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Use of fibrolytic enzymes additives to enhance *in vitro* ruminal fermentation of corn silage

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ABSTRACT

Two experiments were conducted to evaluate the effect of four enzyme additives on ruminal fermentation of corn silage using a 48 h batch culture *in vitro* assay with buffer and ruminal fluid. Experiment 1 (Exp. 1) and Experiment 2 (Exp. 2) were conducted as completely randomized designs each with two runs and four replicates. The enzyme additives (E1, E2, E3, and E4) were commercial products that provided a range in endoglucanase, exoglucanase, and xylanase activities. For both xylanase (birch wood and oat spelt substrate) and endoglucanase (carboxymethylcellulose substrate), the enzyme products (per ml) were ranked E4 > E1 > E2 > E3. In Exp. 1, the four enzymes were added at 0, 2, 4, and 8 μl/g of corn silage dry matter (DM), whereas in Exp. 2 enzymes were added at 0, 0.5, 1, 2, and 4 μl/g DM. Gas production (GP) was measured at 3, 6, 12, 18, 24, and 48 h after incubation. Disappearance of DM (DMD), neutral detergent fiber (NDFD), and acid detergent fiber (ADFD), and volatile fatty acid concentrations (VFA; total and individual molar proportions) were determined after 24 and 48 h. In Exp. 1, E1 and E2 had higher NDFD and ADFD at 24 and 48 h of incubation ($P < 0.001$) compared with E3 and E4. Increasing dose rate increased NDFD and ADFD for all enzymes (except ADFD for E4 at 48 h), with the optimum dose rate dependant on the enzyme additive (dose × enzyme; $P < 0.01$). There were some treatment effects on DMD and total GP at 24 and 48 h, but these responses were not consistent with responses in NDFD and ADFD. Experiment 2 was conducted to confirm the effects and optimum dose rate of each enzyme additive. In Exp. 2, DMD was not affected by enzyme after 24 and 48 h incubation. There were no enzyme × dose interactions for DMD, NDFD, or ADFD after 24 or 48 h of incubation (except for ADFD at 48 h). After 24 h, DMD, NDFD, and ADFD increased linearly with increasing dose ($P < 0.05$); after 48 h DMD increased linearly, whereas NDFD increased quadratically with increasing enzyme dose ($P < 0.05$). The ADFD increased linearly after 48 h for E3 and E4, but after 48 h ADFD increased quadratically for E1 and E2. Total GP was consistently lowest for E4 at both incubation times ($P < 0.05$). There were no enzyme × dose interactions ($P > 0.05$) for any of the fermentation variables at either 24 or 48 h of incubation in Exp. 2. There were differences amongst the additives for total VFA at 24 and 48 h ($P \leq 0.05$); increasing enzyme dose decreased total VFA after 24 h but increased total VFA at 48 h, such that all doses were higher than the control ($P < 0.001$). Overall, the enzyme additives increased NDFD and ADFD of corn silage *in vitro*; however, E1 and E2 were more effective than E3 or E4. Responses to increasing dose of enzyme

Abbreviations: ADF, acid detergent fiber; ADFD, acid detergent fiber disappearance; DM, dry matter; GP, gas production; NDF, neutral detergent fiber; NDFD, neutral detergent fiber disappearance; TGP, total cumulative gas production; VFA, volatile fatty acid

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were generally linear or curvilinear, and the optimum dose rate differed amongst the products evaluated. Evaluation of the enzymes at 24 and 48 h generally led to the same ranking of the additives, and the degradation of NDF and ADF was more useful in differentiating the enzymes compared with DM and total GP.

1. Introduction

Fibrolytic enzyme feed additives have potential to improve fiber digestion and productivity of ruminants. Forages are high in fiber content, which can limit intake and digestibility of feed by ruminants (Jung and Allen, 1995). Rumen microorganisms produce enzymes that hydrolyze fiber; however, the complex cell wall structure and limited residence time of forage in the rumen limit the extent of fiber digestion by ruminants (Wang and McAllister, 2002). Many studies have evaluated the use of fibrolytic enzyme additives to overcome this limitation (as reviewed by Beauchemin et al. (2003)), with most research focused on cellulases and xylanases that degrade cellulose and hemicellulose, respectively, the major constituents of plant cell walls.

Supplemental fibrolytic enzyme additives have been shown to improve *in vitro* fiber digestion and enhance the nutritive value of both low (Yang and Xie, 2010) and high (Eun et al., 2007) quality forages. Eun and Beauchemin (2007) and Eun et al. (2007) used alfalfa and corn silage as substrates and reported that supplemental enzymes increased digestion of dry matter (DM) and fiber when assessed *in vitro*, which was also observed in continuous culture (Colombatto et al., 2003) and *in vivo* (Rode et al., 1999; Yang et al., 2000). In a study that used grass hay:concentrate (600:400 g/kg DM) as the substrate, fibrolytic enzymes increased total bacterial numbers (Giraldo et al., 2007), and cellulolytic bacteria were increased in rumen simulation (*i.e.*, Rusitec) fermenters using barley grain and alfalfa hay as substrates (Wang et al., 2001). *In vivo* studies have also shown positive responses when supplemental fibrolytic enzymes were fed to ruminants (Arriola et al., 2011; Holtshausen et al., 2011). Although some studies have demonstrated positive effects when using fibrolytic enzymes in ruminant feeds, many other studies have shown inconsistent effects, or no effects on *in vivo* digestibility or animal performance (Knowlton et al., 2002; Lewis et al., 1999; Vicini et al., 2003).

It has been suggested that enzyme additives vary in effectiveness depending upon factors such as enzyme activity, type and dose of enzyme, type of diet, enzyme application method, and animal physiological status (Beauchemin et al., 2003). Thus, a major limitation to widespread commercial use of enzyme technology for ruminants is the uncertainty of effectiveness of enzyme products, as well as the variability in response for a given product depending upon the diet and feeding conditions. It is not yet possible to predict the potential effects of feed enzymes from their biochemical characterization alone (Beauchemin et al., 2004). Thus, conducting an *in vitro* bioassay that reflects the conditions of the rumen can be a useful means of identifying ideal enzyme candidates for use in feeding trials (Beauchemin et al., 2004).

Our project focused on corn silage because it is fed to cattle in many parts of the world and has relatively high nutritive value. While some previous feeding studies have evaluated supplemental enzymes using corn silage based diets (*e.g.*, Arriola et al., 2011), it is not clear what enzyme activities and doses are most effective. It is important to establish optimum dose rate of specific enzyme additives because dose rate directly affects the cost:benefit ratio of feeding enzymes to dairy cows to improve forage digestibility.

Therefore, the objective of the present study was to evaluate *in vitro* the effectiveness and optimum dose rate of various enzyme additives for corn silage. A 24 and 48 h *in vitro* batch culture method was used to examine the effects of four commercial enzyme additives on the ruminal disappearance and rumen fermentation profile of corn silage.

2. Materials and methods

Experiment 1 was conducted as a completely randomized design with two runs (batches) and four replicates per run with 16 treatments arranged as a factorial (4 enzyme additives × 4 doses). Experiment 2 was conducted as a completely randomized design with two runs and four replicates per run with 20 treatments arranged as a factorial (4 enzyme additives × 5 doses). In both experiments, the runs were conducted on separate days and the same four enzyme additives and the same corn silage substrate were used.

2.1. Substrate and enzyme product

A laboratory standard corn silage (neutral detergent fiber [NDF], 39.82%; acid detergent fiber [ADF], 19.08%; DM basis) was used as the substrate. Four enzyme additives were evaluated: a 75:25 combination of Cellulase Plus and Xylanase Plus (E1; source organism *Trichoderma longibrachiatum*; Dyadic International, Florida, USA); Rovabio Excel LC2 (E2; source organism *Penicillium funiculosum*; Adisseo France SAS, Antony, France); Rovabio Rips (E3; source organism *P. funiculosum*; Adisseo France SAS, Antony, France), and Econase RDE (E4; *T. longibrachiatum*; AB Vista, Marlborough, UK). The same lot of E1 (3.4 mg/g of total mixed ration DM; corn silage and alfalfa hay were the main forages in the diet) increased fiber digestibility and the efficiency of milk production when fed to dairy cows (Arriola et al., 2011). Similarly, the same lot of E4 had been