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Effects of an exogenous fibrolytic enzyme preparation on *in vitro* ruminal fermentation of three forages and their isolated cell walls☆

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Abstract

This study was completed to investigate whether a fibrolytic enzyme preparation with xylanase and cellulase activities (Fibrozyme™, Alltech Inc., Nicholasville, KY, USA), could stimulate *in vitro* rumen fermentation of alfalfa hay, grass hay and barley straw, and their isolated cell walls as neutral detergent fibre (NDF). Samples (500 mg) of each substrate were incubated in 120 ml bottles with 50 ml of buffered rumen fluid from sheep at 39 °C. The enzyme preparation was added directly to the bottles at the beginning of the incubation at levels of: 0 (control; CON), 50 mg/g substrate DM (LOWFIB) and 100 mg/g substrate DM (HIGHFIB). Effects on volatile fatty acid (VFA) production depended on substrate and incubation time. After incubation for 5 h, enzyme addition increased ($P<0.05$) production of VFA and acetate compared to CON for both alfalfa hay and its isolated cell wall, and increased ($P<0.05$) propionate and butyrate production in isolated cell wall, but reduced ($P<0.05$) them in alfalfa hay. After incubation for 10 h, addition of both doses of enzyme preparation increased ($P<0.05$) VFA and propionate production *versus* CON for both alfalfa hay and its isolated

Abbreviations: aNDF, neutral detergent fibre; ADF, acid detergent fibre; DM, dry matter; ND, neutral detergent; VFA, volatile fatty acids

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cell wall, and decreased (P<0.05) acetate to propionate ratios with both substrates, with intermediate values for LOWFIB and highest for HIGHFIB. After 24 h of incubation, there were no effects due to enzyme treatment or enzyme dose on rumen variables. Addition of both doses of supplemental enzyme increased (P<0.05) VFA, acetate and propionate production after 5 and 10 h incubation in both grass hay and its isolated cell wall, with increases being greater (P<0.05) for HIGHFIB *versus* LOWFIB in all variables. Both doses of enzyme addition reduced (P<0.05) the acetate to propionate ratio at 5 and 10 h of incubation. After 24 h of incubation, enzyme addition increased (P<0.05) VFA, acetate and butyrate production in both grass hay and its isolated cell wall *versus* CON, with some dose effects on both substrates. Enzyme supplementation also increased (P<0.05) VFA, acetate and propionate production *versus* CON in barley straw and its isolated cell wall at 5 and 10 h of incubation, with dose dependent effects after incubation for 5 h with higher (P<0.05) increases for HIGHFIB *versus* LOWFIB in both substrates. Both enzyme doses also reduced (P<0.05) the acetate to propionate ratio in both barley straw and its cell wall after incubation for 5 h. Production of total VFA and butyrate increased (P<0.05) with both doses of enzyme after incubation for 24 h. Final pH, ammonia-N and disappearance of aNDF and ADF did not differ among enzyme treatments after incubation of grass hay and barley straw or their isolated cell wall for 24 h. Results indicate that this fibrolytic enzyme preparation stimulated *in vitro* fermentation of substrates at short (5 and 10 h), but not at long (24 h) incubation times, and that effects depended on both the dose used and the presence of neutral detergent soluble components in the substrate.

Keywords: Exogenous fibrolytic enzymes; Cell walls; Batch cultures; Ruminal fermentation

1. Introduction

Effects of exogenous fibrolytic enzymes on ruminant productivity have been actively researched in the past 15 years. Improvements in ruminant production with supplemental fibrolytic enzymes are generally attributed to increased ruminal fibre digestion, but the mechanism by which this increase occurs is not understood. Numerous potential mechanisms have been proposed (McAllister et al., 2001; Beauchemin et al., 2001, 2003) including preingestive and ruminal effects such as direct hydrolysis, structural changes in the fibre, increased ruminal microbial attachment, stimulation of ruminal microbial populations and synergism with ruminal microbial enzymes. Differences in fibre level, composition and structure among forages are likely reasons for the widely recognized specificity between exogenous fibrolytic enzymes and feed composition (Wallace et al., 2001; Colombatto et al., 2003a,b). Most evidence for the specificity between enzyme activity and feed composition comes from studies that compared digestion characteristics of various intact forages *in vitro* (Eun et al., 2006; Giraldo et al., 2007, in press). However, the potential role of neutral detergent (ND) soluble components in this interaction has not been considered.

The objectives for this study were to evaluate the importance, if any, of readily fermentable, ND soluble components on supplemental enzyme efficacy by characterizing enzyme effects in alfalfa hay, grass hay and barley straw, as well as their respective isolated cell walls devoid of ND soluble components. Enzyme was added without prior incubation with feed in order to avoid prefeeding effects.