



## Improving the performance of dairy cattle with a xylanase-rich exogenous enzyme preparation

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### ABSTRACT

The objective of this experiment was to examine effects of adding 2 exogenous fibrolytic enzymes (EFE) to the total mixed ration (TMR) on the performance of lactating dairy cows (experiment 1) and the kinetics of ruminal degradation of the diet (experiment 2). Twelve EFE had been screened in a series of *in vitro* assays that identified the most potent EFE and their optimal doses for increasing the digestibility of bermudagrass. In experiment 1, 66 Holstein cows ( $21 \pm 5$  d in milk) were grouped by previous milk production and parity (45 multiparous and 21 primiparous) and assigned randomly to 1 of the following 3 treatments: (1) control (CON, untreated), (2) Xylanase Plus [2A, 1 mL/kg of TMR dry matter (DM); Dyadic International, Jupiter, FL], and (3) a 75:25 (vol/vol) mixture of Cellulase Plus and Xylanase Plus EFE (3A, 3.4 mL/kg of TMR DM; Dyadic International). The EFE were sprayed twice daily onto a TMR (10% bermudagrass silage, 35% corn silage, 5% alfalfa-orchardgrass hay mixture, and 50% concentrates; DM basis) and fed for a 14-d training and covariate period and a 70-d measurement period. Experiment 2 aimed to examine the *in situ* DM ruminal degradability and ruminal fermentation measurements of the diets fed in experiment 1. Three ruminally fistulated lactating Holstein cows were assigned to the diets. The experiment had a  $3 \times 3$  Latin square design with 23-d periods. In experiment 1, application of 2A increased intakes (kg/d) of DM (23.5 vs. 22.6), organic matter (21.9 vs. 20.9), and crude protein (3.9 vs. 3.7) and tended to increase yields (kg/d) of fat-corrected milk (41.8 vs. 40.7) and milk fat (1.48 vs. 1.44). In particular, 2A increased milk yield (kg/d) during wk 3

(41.2 vs. 39.8, tendency), 6 (41.9 vs. 40.1), and 7 (42.1 vs. 40.4), whereas 3A increased milk yield (kg/d) during wk 6 (41.5 vs. 40.1, tendency), 8 (41.8 vs. 40.0), and 9 (40.9 vs. 39.5, tendency). In experiment 2, EFE treatment did not affect ruminal DM degradation kinetics or ruminal pH, ammonia-N, and volatile fatty acid concentration. Application of 2A to the bermudagrass-based TMR increased DM intake and milk production, implying that this EFE could be used to increase the performance of lactating dairy cows fed diets containing up to 10% bermudagrass.

**Key words:** fibrolytic enzyme, dairy cattle, bermudagrass

### INTRODUCTION

Several studies have examined the efficacy of using exogenous fibrolytic enzymes (EFE) to improve forage quality and ruminant animal performance but the results have been equivocal (Beauchemin et al., 2003; Adesogan et al., 2014; Meale et al., 2014). Supplementing EFE to nonruminant livestock can improve feed efficiency and allows for the inclusion of ingredients with a higher fiber concentration than corn (Bedford and Partridge, 2010). However, EFE use in ruminant diets has been limited due to inconsistent animal performance responses. This inconsistency is due partly to the wide array of conditions under which EFE are tested and the limited understanding of their mode of action (Beauchemin and Holtshausen, 2010). In most cases, enzymatic activities supplied by EFE are not novel to the rumen and therefore EFE act on the same plant cell wall targets as endogenous ruminal enzymes (Wang and McAllister, 2002). Consequently, EFE effects seem greater when the endogenous capacity of the rumen to digest fiber is decreased. This occurs in dairy cows in early lactation (Schingoethe et al., 1999; Knowlton et al., 2002), which typically have relatively low ruminal fiber digestion due to factors such as low ruminal pH and high total-tract rate of passage (Mouriño et al.,

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2001; Cochran et al., 2007). Furthermore, EFE seem to be more effective at improving the productivity of cattle fed ad libitum relative to their effects on cattle fed for restricted intake (Yang et al., 2000; Beauchemin and Holtshausen, 2010), where the longer ruminal retention times result in more complete digestion of fiber.

Previous studies have examined effects of applying EFE to major forage crops such as corn silage and alfalfa and identified promising candidates for increasing in vitro NDF digestibility (NDFD; Eun and Beauchemin, 2007; Lynch et al., 2014). However, in tropical and subtropical regions such as the southeastern United States, perennial warm-season grasses are more agronomically and economically suited than alfalfa (Bernard et al., 2010) but their high fiber content and low digestibility limit dairy cow productivity (Hanna and Sollenberger, 2007). Consequently, such forages and the diets that include them are potentially good candidates for improvement by EFE. Arriola et al. (2011) reported that adding an EFE to a corn silage and alfalfa hay-based TMR for dairy cows increased NDF digestibility by 6%, and increased feed efficiency (FCM:DMI) by about 16%. However, when the same EFE (Queiroz et al., 2011) and another one (Bernard et al., 2010) were applied to a bermudagrass silage-based TMR, none of the performance measures was increased. Consequently, strategies that increase effectiveness of EFE at enhancing the digestion of bermudagrass and the performance of cows fed bermudagrass-based diets are needed. For this purpose, we developed a strategy to identify promising EFE candidates and the optimal conditions for their use. To choose the best EFE to test on a bermudagrass-based TMR for dairy cows, we conducted a series of in vitro experiments. The aims were to determine the most promising EFE for increasing the NDFD of bermudagrass haylage and its optimal dose, and to examine whether its efficacy would be improved by metal ion cofactors. These experiments (1) identified 5 of 12 EFE that increased the NDFD of bermudagrass haylage by at least 8% (Romero et al., 2015c), (2) determined the lowest doses of each of the 5 selected EFE that maximized the NDFD of bermudagrass haylage (Romero et al., 2015b), and (3) showed that metal ion cofactors such as manganese synergistically increased the effects some EFE on the NDFD of bermudagrass haylage but did not affect those of others (Romero et al., 2015a). These studies revealed that the most promising EFE for increasing the NDFD of bermudagrass haylage was EFE 2A (Xylanase Plus, Dyadic International, Jupiter, FL), and unlike others, its efficacy was not synergistically improved by adding cofactors. A final in vitro assay (Romero et al., 2014) validated the promise of EFE 2A by showing that it was more effective at increasing the NDFD of corn silage, an alfalfa-orchardgrass hay

mixture, or bermudagrass haylage than EFE 3A [75:25 (vol/vol) mixture of Cellulase Plus and Xylanase Plus, respectively, Dyadic International], which improved the feed efficiency of dairy cows (Arriola et al., 2011).

The objective of the present experiment was to examine effects of adding 2 EFE (2A or 3A) to a TMR containing bermudagrass haylage, corn silage, and an alfalfa-orchardgrass hay mixture, on DMI, ruminal fermentation, kinetics of ruminal digestion, and performance of lactating dairy cattle. We hypothesized that adding either of the EFE to the diet would increase the performance of cows, but EFE 2A would produce a greater response because it increased the NDFD of the dietary forages to a greater extent than EFE 3A in the screening studies.

## MATERIALS AND METHODS

### Location, Housing, and Weather

The study was conducted at the University of Florida Dairy Unit (Hague) from February to August 2013 (26 wk). Cows were housed in a freestall, open-sided barn fitted with 2 rows of fans (1 fan/6 linear meters) for cooling. Fans were equipped with low-pressure water nozzles, and both fans and nozzles were activated once ambient temperature reached 21.1°C. During the experiment, the mean temperature and relative humidity were 21.4°C and 80.9%, with minima of 3.2°C and 49.0% and maxima of 28.3°C and 97.0%, respectively (FAWN, 2013). Stalls (1.14 × 2.31 × 1.21 m) were bedded with sand for alleviation of hoof and leg stress. The areas between feed bunks and freestalls were flushed out twice daily by an automated flushing system.

### Animals and Treatments

The University of Florida Institute of Food and Agricultural Sciences Animal Research Committee approved the protocol for this study. In experiment 1, 66 lactating Holstein cows in early lactation (21 ± 5 DIM) were grouped by milk production 5 d before enrollment (16–20 ± 5 DIM) and parity (45 multiparous and 21 primiparous). Cows were assigned randomly to 1 of the following 3 treatments: (1) Control (CON, untreated), (2) Xylanase Plus (2A, 1 mL/kg of TMR DM), and (3) a 75:25 (vol/vol) mixture of Cellulase Plus and Xylanase Plus EFE (3A, 3.4 mL/kg of TMR DM). The 3A and 2A EFE were called EFE 3A and 2A, respectively, in the laboratory screening studies (Romero et al., 2015b,c), and they were sourced from nonrecombinant *Trichoderma reesei* (Dyadic International). Commercial enzyme product names for crude extracts should be taken as proper nouns and not as specific Enzyme

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