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Use of an *in vitro* fermentation bioassay to evaluate improvements in degradation of alfalfa hay due to exogenous feed enzymes

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Abstract

An *in vitro* batch culture assay was used to determine the efficacy of five developmental feed enzyme products in terms of improving ruminal degradation of alfalfa hay. A second objective was to establish whether the length of time that alfalfa hay was incubated with ruminal fluid and buffer in a batch culture *in vitro* system influenced enzyme efficacy. The experiment was conducted as a completely randomized design in four replications with treatments arranged as a 3 (incubation times) × 5 (enzyme products) factorial. Two of the enzyme products (P1 and P2) were proteases, while the other three products (F1, F2, and F3) contained differing proportions of endoglucanases and xylanases. Milled alfalfa hay was incubated with buffer, ruminal fluid and enzyme product (1.5 mg/g of dry matter; DM). Gas production (GP) and degradability of DM and fibre were measured after terminating the incubation at 12, 18 and 24 h. At all incubation times, P1 increased GP by 5.6–7.9%, but GP was not affected by P2. Of the polysaccharidases evaluated, F1 and F2 increased GP by 3.7–10.6% at all incubation times, while F3 had no effect. Improvements in fibre degradability depended upon enzyme product and incubation time. Degradation of neutral detergent fibre (aNDF) was increased at 12 h of incubation using F1 and F2 (12–13% increase), at 18 h of incubation using P1 (11%) and F1 (7%), and at 24 h by F1 (10.5%), F2 (16.5%), and F3 (11.3%). Improvements in acid detergent fibre degradation ranged from 17.5 to 44.4% for these enzymes, depending upon incubation time.

Abbreviations: ADF, acid detergent fibre; DM, dry matter; GP, gas production; aNDF, neutral detergent fibre; NH_3N, ammonia N; VFA, volatile fatty acids

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Three of the enzyme products (F1, F2 and F3) decreased acetate to propionate ratio, suggesting that improvements in fibre degradation would increase availability of glucose precursors to the animal. Larger improvements in degradability of alfalfa hay occurred for enzyme products containing mainly fibrolytic, rather than proteolytic, activity. An *in vitro* bioassay that consists of incubating forage samples in the presence of ruminal fluid, buffer and enzyme can be a useful means of screening efficacy of exogenous feed enzymes for ruminants. Cost and labour of the assay can be reduced by using a single incubation time of 24 h to assess enzyme effects on GP and degradability of DM and fibre.

Keywords: Feed enzymes; Gas production; *In vitro* degradability; Forage digestibility

1. Introduction

There is increasing evidence that exogenous feed enzymes enhance forage digestion by ruminants (Schingoethe et al., 1999; Eun and Beauchemin, 2005). Therefore, use of exogenous enzymes in ruminant diets holds promise as a means of increasing feed utilization and improving production efficiency (Beauchemin et al., 2003). Responses to enzyme supplementation are expected to be largest for dairy cows in early lactation due to the increased need for digestible energy (Schingoethe et al., 1999). However, dairy cattle response to enzyme supplementation of diets has been variable (Kung et al., 2002; Vicini et al., 2003), which limits the cost effectiveness of the technology. There is a need to predict the effectiveness of enzyme products prior to conducting animal feeding studies.

For enzymes to improve forage degradation, the array of enzyme activities supplemented must be specific to the chemical composition of the targeted forage, due to the specificity of enzymes for their substrate (White et al., 1993). Furthermore, exogenous enzymes work in synergy with rumen microbial enzymes, which increases their hydrolytic potential within the rumen (Morgavi et al., 2000). To date, it has not been possible to predict the potential of exogenous feed enzymes to increase cell wall degradation in the rumen based on their biochemical characterization alone (Colombatto et al., 2003). Consequently, *in vitro* systems including batch culture incubations that permit measurements of fibre degradability and gas production (GP) have been used to identify effective enzyme candidates (Eun and Beauchemin, 2005). *In vitro* methods are less expensive, less time consuming, and allow more control of experimental conditions than *in vivo* experiments. Furthermore, *in vitro* systems can accommodate a large number of enzyme candidates.

Alfalfa forage is fed to dairy cattle in many countries, yet limited information is available on the potential to improve its utilization by using exogenous enzymes. Beauchemin et al. (1995) reported that adding an enzyme product containing xylanase and cellulase activities to alfalfa hay increased average daily gain of growing beef cattle by up to 30%. Nsereko et al. (2000) reported that enzyme products containing xylanases and esterases had stimulatory effects on fibre degradation of alfalfa hay *in vitro*, whereas Colombatto et al. (2003) indicated that some xylanases and proteases improved *in vitro* degradation of alfalfa hay. However, further research is warranted to establish the effectiveness of exogenous enzymes in improving alfalfa utilization by ruminants.