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Screening exogenous fibrolytic enzyme preparations for improved in vitro digestibility of bermudagrass haylage

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ABSTRACT

Our objectives were to evaluate the effects of 12 exogenous fibrolytic enzyme products (EFE) on ruminal in vitro neutral detergent fiber digestibility (NDFD) and preingestive hydrolysis of a 4-wk regrowth of bermudagrass haylage (BH), to examine the accuracy of predicting NDFD with EFE activity measures, and to examine the protein composition of the most and least effective EFE at increasing NDFD. In experiment 1, effects of 12 EFE on NDFD of BH were tested. Enzymes were applied in quadruplicate to culture tubes containing ground BH. The suspension was incubated for 24 h at 25°C before addition of rumen fluid media and further incubation for 24 h at 39°C. The experiment was repeated twice. In addition, regression relationships between EFE activity measures and NDFD were examined. Compared with the values for the control, 9 EFE-treated substrates had greater NDFD (37.8 to 40.4 vs. 35.6%), 6 had greater total VFA concentration (59.1 to 61.2 vs. 55.4 m*M*), and 4 had lower acetate-to-propionate ratios (3.03 to 3.16 vs. 3.24). In experiment 2, EFE effects on preingestive fiber hydrolysis were evaluated by incubating enzyme-treated and untreated bermudagrass suspensions in quadruplicate for 24 h at 25°C and examining fiber hydrolysis measures. Compared with values for the control, 3 EFE reduced neutral detergent fiber concentration (62.8 to 63.7 vs. 67.3%), 10 increased release of water-soluble carbohydrates (26.8 to 58.5 vs. 22.8 mg/g), and 8 increased release of ferulic acid (210 to 391 vs. 198 μg/g). Regression analyses revealed that enzyme activities accurately [coefficient of determination (R^2) = 0.98] predicted preingestive hydrolysis measures (water-soluble carbohydrates, ferulic acid), moderately (R^2 = 0.47) predicted neutral detergent fiber hydrolysis, but poorly ($R^2 \leq 0.1$) predicted dry matter and NDFD. In experiment 3, proteomic tools were used to examine the protein composition of the most and least effective EFE at improving NDFD. Relative to the least effective, the most effective EFE at increasing NDFD contained 10 times more endoglucanase III, 17 times more acetylxylan esterase with a cellulose-binding domain 1, 33 times more xylanase III, 25 times more β-xylosidase, and 7.7 times more polysaccharide monooxygenase with cellulose-binding domain 1 and 3 times more swollenin. The most effective EFE had a much greater quantity of fibrolytic enzymes and key proteins necessary for hemicellulose and lignocellulase deconstruction. This study identified several EFE that increased the NDFD and in vitro fermentation of 4-wk BH and revealed why some EFE are more effective than others.

Key words: enzyme, digestibility, bermudagrass, fermentation

INTRODUCTION

In the southeastern United States, warm-season grasses are used extensively in cattle-production systems, but their high fiber content and low digestibility can limit animal productivity and consequently profitability (Hanna and Sollenberger, 2007). Improving the quality of warm-season grasses is a major concern for the dairy industry in the southeast. Bermudagrass (*Cynodon dactylon*) is the most planted warm-season perennial grass in the southeastern United States (10 to 12 million ha; Newman, 2007). Among bermudagrasses, the Tifton 85 cultivar is preferred by southeastern dairy producers because it provides the best combination of high yields, high quality, and pest resistance and it is often considered a replacement for expensive imported alfalfa hay (Bernard et al., 2010). Exogenous fibrolytic enzyme (**EFE**) application has improved fiber digestion and animal performance in some studies, but the results have not been consistent (Beauchemin and Holtshausen, 2010). Previous studies reported that adding a fibrolytic enzyme to a corn silage and alfalfa hay-based TMR containing high (48%) or low (33%)

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concentrate inclusion levels increased total tract NDF digestibility by 6 and 7% and increased feed efficiency by about 6 and 16%, respectively (Arriola et al., 2011). However, when the same enzyme mixture was applied to a bermudagrass silage-based TMR, feed efficiency was unaffected (Bernard et al., 2010; Queiroz et al., 2011). This highlighted the need for research to develop strategies to increase the hydrolysis of bermudagrass by EFE to improve its quality as a feed for dairy cattle. The objective of the first study was to identify the most promising EFE preparation for increasing the NDF digestibility (**NDFD**) and preingestive fiber hydrolysis of 4-wk regrowth bermudagrass haylage (**BH**). Additional objectives were to use proteomic tools to identify differences in the composition of the EFE and to determine the accuracy of predicting NDFD and measures of preingestive hydrolysis from EFE activity. Our hypothesis was that the EFE treatment will increase the in vitro digestibility, fermentation, and preingestive fiber hydrolysis of BH, but the magnitude of the response will differ with EFE.

MATERIALS AND METHODS

BH Substrate

An established stand of Tifton 85-bermudagrass (*Cynodon dactylon*) in Alachua County, Florida, was staged in June 2010 by mowing to 4 cm of stubble and removing the residue. The field was subsequently fertilized with N (95 kg/ha) and a 4-wk regrowth was harvested on July 7, 2010, by mowing within 1 d to 4 cm of stubble with a Claas 3500 mower conditioner (Claas North America, Omaha, NE). The grass was wilted for 2.5 h in the windrow, rolled into round bales without inoculant addition (John Deere 468 baler, John Deere Co., Moline, IL), wrapped with 7 layers of 6-mm plastic, and ensiled for 53 d. Ensiled bermudagrass was chosen over hay because it is more widely used by the Florida dairy industry due to the humid weather and frequent rainfall hinders proper drying of hay before baling. Representative haylage samples were cored from the bales, dried at 60°C for 48 h, and ground to pass a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA). The OM, NDF, ADF, ADL, and CP concentrations of the haylage were 93.5, 68.1, 34.2, 3.7, and 18.7%, respectively (DM basis) and the DM was 49.4%.

Enzymes

Effects of 12 EFE from 3 companies on in vitro 24-h NDFD were examined in quadruplicate in each of 3 in vitro runs. The enzymatic designations, activities, protein concentrations, application doses, and microbial sources of the EFE are shown in Table 1.

Application rates were suggested by the respective manufacturers. Endoglucanase (**EN**; EC 3.2.1.4), exoglucanase (**EX**; EC 3.2.1.91), xylanase (**XY**; EC 3.2.1.8), and β-glucosidase (**BG**; EC 3.2.1.21) activities were quantified using carboxymethylcellulose, avicel, oat-spelt xylan, and cellobiose as the substrates, respectively (Wood and Bhat, 1988). Ferulic acid esterase (**FE**; EC 3.1.1.73) activity was measured using ethyl ferulate as the substrate (Lai et al., 2009). All activities were measured at 39°C and pH of 6 to mimic ruminal conditions in a lactating dairy cow fed a typical TMR in the United States. In addition, activities were determined at 20°C to approximate room temperature and the conditions of the preingestive hydrolysis study. The same lots of substrates and reagents were used for all enzymatic activity determinations. Protein concentration was measured using the Bio-Rad Protein Assay (Bradford, 1976) with BSA as the standard (Bio-Rad Laboratories, Hercules, CA).

EFE Effects on In Vitro Ruminal Digestibility (Experiment 1)

All EFE were evaluated with a 24-h in vitro ruminal digestibility assay (Goering and Van Soest, 1970) using BH as the substrate. The intent was to attempt to simulate application of the EFE to the bermudagrass before feeding. As described by Krueger and Adesogan (2008), amounts of EFE corresponding to the respective application doses (Table 1) were diluted in 2 mL of 0.1 *M* citrate-phosphate buffer (pH 6) and added to 0.5 g of substrate (in quadruplicate) in a 100-mL polypropylene tube fitted with a rubber stopper containing a one-way gas release valve. The control treatment consisted of only the buffer. Tubes were tapped gently to ensure proper mixing of the EFE solution with the substrate. The mixtures were preincubated at 25°C for 24 h before the addition of rumen fluid media. All tubes and Goering and Van Soest (1970) medium were prewarmed (39°C) and the medium was gassed with CO_2 before ruminal fluid addition. The ruminal fluid collection protocol was approved by the University of Florida Animal Care Research Committee. The ruminal fluid was representatively aspirated from 2 nonlactating, nonpregnant, ruminally cannulated Holstein cows 3 h after consuming a ration of coastal bermudagrass ad libitum supplemented with corn (0.45 kg), cottonseed hulls (0.46 kg), soybean meal (0.90 kg), and a vitamin-mineral mix (35.8 g, DM basis). Immediately after collection, the ruminal fluid was filtered through 4 layers of cheesecloth, gassed with CO_2, and 52 mL of media containing rumen fluid inoculum and Goering and Van