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# Rumen disappearance kinetics and chemical characterization of by-products from cellulosic ethanol production

S.L. Scott[a,*], R.S. Mbifo[a,1], J. Chiquette[b], P. Savoie[c], G. Turcotte[d,2]

[a] Université Laval, Department of Animal Science, Québec, QC, Canada G1K 7P4
[b] Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre, 2000 College Street, Sherbrooke, QC, Canada J1M 1Z3
[c] Agriculture and Agri-Food Canada, Crops and Soils Research and Development Centre, 2560 Hochelaga Blvd., Québec, QC, Canada G1V 2J3
[d] Université Laval, Department of Food Science and Nutrition, Québec, QC, Canada G1K 7P4

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ABSTRACT

Cellulose-rich biomasses can be used as an alternative to starch-rich corn grain as a source of sugars for ethanol (EtOH) production. However, converting cellulosic biomass to ethanol produces large quantities of co-products which need to be disposed of, preferably in a value-added process, possibly as animal feed. In order to estimate the feeding value of these co-products for ruminants, the chemical composition and ruminal *in sacco* disappearance of five mature forages (*i.e.*, reed canary grass hay, timothy hay, alfalfa hay, corn stover, barley straw) were determined following no treatment (control), or treatment with ammonia fiber explosion (AFEX) or AFEX plus enzymatic hydrolysis of cellulose and hemicellulose (AFEX + ENZ). AFEX treatment alone doubled the N content of forages compared with controls. AFEX + ENZ-treated forages contained a similar level of N as the AFEX-treated forages. Acid-detergent insoluble N (ADIN) was twice as high in AFEX + ENZ-treated as in the AFEX-treated forages. The AFEX + ENZ-treated forages had 32% less neutral detergent fiber (NDF) and 18% less ADF than the controls. Effective ruminal disappearance of dry matter (DM), NDF, and N were higher ($P<0.05$) for AFEX + ENZ-treated forages than for untreated forages (719 g/kg *versus* 322 g/kg, 501 g/kg *versus* 200 g/kg, and 839 g/kg *versus* 437 g/kg, respectively). AFEX + ENZ treatment increased the N content and disappearance of plant constituents, but reduced the content of the major structural carbohydrates (ADF and NDF). The AFEX + ENZ-treated forages could therefore be considered for use as a non-protein N supplement in combination with high energy diets low in ruminally degradable protein.



## 1. Introduction

Interest in production of "second generation" biofuels from non-food, lignocellulosic biomasses has increased in recent years (Klein and LeRoy, 2007; Sims et al., 2008). Cellulose and hemicelluloses, which comprise a substantial proportion of

*Abbreviations:* ADF, acid detergent fiber; ADIN, acid detergent insoluble N; AFEX, ammonia fiber explosion; DM, dry matter; ED, effective disappearance; EtOH, ethanol; NDF, neutral detergent fiber.

* Corresponding author. Current address: Agriculture and Agri-Food Canada, Brandon Research Centre, P.O. Box 1000A, RR#3, Brandon, MB, Canada R7A 5Y3. Tel.: +1 204 578 3605; fax: +1 204 728 3858.
*E-mail address:* shannon.scott@agr.gc.ca (S.L. Scott).
[1] Current address: Canadian Food Inspection Agency, 310 Baig Blvd., Moncton, NB, Canada E1E 1C8.
[2] Current address: Department of Chemical Engineering, Ryerson University, Toronto, ON, Canada M5B 2K3.

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lignocellulosic biomass, can be hydrolyzed to simple sugars by a process called saccharification, and, in turn, these sugars can be fermented to ethanol (Lynd, 1996).

Forage crops (*e.g.*, reed canary grass, timothy grass, alfalfa, as well as barley, triticale, pearl millet, and sweet sorghum hays), and crop residues (*e.g.*, corn stover, bagasse, as well as wheat, barley, triticale, and rice straws), have been identified as potential sources of lignocellulose for bioethanol production (Michaud et al., 1997; Chen et al., 2007). For example, Champagne (2007) estimated that in Canada, there are approximately 17.8 million tonnes of crop residue biomass dry matter (DM) left on fields per year. A large fraction of this residue must be left on fields for protection against erosion and replenishment of soil organic matter. However, a certain portion of this residue is available from well-managed and low sloping land (Graham et al., 2007). If that portion were half of the residues produced, the resulting biomass could provide 4.3 million tonnes of carbon per year. That mass of carbon could potentially translate into production of 0.15 exajoules (EJ; $10^{18}$ J) of energy per year via ethanol production, equivalent to 0.018 of fossil fuel use in Canada.

One of the challenges associated with production of lignocellulosic ethanol is the need to pretreat the feedstocks in order to maximize conversion of cellulose and hemicelluloses into simple sugars and then ethanol (Wyman et al., 2005). Ammonia fiber explosion (AFEX) has been shown to be an effective and economical pretreatment to increase yields of fermentable sugars from lignocellulosic biomasses (Eggeman and Elander, 2005; Teymouri et al., 2005). In the AFEX pretreatment process, a biomass is mixed with liquid anhydrous ammonia at moderate temperatures (80–150 °C) under high pressure (1.4–2.8 MPa) for 5–30 min, followed by rapid pressure release which causes the ammonia to freeze and rupture the fibrous structure of the plant (Bals et al., 2010). The crystalline structure of cellulose so fractured facilitates subsequent conversion of cellulose and hemicellulose into simple sugars by specialized enzymes from fibrolytic fungi (Teymouri et al., 2005). AFEX pretreatment followed by enzymatic hydrolysis results in conversion of about 50–60% of biomass DM into fermentable sugars which can be used for ethanol production, leaving the other 40–50% of DM as a non-hydrolyzed solid residue (Savoie et al., 1998; Wyman et al., 2005).

One way of improving the economic efficiency of lignocellulose conversion is to use this non-hydrolyzed solid co-product as an animal feed. While there are several reports characterizing the feeding value of co-products from grain-based ethanol production for ruminants (Mustafa et al., 2000a,b; Klopfenstein et al., 2008), there have been few reports on the feeding value of the co-products of cellulosic ethanol production, especially those produced from AFEX pretreatment.

The objective of this study was to evaluate the feeding value for ruminants of solid co-products resulting from cellulosic ethanol production from five forages (reed canary grass hay, timothy hay, alfalfa hay, corn stover, barley straw). The approach taken to achieve this objective was to subject lignocellulosic biomass to AFEX pretreatment and enzymatic hydrolysis followed by evaluation of the chemical composition and ruminal *in sacco* disappearance of the co-products produced.

## 2. Materials and methods

### 2.1. Feedstuffs

Three forage species, reed canary grass (*Phalaris arundinacea* 'Vantage'), alfalfa (*Medicago sativa* 'Apica') and timothy grass (*Phleum pratense* 'Basho'), were harvested at late maturity in July and stored as baled hay. Two crop residues, barley (*Hordeum vulgare*) straw and corn (*Zea mays*) stover, were harvested after combining the grain in August and October, respectively. These species were chosen for their relative abundance and importance in the province of Québec (Canada). All forages were collected in the fall at an average DM content of 920 g/kg and stored in a cold shed until the following March or May, when AFEX pretreatment and enzymatic hydrolysis were carried out. Forages were chopped with a stationary chopper (Forano, Plessisville, QC) to obtain an average particle size of 3 mm for forage crops and 5 mm for crop residues. The particle size of chopped forages was determined by screening using the ASAE standard method S424 (ASABE, 2007).

### 2.2. AFEX treatment and enzymatic hydrolysis

A completely randomized design with a 5 × 3 factorial arrangement of treatments was used (5 forages × 3 treatments). Treatments consisted of original forages with no processing (control), with AFEX treatment, and with AFEX pretreatment followed by enzymatic hydrolysis. AFEX treatment was applied once to a sufficient quantity of each of the five forages to produce both the AFEX and AFEX + ENZ samples.

AFEX pretreatment was as described by Savoie et al. (1998). Briefly, 550 g samples of chopped forages (average of 506 g of DM) were wetted with 112 g of water for 24 h and placed in a stainless steel autoclave (Autoclave Engineers Inc., Erie, PA, USA). Anhydrous ammonia (0.995 purity, Prodair, Quebec, QC, Canada) was poured into the autoclave at a mass ratio of 1:1 (g ammonia:g dry biomass). The temperature was raised to 90 °C at a pressure of 3.65 MPa and maintained for 30 min; similar to time and temperature conditions used by Bals et al. (2010) for 11 forages. The pressure was then rapidly released to the atmosphere by opening a large ball valve. Residual ammonia in the fiber was allowed to evaporate overnight under a fume hood, then the treated material was transferred to an aerated area to finish drying. The final DM content of the treated material was generally 900–940 g/kg (Savoie et al., 1998).

The AFEX-treated biomass was mixed at 50 g/L in a buffer solution containing 3 g/L of sodium acetate and 2.6 g/L of acetic acid and which had a final pH of 4.85. The samples were enzymatically hydrolyzed for 30 h at 50 °C with shaking at