J. Dairy Sci. 98:406–417
http://dx.doi.org/10.3168/jds.2014-8285

Effect of the dose of exogenous fibrolytic enzyme preparations on preingestive fiber hydrolysis, ruminal fermentation, and in vitro digestibility of bermudagrass haylage

J. J. Romero,[1] M. A. Zarate, and A. T. Adesogan[2]
Department of Animal Sciences, Institute of Food and Agricultural Sciences, University of Florida, Gainesville 32608

ABSTRACT

Our objectives were to evaluate the effects of the dose rates of 5 *Trichoderma reesei* and *Aspergillus oryzae* exogenous fibrolytic enzymes (EFE; 1A, 2A, 11C, 13D, and 15D) on in vitro digestibility, fermentation characteristics, and preingestive hydrolysis of bermudagrass haylage and to identify the optimal dose of each EFE for subsequent in vitro and in vivo studies. In experiment 1, EFE were diluted in citrate-phosphate buffer (pH 6) and applied in quadruplicate in each of 2 runs at 0× (control), 0.5× , 1× , 2×, and 3×; where 1× was the respective manufacturer-recommended dose (2.25, 2.25, 10, 15, and 15 g of EFE/kg of dry matter). The suspension was incubated for 24 h at 25°C and for a further 24 h at 39°C after the addition of ruminal fluid. In experiment 2, a similar approach to that in experiment 1 was used to evaluate simulated preingestive effects, except that sodium azide (0.02% wt/vol) was added to the EFE solution. The suspension was incubated for 24 h at 25°C and then 15 mL of water was added before filtration to extract water-soluble compounds. For both experiments, data for each enzyme were analyzed separately as a completely randomized block design with a model that included effects of EFE dose, run, and their interaction. In experiment 1, increasing the EFE dose rate nonlinearly increased the DM digestibility of 1A, 2A, 11C, and 13D and the neutral detergent fiber digestibility (NDFD) of 1A, 2A, 11C, and 13D. Optimal doses of 1A, 2A, 11C, 13D, and 15D, as indicated by the greatest increases in NDFD at the lowest dose tested, were 2×, 2×, 1×, 0.5×, and 0.5×, respectively. Increasing the dose rate of 2A, 11C, and 13D nonlinearly increased concentrations of total volatile fatty acids and propionate (m*M*), decreased their acetate-to-propionate ratios and linearly decreased those of samples treated with 1A and 15D. In experiment 2, increasing the dose rate of each EFE nonlinearly decreased concentrations of netural detergent fiber; also, increasing the dose rate of 1A, 2A, 11C, and 1D nonlinearly increased concentrations of water-soluble carbohydrates and free ferulic acid (μg/g). Application of increasing doses of the EFE increased NDF hydrolysis, NDFD, and ruminal fluid fermentation of bermudagrass haylage, but the optimal dose varied with the EFE.

Key words: fibrolytic enzyme, dairy cattle, bermudagrass, in vitro digestibility, dose

INTRODUCTION

Warm-season grasses are extensively used for cattle production in the southeast United States. Bermudagrass is the most important of such grasses that is used for cattle production (Newman, 2007), but as with other warm-season grasses, the quality of bermudagrass is low (Hanna and Sollenberger, 2007). Exogenous fibrolytic enzyme (**EFE**) treatment has been proposed as a method to improve forage quality and animal performance, but results of published studies have been equivocal (Adesogan et al., 2014). Various enzyme, animal, feed, and management factors influence the efficacy of fibrolytic EFE (Beauchemin et al., 2003; Adesogan et al., 2014), many of which are challenging to control. One factor that is easily controlled is the dose of the EFE. To our knowledge, only 2 studies (Dean et al., 2005; Krueger et al., 2008) have been conducted on effects of the dose of EFE on the nutritive value of bermudagrass. Dean et al. (2005) reported that 48-h in vitro NDF digestibility (**NDFD**) increased quadratically with increasing doses of 1 of 3 cellulase-xylanase EFE applied at the point of ensiling to a 5-wk regrowth of Tifton 85 bermudagrass. Krueger et al. (2008) reported that applying increasing doses of an EFE with high esterase activity to Coastal or Tifton 85 bermudagrass hay had no effect on 6-, 24-, and 48-h in vitro NDFD, except for a linear increase in 6-h NDFD of the Tifton 85 cultivar. More studies are needed to examine effects of EFE dose rates on the quality of bermudagrass hay, silage, and haylage due to the important role of these forages in the diets of dairy and beef cattle in the southeast United States. This is

Received April 24, 2014.
Accepted September 4, 2014.
[1]Current address: Department of Crop Science, North Carolina State University, Raleigh, 1104A Williams Hall, Raleigh, NC 27695-7620.
[2]Corresponding author: adesogan@ufl.edu

because EFE can be ineffective if applied in insufficient or excessive amounts (Sanchez at al., 1996; Beauchemin et al., 2004). Low doses do not fully exploit the hydrolytic potential of EFE, especially during short incubation times. In contrast, excessively high doses decrease availability of substrates for catalysis or accessibility of substrates to these sites by crowding the substrate surface, which reduces the enzymatic hydrolysis rate (Bommarius et al., 2008). In the rumen, competition between excessively high doses of EFE and ruminal endogenous cellulolytic bacterial enzymes for substrates can decrease fiber digestibility (Nsereko et al., 2002) and consequently reduce animal performance (Kung et al., 2000). Therefore, optimization of the EFE dose is critical for using EFE to improve the digestibility of forages.

The objective of our study was to determine the optimum dose of 5 EFE (1A, 2A, 11C, 13D, and 15D) that were selected as the most promising of 12 candidates from 3 companies at improving the NDFD of bermudagrass haylage (**BH**; Romero et al., in press). The hypothesis was that increasing the dose of each EFE would increase the NDFD of BH in a quadratic manner.

MATERIALS AND METHODS

Bermudagrass Substrate

An established stand of bermudagrass (*Cynodon dactylon* cultivar Tifton 85) in Alachua, Alachua County, Florida, was staged in June 2010, by mowing to a 4-cm stubble and removing the residue. The field was fertilized subsequently with N (95 kg/ha) and the grass was allowed to regrow for 4-wk such that the harvest day was July 7, 2010. On harvest day, the grass was mowed within 1 d to a 4-cm stubble with a Claas 3500 mower conditioner (Claas North America, Omaha, NE). The grass was wilted for 2.5 h in the windrow and then rolled into round 280-kg round bales without inoculant addition. Bales were wrapped with 7 layers of 6-mm plastic and ensiled for 53 d. Ensiled bermudagrass was chosen over hay because it is more typically used in this form by dairy producers due to the high humidity and frequent summer rainfall in Florida (Staples, 2003). Representative haylage samples were collected as substrate for our study, dried at 60°C for 48 h, and ground to pass the 1-mm screen of a Wiley mill (Arthur H. Thomas, Philadelphia, PA). The haylage had 49.4% of DM and 93.5, 68.1, 34.2, 3.7, and 18.7% of OM, NDF, ADF, ADL, and CP, respectively (DM basis).

Enzymes

Five previously selected (Romero et al., in press) commercial and experimental EFE preparations provided by 3 manufacturers were examined at 4 doses (0×, 0.5×, 1×, and 2×, where 1× was the manufacturer's recommended dose). Table 1 lists the enzymatic activities and protein concentrations, form, doses, and biological sources of the EFE preparations. Endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91), xylanase (EC 3.2.1.8), and β-glucosidase (EC 3.2.1.21) activities were quantified using carboxymethyl cellulose, avicel, oat-spelt xylan, and cellobiose as artificial substrates, respectively (Wood and Bhat, 1988). Enzymes were incubated with the respective substrates for 15, 120, 5, and 30 min as suggested by Colombatto and Beauchemin (2003). Glucose was used as the standard for measuring endoglucanase, exoglucanase, and β-glucosidase activity, whereas xylose was used as that for measuring xylanase activity. Ferulic acid esterase (EC 3.1.1.73) activity was measured using ethyl ferulate as the substrate with an incubation period of 5 min with the enzymes (Lai et al., 2009). All activities were measured at 39°C and a pH of 6 to mimic conditions in the rumen as previously recommended for enzyme studies for lactating dairy cows (Colombatto and Beauchemin, 2003). Activities measured at 20°C and pH 6 were included as a reference for the simulated preingestive hydrolysis assay that was conducted at 25°C. Protein concentration was measured using the Bio-Rad protein assay (Bradford, 1976) with BSA as the standard (Bio-Rad Laboratories, Hercules, CA).

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. As described by Krueger and Adesogan (2008), EFE were diluted in 0.1 *M* citrate-phosphate buffer (pH 6) and 2 mL of the solution containing the requisite EFE dose was applied to 0.5 g of substrate. The 0× (control) treatment consisted only of the citrate-phosphate buffer and the substrate. Treatments were applied in quadruplicate to the substrate in 100-mL polypropylene tubes capped with a rubber stopper fitted with a one-way gas-release valve. Two blank tubes per treatment, containing no substrate, were used to correct for the substrate contribution from the ruminal inoculum. Tubes were tapped gently to ensure proper mixing of EFE solution with the substrate and the suspensions were subsequently incubated at 25°C for 24 h before addition of buffered ruminal fluid. The ruminal fluid was representatively collected by aspiration 3 h after feeding (0800 h) from 2 nonlactating, nonpregnant, ruminally cannulated Holstein cows consuming a ration consisting of coastal bermudagrass hay ad libitum supplemented with corn (0.45 kg), cottonseed hulls (0.46 kg), soybean meal (0.90 kg), and a