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Effects of different mixtures of fibrolytic enzymes on digestion and fermentation of bahiagrass hay☆

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Abstract

This study was designed to determine the best combination of ferulic acid esterase (FAE), cellulose (CEL), and xylanase (XYL) for hydrolyzing mature bahiagrass in the absence of rumen fluid, and to evaluate effects of the best multienzyme cocktails on digestibility and fermentation of bahiagrass in the presence of rumen fluid. In Experiment 1, 42 enzyme mixtures of pure CEL, XYL, and FAE were dissolved in a citrate phosphate buffer (pH 6.0) and added to 0.5 g of a 12-week regrowth of bahiagrass (*Paspalum notatum*) hay. In each mixture, each enzyme was applied at 0, 0.5, 1, or 2 g/100 g of dry matter (DM) except that FAE was not applied at 2 g/100 g DM. Dry matter (DM) disappearance was measured after the solutions were incubated at 39 °C for 24 h. Each enzyme was applied in triplicate and the experiment was repeated thrice. The highest DM disappearance was with enzyme cocktails containing 2, 2, and 0 (2–2–0) or 2, 2, and 1 (2–2–1) g of XYL, CEL, and FAE, respectively/100 g of forage DM. In Experiment 2, the hay was treated with nothing (Control), a commercial enzyme preparation (Depol 740, D740L) containing all three enzymes, or the 2–2–0

Abbreviations: 2–2–0, enzyme mixture containing 2, 2, and 0 g/100 g of forage DM of cellulase, xylanase, and ferulic acid esterase, respectively; 2–2–1, enzyme mixture containing 2, 2, and 1 g/100 g of forage DM of cellulase, xylanase, and ferulic acid esterase, respectively; ADF, acid detergent fiber; *B*, extent of gas production; BAH, Pensacola bahiagrass; *c*, rate of fermentation of *B*; CEL, cellulase; CP, crude protein; D740L, Depol 740L enzyme; DM, dry matter; DMD, *in vitro* DM digestibility; FA, ferulic acid; FAE, ferulic acid esterase; *L*, lag time; NDF, neutral detergent fiber; NDFD, *in vitro* NDF digestibility; *P*, gas production at time *t*; *t*, time incubated in ruminal fluid; VFA, volatile fatty acids; XYL, xylanase

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and 2–2–1 enzyme cocktails from Experiment 1. The enzyme mixtures were dissolved in 1 ml of citrate–phosphate buffer (pH 6.0) and sprayed on 0.5 g of bahiagrass hay in triplicate in each of two runs. Samples were fermented in buffered-rumen fluid using a wireless automated gas production system for either 24 or 96 h in duplicate runs, and residues were analyzed for DM and volatile fatty acid concentrations. The kinetics of 96 h fermentation were determined by fitting a non-linear model to the data. For the 24 h fermentations, enzyme application did not increase DM or NDF digestibility but all enzyme mixtures decreased acetate concentration ($P<0.001$) and increased ($P<0.04$) propionate and butyrate concentrations. Consequently, all enzyme mixtures decreased ($P<0.001$) the acetate to propionate ratio. For the 96 h fermentations, all enzyme mixtures decreased the lag phase ($P<0.05$) but did not impact DM or NDF digestibility, gas pool size or fermentation rate. This study identified certain mixtures of XYL, CEL, and FAE that increased 24-h DM disappearance of bahiagrass in the absence of rumen fluid. Application of these multienzyme cocktails reduced the lag phase and improved efficiency of fermentation of mature bermudagrass in rumen fluid but did not improve extent of DM digestion.

Keywords: Ferulic acid esterase; Xylanase; Cellulase; Gas production; Fermentation

1. Introduction

Tropical C_4 grasses are the mainstay of ruminant livestock production in warm climates, but the nutritive quality of such grasses is typically poor because they contain higher concentrations of phenolic compounds and lignin than temperate grasses (Akin, 1986). Cross-linking of lignin to cell wall polysaccharides through ferulic acid bridges is a key mechanism by which lignin limits cell wall digestion in plants (Jung and Allen, 1995). Ferulic acid esterases (FAEs) can release ferulic acid (FA) bound to arabinose side chains of hemicellulose (Faulds and Williamson, 1994), allowing further degradation of the cell wall by other polysaccharidases. Addition of FAE to other cell wall-degrading enzymes, like xylanase and cellulase, produces a synergistic effect on degradation of plant cell walls (Faulds and Williamson, 1995; Bartolome et al., 1997; Yu et al., 2002a, 2003, 2005), due to increased accessibility to digestible cell wall components by rumen microorganisms. Previous research showed that commercially available mixtures of FAE, and other enzymes, increased the initial phase of the digestion of C_4 grasses, but did not substantially increase the extent of digestion (Krueger et al., 2003, 2008). There is a need to determine the best ratio of FAE to other fibrolytic enzymes for optimizing the digestion and utilization of C_4 grasses in ruminant livestock diets. The objectives of this study were to determine the best two combinations of FAE, cellulase and xylanase for hydrolyzing mature bahiagrass in the absence of rumen fluid, and to evaluate effects of such enzyme cocktails on digestibility and fermentation of the forage in the presence of rumen fluid.

2. Materials and methods

2.1. Forage and enzymes

A representative sample (3 kg) of hay produced from a 12-week regrowth of Pensacola bahiagrass (BAH; *Paspalum notatum*) was sampled from 450-kg round bales at the Univer-