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Assessment of the efficacy of varying experimental exogenous fibrolytic enzymes using in vitro fermentation characteristics

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Abstract

This study evaluated a series of recombinant, single activity experimental enzyme products including 13 endoglucanases (END) and 10 xylanases (XY), for their potential to improve in vitro ruminal degradation of alfalfa hay in two experiments. Based on the endoglucanase or xylanase enzymatic activities measured using complex substrates at the optimal conditions (pH 5.4, 37 °C) for the enzymes, a dose level (1 unit/g dry matter [DM]) was chosen for addition of enzymes to substrate. Enzyme products, re-suspended with water, were added to alfalfa hay (0.5 or 1.0 g DM) in culture vials in six replications. Anaerobic buffer medium (20 or 40 ml) adjusted to pH 6.0 and strained ruminal fluid (5 or 10 ml) were sequentially added to the vials and incubated for 18 h. Headspace gas production (GP) was measured throughout the incubation, and degradability of organic matter (OMD) and fibre and volatile fatty acid (VFA) concentrations were determined after 18 h of incubation. The enzyme products had a wide range of added endoglucanase or xylanase activities when determined using

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Abbreviations: ADF, acid detergent fibre; ADFD, ADF degradability; DM, dry matter; DMD, DM degradability; END, experimental endoglucanse enzyme; GP, gas production; aNDF, neutral detergent fibre; aNDFD, aNDF degradability; NH_3N, ammonia N; OM, organic matter; OMD, OM degradability; VFA, volatile fatty acids; XY, experimental xylanase enzyme

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pure substrates and physiological conditions typical of the rumen (pH 6.0, 39 °C). In experiment 1, many END, and some XY, products increased GP and OMD. The correlation between added endoglucanase activity determined at ruminal conditions and OMD improvement was high ($r = 0.71$; $P<0.01$), whereas added activity of xylanase was not associated with OMD improvement. Two END and two XY products selected from experiment 1 were further assessed because they substantially improved GP and OMD. In experiment 2, all enzyme treatments, alone or in combination, increased total GP and DM and fibre degradabilities ($P<0.05$). However, the combinations of END and XY did not increase degradation of alfalfa beyond that of the component enzymes. Total VFA production was not affected by enzyme treatments although some products changed the acetate to propionate ratio. Experimental exogenous enzyme products with either endoglucanase or xylanase activity substantially improved in vitro ruminal degradation of alfalfa hay, but further improvement by combining these activities did not occur.

Keywords: Endoglucanase; Xylanase; Alfalfa Hay

1. Introduction

Use of exogenous enzymes in ruminant feeds has increased forage utilization, improved production efficiency, and reduced nutrient excretion (Beauchemin et al., 2003). However, the effectiveness of enzyme products is highly variable (Colombatto et al., 2003a). Part of this variability may be due to the types and activities of enzymes, which can vary widely depending on the source organism, growth substrate, and culture conditions employed (Considine and Coughlan, 1989; Gashe, 1992). To date, it has not been possible to predict the effectiveness of enzyme products for ruminants based on their enzymatic activities (Colombatto et al., 2003a).

There is a need to identify the key enzymatic activities, and the optimal concentrations, to improve feed digestion in ruminants. Most fibrolytic enzyme products are commercially produced for non-feed applications, which include use by the food, pulp and paper, textile, fuel and chemical industries (Bhat and Hazlewood, 2001). In ruminant applications, the enzymes must act synergistically with the endogenous enzyme activities of the rumen microbes (Morgavi et al., 2000). Thus the key activities needed to improve forage fibre degradation likely differ from those needed for other applications (Wallace et al., 2001; Colombatto et al., 2003a).

The focus of most enzyme-related research for ruminants has been on plant cell wall degrading enzymes, cellulases and hemicellulases, which degrade the major plant structural polysaccharides, cellulose and hemicellulose. We hypothesized that because cellulases and hemicellulases act synergistically to hydrolyze plant cell wall, enzymes with cellulase and hemicellulase activities may be more efficacious in degrading forage compared with enzymes with either single activity. This study assessed 13 experimental endoglucanases (END) and 10 experimental xylanases (XY) containing single enzyme activity for their effects on in vitro ruminal degradation of alfalfa hay. Among them, two END and two XY products were selected based on initial screening, and their combined effects on in vitro degradation of alfalfa hay were determined.