. Degassed HPLC water with a flow rate of 0.6 mL/min was used as the mobile phase, while the temperature in the column was kept constant at 85 °C.

2.6. *Statistical analysis*

Only differences between treated and untreated samples were considered in this study, not differences between different forages. Two experiments, the rate of digestion for corn stover and switchgrass and the impact of varying AFEX treatment conditions for switchgrass, were performed in duplicate for each sample or time point. All other nitrogen and aNDFom values were determined in triplicate. A two-tailed Student's *t*-test was used to determine if differences between untreated and treated samples, and all statements of significance were based on a probability level of 0.05, while statements regarding trends were based on a probability $0.05<p<0.10$.

The rate of digestion for corn stover and late-harvest switchgrass was determined for each treatment using the following first order degradation model (Robinson et al., 1986):

$$\mathrm{NDF} = A\,e^{-kt} + U$$

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where NDF is the amount of undigested aNDFom remaining, A is the amount of digestible aNDFom in the forage (g/kg dry matter), k is the rate constant (h^{-1}), t is the time (h), and U is the amount of indigestible aNDFom in the forage (g/kg dry matter). Constants were determined using the Gauss–Newton method to minimize the sums of squares for the error.

An analysis of variance was performed for the rate of digestion model. A sum of squares reduction test was used to compare each pair of treatments (Seber and Wild, 1989). In this case, the comparison is between the full model and a reduced model with the following constraints:

$$A_i = A_j$$

$$k_i = k_j$$

$$U_i = U_j$$

where i and j are either no treatment, AFEX treatment, or ammonia treatment. The test statistic is calculated as

$$F_{obs} = \frac{(SSR_{reduced} - SSR_{full})/(df_{full} - df_{reduced})}{MSE_{full}}$$

using an F distribution with the numerator degrees of freedom as the difference in degrees of freedom between the full and reduced models and the denominator degrees of freedom as the residual degrees of freedom in the full model. In addition, each pair of parameters was also compared for significance using the same method. No differences between switchgrass and corn stover were tested. All statistical analysis was performed using SAS 9.1 software (Cary, NC, USA).

3. Results

3.1. Characteristics of AFEX-treated forages

Ammoniation darkened the color of all forages, and the color was darker for AFEX treatment compared to traditional ammoniation for corn stover and Alamo switchgrass. Despite the elevated temperatures, no burning or charring was observed. No soluble materials are removed during the AFEX process, and thus the only changes in dry matter composition are due to ammonia addition and hydrolysis, such as the cleavage of acetyl bonds. From previous research, these reactions result in only a slight increase in mass (Teymouri et al., 2005).

Nitrogen and aNDFom values for all samples are shown in Table 2. AFEX treatment decreased aNDFom concentration in all samples, ranging from 48 to 195 g/kg dry matter, with an average of 110 g/kg dry matter. A slight linear trend was observed between untreated initial aNDFom and the amount removed for AFEX-treated samples (R^2 = 0.348, p=0.052). This trend can partially be explained as forages with a higher fiber concentration have more reactive sites for the AFEX treatment to disrupt. The aNDFom loss for Alamo switchgrass was significantly higher for AFEX than conventional for ammoniation (136 g/kg *vs*. 38 g/kg dry matter for switchgrass) in this study, although there was no difference in aNDFom loss between AFEX and convential ammoniation for corn stover.

Table 2
Neutral detergent fiber (aNDFom) and nitrogen concentration in treated and untreated forages.

	aNDFom (g/kg dry matter)					Nitrogen (g/kg dry matter)				
	Untr.[a]	S.E.M.[b]	Treat	S.E.M.	Diff.[c]	Untr.	S.E.M.	Treat	S.E.M.	Diff.
Corn silage	481	10.5	399	5.7	82	18.2	0.13	33.9	0.38	15.7
Alfalfa hay	492	11.7	426	5.0	67	26.2	0.24	48.2	0.32	22.0
Orchardgrass hay	678	10.0	611	7.0	105	21.1	0.83	40.1	0.09	18.9
Rice straw	711	2.6	663	13.0	48	6.5	0.09	23.8	0.08	17.3
Cave-in-rock switchgrass (early harvest)	769	8.1	593	6.2	176	11.0	0.10	25.2	0.25	14.1
Forage sorghum	781	4.4	684	5.3	97	7.1	0.12	16.0	0.46	8.9
Corn stover	806	15.3	717	0.2	89	10.8	0.17	27.4	0.70	16.7
Corn stover[d]	806	15.3	748	19.2	58[e]	10.8	0.17	18.3	0.18	7.5
Alamo switchgrass (late harvest)	819	11.1	683	7.1	136	3.6	0.02	23.3	0.09	19.7
Alamo switchgrass[d]	819	11.1	781	0.8	38	3.6	0.02	18.4	0.08	14.8
Wheat straw	821	9.7	739	9.9	82	4.1	0.05	19.0	0.22	14.9
Sugarcane bagasse	835	21.0	692	10.0	143	3.6	0.11	19.1	0.12	15.4
Miscanthus	909	7.1	713	22.4	195	2.5	0.06	20.8	0.07	18.3

[a] Untreated samples.
[b] Standard error of the mean (three replicates for each sample).
[c] Difference between treated and untreated samples.
[d] Conventional ammoniation treatments. All other treatments are AFEX treated.
[e] Denotes no significance between untreated and treated samples. All other treatments were significantly different from untreated samples.

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Table 3
aNDFom digested after 48 h of *in vitro* fermentation for eleven forages.

	Digested (g/kg)[a]						Total digested (g/kg)[b]			
	Untr.[c]	S.E.M.[d]	Treat	S.E.M.	Diff.[e]	% Incr.[f]	Treat	S.E.M.	Diff.	% Incr.
Corn silage	208	23	275	10	67[h]	32	357	11	149	72
Alfalfa hay	211	16	197	11	−14[h]	−7	264	12	53	25
Orchardgrass hay	349	13	354	13	4[h]	1	459	14	110	31
Rice straw	347	4	507	16	160	46	555	20	208	60
Cave-in-rock switchgrass (early harvest)	492	11	483	9	−10[h]	−2	659	11	167	34
Forage sorghum	179	6	237	17	59	32	334	18	155	87
Corn stover	370	17	564	9	194	52	653	9	283	76
Corn stover[g]	370	17	325	20	−45[h]	−12	383	28	13[h]	3
Alamo switchgrass (late harvest)	174	12	397	9	223	128	533	12	359	206
Alamo switchgrass[g]	174	12	233	19	59	34	271	19	97	56
Wheat straw	315	12	512	14	197	63	594	17	279	89
Sugarcane bagasse	212	22	356	17	143	68	499	20	287	135
Miscanthus	51	9	132	32	81	159	327	39	276	542

[a] aNDFom digested during rumen digestion only, given as g NDF/kg dry matter.
[b] Total aNDFom removed for treated samples, including both the amount removed due to AFEX or ammonia treatment and the amount digested during *in vitro* fermentation, given as g NDF/kg dry matter.
[c] Untreated samples.
[d] Standard error of the mean.
[e] Difference between treated and untreated samples.
[f] Percent increase in treated sample over untreated sample.
[g] Conventional ammoniation treatments. All other treatments are AFEX treated.
[h] Denotes no significance between untreated and treated samples. All other treatments were significantly different from untreated samples.

Ammonia treatment increased the nitrogen concentration for all forages, ranging from 8.9 g/kg dry matter increase for sorghum to 22.0 g/kg increase in alfalfa. Untreated nontraditional forages all had low nitrogen concentration. With the ammonia addition, these samples are more comparable to the untreated traditional forages. Furthermore, nitrogen addition was significantly greater for AFEX treatment compared to ammoniation for both switchgrass and corn stover, with AFEX increasing nitrogen over conventional ammoniation by 4.9 g/kg for switchgrass and 9.1 g/kg for corn stover. It is believed that most of the additional nitrogen is in the form of acetamide (Weimer et al., 1986).

3.2. *In vitro* digestibility of AFEX-treated feeds

AFEX improved the 48 h *in vitro* aNDFom digestibility of most of the forages tested, as seen in Table 3. However, AFEX treatment did not increase *in vitro* digestibility of aNDFom for four forages: the three conventional forages and the early harvest switchgrass. AFEX treatment of Alamo switchgrass and wheat straw increased rumen digestion by 223 and 197 g aNDFom/kg dry matter respectively, the largest increases among all forages. AFEX treatment increased digestibility of several nontraditional forages to levels that are greater than untreated traditional forages. AFEX-treated corn stover had the greatest amount of fiber digested (564 g/kg dry matter) of any treated or untreated forage in this study. This accounts for a 52% increase in fiber digestibility compared to untreated samples. Likewise, later harvest switchgrass increased digestibility by 128%, the second largest percentage increase of the forages tested. AFEX treatment of forage sorghum and miscanthus improved aNDFom digestibility, but the overall amount of fiber digested was still poor compared to other untreated traditional forages. When taking into account both loss due to treatment and microbial digestion, AFEX increases the total fiber loss for all forages.

Also of note is the proportion of fiber digested for AFEX-treated samples. AFEX treatment increased digestibility by 0.25–0.35 for multiple forages. The digestibility of AFEX-treated corn stover was 0.8, while digestibility of AFEX-treated wheat straw was nearly 0.7. In comparison, aNDFom digestibility of untreated traditional forages ranged from 0.4 to 0.5, with early harvest switchgrass being the most highly digestible untreated forage (0.64). Thus, these treated samples have both more digestible aNDFom per ton of NDF as well as per ton of dry matter compared to untreated traditional forages.

AFEX treatment appears to have a greater effect on both the rate and extent of fiber digestion over both untreated and conventional ammonia-treated samples, as seen in Fig. 1. The amount of aNDFom remaining after 168 h was more than twice as high for untreated switchgrass *vs.* AFEX treated (574 g/kg *vs.* 267 g/kg), and conventional ammoniation (420 g/kg) trended slightly higher than AFEX-treated grass (p=0.057). AFEX treatment of corn stover resulted in even greater digestion compared to both untreated (145 g/kg *vs.* 365 g/kg) and ammoniated (266 g/kg) samples. A first order degradation model was determined for each treatment, and the parameters are shown in Table 4. For corn stover, there was a slight trend between ammonia treatment and no treatment (p=0.100). However, AFEX treatment significantly improved the degradation of aNDFom compared to either ammonia treatment or untreated samples. For switchgrass, all three treatments were significantly different from each other.

The primary impact of AFEX treatment is to decrease the amount of indigestible aNDFom. For both corn stover and switchgrass, AFEX has significantly less indigestible fiber than untreated samples, and less indigestible fiber than ammonia-

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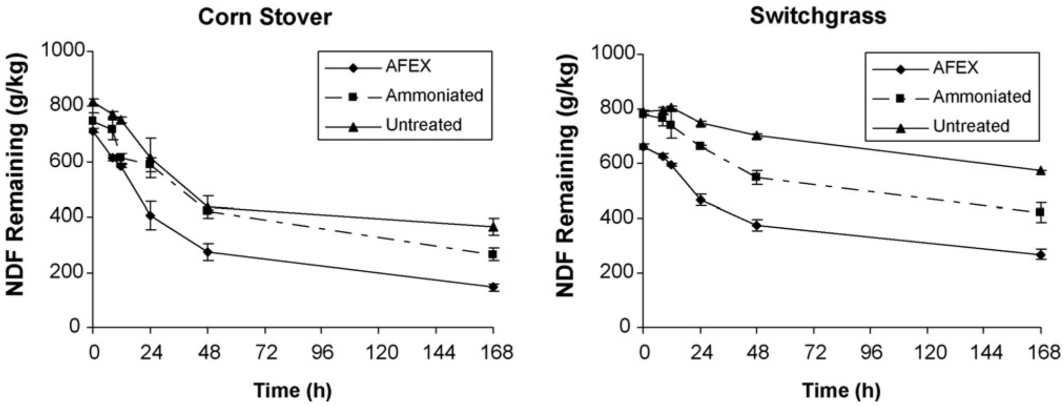

Fig. 1. Effect of conventional ammoniation and AFEX treatment compared to untreated corn stover (left) and switchgrass (right) on aNDFom remaining over time during NDF digestion. Error bars represent the high and low values for duplicate samples.

Table 4
Comparison of the rate of fiber removal for corn stover and Alamo switchgrass.

		Parameters[a]					
		A	SE[b]	*k*	SE	*C*	SE
Corn stover	AFEX[c]	593[c]	44	0.029[c]	0.005	136[c]	37
	Ammonia[d]	512[c]	48	0.020[c]	0.005	246[c,d]	46
	Untreated[d]	471[c]	45	0.023[c]	0.006	339[d]	41
Alamo switchgrass (late harvest)	AFEX[c]	427[c]	26	0.026[c]	0.004	257[c]	23
	Ammonia[d]	405[c]	29	0.018[c,d]	0.004	398[d]	29
	Untreated[e]	319[c]	103	0.008[d]	0.005	490[d]	110

Different letters (c, d and e) denote significant differences (p<0.05) among the parameters and treatments using the sum of squares reduction test. Significant differences are tested only between treatments and not between forages.

[a] Parameters obtained using a least squares nonlinear regression on the rate of aNDFom removal (data from Fig. 1). The regression equation is $NDF = A\,e^{-kt} + U$, where NDF is the aNDFom remaining in the biomass (g/kg dry forage), t is the time after inoculation (h), A is the amount of digestible aNDFom (g/kg dry forage), k is the rate constant (h^{-1}), and U is the amount of indigestible aNDFom (g/kg dry forage).

[b] Approximate standard error.

treated samples for switchgrass. In addition, AFEX treatment significantly improves the rate of digestion for switchgrass compared to no treatment.

As previously stated, the effectiveness of AFEX pretreatment depends on the conditions present during the reaction. Much literature has been published for optimizing AFEX conditions based upon theoretical ethanol production (Teymouri et al., 2005; Bradshaw et al., 2007); these conditions are likely to be optimal for ruminant feed as well. As expected, there is a significant correlation between enzymatic digestion using commercial cellulases and *in vitro* rumen digestion, as seen in Fig. 2. AFEX increases the accessibility of cellulose and hemicellulose to enzymatic attack, which should affect fiber digestibility using both methods. The amount of aNDFom digested during *in vitro* studies is approximately twice as high as sugar released by commercial cellulases. This is due partly to different conditions, as a low enzyme loading was used and without the complete array of enzymes present in rumen microbes, and partly to a difference in analyses, as the sugar analysis does not include oligomers or solubilized lignin.

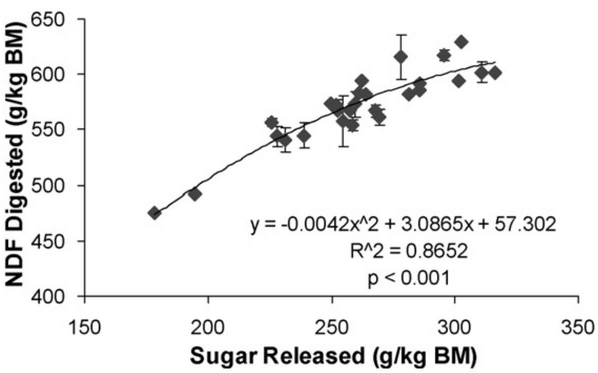

Fig. 2. Relationship between monomeric sugars released during enzymatic hydrolysis with commercial cellulases (x-axis) and *in vitro* aNDFom digestion at 48 h (y-axis) for multiple AFEX conditions of late-harvest switchgrass. The line represents a quadratic curve obtained from an ordinary least squares regression. AFEX conditions ranged from 0.4 to 2.0 g water/g dry matter, 0.4–2.0 g ammonia/g dry matter, 5–30 min residence time, and 80–150 °C.

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4. Discussion

Based upon our results, it appears that AFEX pretreatment is an effective treatment for improving the digestibility of some forages for ruminant feeding. Many types of forages are viable candidates for AFEX-treated animal feeds. In general, forages harvested at early maturities are not appropriate for AFEX treatment, because only modest improvements in digestibility are observed. In addition, aNDFom digestibility of miscanthus remained low after AFEX treatment, and thus further research is required in order to make it viable for animal feed. Corn stover and late-harvest switchgrass are of particular interest because they are leading potential feedstocks for cellulosic ethanol while simultaneously offering large improvements in aNDFom digestibility (Parrish and Fike, 2005; Wyman et al., 2005a). Also of interest is the improvement in rice straw and sugarcane bagasse digestibility, as this process could be applied in nations such as China and Brazil, respectively. China in particular has a history of promoting ammoniated straw as a feedstuff for beef, citing improved efficiency and economic development (Bingsheng, 2002). Brazil is expected to continue expanding its sugarcane production for ethanol, thus finding value for its residues will become increasingly important.

The different cell wall compositions among the various forages likely account for differences in response to AFEX treatment among forages as well. Ammoniation of forages, particularly AFEX treatment, serves primarily to cleave the ester linkages within lignin and hemicellulose as well as the linkages between lignin and polymeric carbohydrates (Buranov and Mazza, 2008). Furthermore, significant amount of oligomeric carbohydrates are formed during AFEX treatment (unpublished research), and that these oligomers are available to enzymes for further breakdown. Thus, while some fiber is removed during AFEX treatment, much of the removed material may still be available as a source of energy. Further research into the mechanisms of AFEX, including the breakdown of cell wall materials and the formation of ammonia-based products such as acetamide, are ongoing.

The data presented shows a greater disruption of cell wall material for AFEX treatment compared to conventional ammoniation for corn stover and switchgrass, likely due to the higher temperature and ammonia loading during treatment. Likewise, literature values for conventional ammoniation also tend to be lower than AFEX treatment. Two references for ammoniation of wheat straw give neutral detergent fiber losses of 63 and 78 g/kg forage, respectively (Solaiman et al., 1979; Birkelo et al., 1986), while 82 g/kg was hydrolyzed by AFEX treatment. Increases in crude protein for AFEX compared to ammoniation were approximately the same as literature values for rice straw (Broudiscou et al., 2003), with 10.8 g/kg nitrogen increase *vs.* 10.5 g/kg cited. For wheat straw, literature values for ammoniation (Solaiman et al., 1979; Birkelo et al., 1986) were slightly higher than AFEX treatment (9.5 and 11 g/kg, respectively, *vs.* 9.3 g/kg reported here).

Numerous other pretreatments have been suggested for preparing cellulosic biomass for ethanol production (Henricks and Zeeman, 2009). However, AFEX may be almost uniquely suited for animal feed operations combined with ethanol production. Most acidic treatments completely hydrolyze the hemicellulose portion of the forage, which would greatly reduce the neutral detergent fiber available. In addition, virtually all other chemical pretreatments use free water, and thus have separate liquid and solid streams. Significant amounts of material are removed from the solid forage in the liquid stream, ranging from 10 to 40% of the carbohydrates present (Wyman et al., 2005b). Potential nutrients such as protein and ash are removed as well, whereas only one stream is present during AFEX, thus insuring all components of the forage are potentially available for subsequent use. In addition, the increase in non-protein nitrogen, assuming it can be utilized by ruminal microbes, may be necessary to compete with high protein forages such as alfalfa silage.

As AFEX technology is currently not commercially available, it is impossible to provide a precise assessment of the costs for this pretreatment. A study in 1998 placed the estimated capital and operating costs at $20–40 per ton of dry matter (Wang et al., 1998). However, improvements over the last decade in ammonia recovery design and understanding of the system have decreased the expected costs. A model for cellulosic ethanol production developed at the National Renewable Energy Laboratory (NREL, Golden, Colorado) and adapted to AFEX at Dartmouth University and Michigan State University show significant gains in process economics (Sendich et al., 2008). By extracting the feed handling and AFEX pretreatment parameters, the capital and operating costs of a stand alone AFEX pretreatment center were estimated to be between $14 and 21 per ton dry matter processed. Capital requirements may be lower than stated in this analysis due to a novel reactor design (Carolan et al., 2007). Estimates for transport costs are generally within $7–10 per ton, and may be lower depending upon the bulk density of the forage and the transportation radius (Kadam et al., 2000). Payments to the farmer for the forage will be dependent on the type of forage, but estimates range from ∼$20 per dry ton for rice straw and $60 per dry ton for switchgrass (Kadam et al., 2000; Sokhansanj et al., 2009). Thus, it is likely that the production of AFEX-treated residues could be achieved at $60–100 per dry ton.

Although AFEX-treated grasses may have the same cost of production as other common forages, its true value as a feed may be much higher. Oba and Allen (1999) performed a meta-analysis of available literature with dairy cattle and found that improved neutral detergent fiber digestibility increased dry matter intake and milk yield. It is believed that increased digestibility leads to increased clearance from the rumen, thereby enabling an increase in nutrient intake for milk production. Although the increase in aNDFom digestibility shown in this paper is greater than the range studied in these analyses, this strongly suggests that AFEX-treated feeds may improve dry matter intake and therefore milk yield in dairy cattle compared to traditional untreated forages. Because of this potential added value, it is impossible to estimate the true value of AFEX-treated grasses without performing *in vivo* studies.

Treating forages for ruminant feeding purposes may also help reduce the risk of cellulosic ethanol production. The high capital costs of ethanol facilities and need for a reliable supply chain are seen as key hurdles to cellulosic ethanol

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development. Multiple Regional Biomass Processing Centers (RBPCs), capable of pretreating ∼100–500 dry tons per day of dry matter, surrounding an ethanol facility could provide the material for both ethanol production and animal feeding. This would reduce the capital costs of the ethanol facility, lowering the investment required to begin operations. In addition, the potential high value of animal feed could subsidize ethanol production costs. Likewise, the high volumes of ethanol production lower the capital cost requirements per ton of material for animal feeding operations due to improved economies of scale. Furthermore, the small scale and wide dispersion of these RBPCs relative to ethanol facilities enables the local rural community to capture more of the added value of pretreated forages, thereby further incentivizing the community to produce suitable forages for both feed and biofuels. Further technical and economic research is necessary to confirm this potential.

5. Conclusions

Ammonia fiber expansion (AFEX) pretreatment increased *in vitro* aNDFom digestibility of a range of forages. The greatest improvements were in moderately digestible forages not commonly used as cattle feed. Of particular interest are corn stover and late-harvest switchgrass, in which AFEX increased digestibility by 53% and 128% over untreated material and 74% and 70% over ammonia-treated samples, respectively. Although some aNDFom is lost during the pretreatment, it is expected that much of that fiber is converted to oligomeric sugars, which is of nutritional value for the ruminants. Crude protein content also increased, with AFEX-treated samples being comparable to common ruminant feeds.

This study strongly suggests that treated agricultural residues such as corn stover or dedicated energy crops such as switchgrass can compete with traditional forages such as alfalfa or orchardgrass for ruminant diets. Such competition will depend upon the cost of AFEX pretreatment and the feeding value of the treated material. Initial information suggests these feeds can be produced at a cost comparable to traditional forages while potentially giving added benefits such as improved intake and milk production. Future research is necessary in order to obtain a more accurate assessment of the value of AFEX for animal operations, particularly in determining the nutritional value of the fiber solubilized during AFEX treatment and the effect of treatment on intake and digestibility *in vivo* must be studied.

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