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The effect of exogenous fibrolytic enzymes and a ferulic acid esterase-producing inoculant on the fibre degradability, chemical composition and conservation characteristics of alfalfa silage

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ABSTRACT

The objective of this study was to determine the effect of applying fibrolytic enzymes at ensiling, either alone or in combination with a ferulic acid esterase-producing bacterial silage inoculant, on the silage conservation characteristics and nutritive value of alfalfa (*Medicago sativa* L.). Second-cut alfalfa (340 g DM/kg fresh crop) was harvested, wilted, chopped and sub-sampled into 24 batches. Samples were randomly allocated in triplicate to one of four enzyme product treatments supplying endoglucanases and xylanases: none (control), EN1, EN2, EN3; applied alone or in combination with a ferulic acid esterase-producing silage inoculant (FAEI). Treatments were arranged in a 4×2 factorial design. All enzyme treatments were applied at 2 ml enzyme product/kg herbage DM, and inoculant was applied at 1×10^5 cfu/g fresh herbage. Samples were packed into laboratory-scale silos and stored for 7, 27 or 70 days, and analysed for dry matter (DM) losses, aerobic stability, chemical composition and *in vitro* ruminal degradability. The use of enzymes did not affect ($P>0.05$) ensilage DM losses or lactic or acetic acid concentrations after 70 days of ensilage, compared to the control silage. Silage produced using EN1 had lesser neutral detergent fibre (aNDF, $P=0.046$) and acid detergent fibre (ADF; $P=0.006$) concentrations than control silage. However, no difference ($P>0.05$) was observed between the control silage and silage produced with EN1 for aNDF or ADF degradability (NDFD, ADFD). Silages produced with FAEI had greater DM losses ($P=0.017$) and pH ($P<0.001$) and lesser NDFD ($P=0.019$), ADFD ($P=0.010$) and proportion of lactic acid in the total fermentation products ($P=0.006$) after 70 days of ensilage, compared to uninoculated silages. The use of fibrolytic enzymes did not have a major effect on the ensilage fermentation of alfalfa, either ensiled alone or with an inoculant. No advantage in ruminal DM or fibre degradability was observed for silages produced with fibrolytic enzymes. The use of a ferulic acid esterase-producing inoculant alone did not improve the nutritive value of alfalfa silage, and did not promote any incremental effects when applied in combination with fibrolytic enzyme products.

Abbreviations: ADF, acid detergent fibre; ADFD, ADF degradability; CP, crude protein; DM, dry matter; DMD, dry matter degradability; FAEI, ferulic acid esterase-producing inoculant; LAB, lactic acid bacteria; MRS, De Man, Rogosa and Sharpe; NA, nutrient agar; aNDF, neutral detergent fibre analysed with a heat stable amylase and expressed inclusive of residual ash; NDFD, aNDF degradability; NA, nutrient agar; SDA, Sabouraud's dextrose agar; WSC, cold water soluble carbohydrates.

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1. Introduction

Fibre digestibility is a primary limiting factor in livestock production efficiency, with often less than 600 g/kg of dietary fibre being digested by the animal (Van Soest, 1994). Therefore, improvements in the fibre degradability of the forage portion of an animal's diet would likely result in an increase in the efficiency and sustainability of the production system. The use of fibrolytic enzyme products, which primarily consist of xylanases and cellulases, in ruminant diets could potentially improve fibre degradability and subsequently increase the intake of digestible energy (Beauchemin et al., 2003).

Alfalfa (*Medicago sativa* L.) is a legume crop that is typically difficult to ensile, primarily due to a high buffering capacity and low water soluble carbohydrate (WSC) concentration (McDonald et al., 1991; McAllister et al., 1998). The application of microbial additives that consist primarily of heterofermentative lactic acid bacteria (LAB) can improve the preservation of alfalfa silage (Denek et al., 2011; Mohammed et al., 2012; Contreras-Govea et al., 2013), although results can be inconsistent (Filya et al., 2007; Contreras-Govea et al., 2011).

Previous studies have focused on the potential benefits of using fibrolytic enzyme products, applied to alfalfa and other forages such as orchardgrass, at ensiling, to improve the silage fermentation by hydrolysing cell wall carbohydrates into substrates utilisable by LAB, which can result in greater lactic acid production, a more rapid reduction in pH and a more favourable fermentation (Jaster and Moore, 1988; Nadeau et al., 1996). In addition, the use of these enzymes may also improve the feed nutritive value by reducing the neutral detergent fibre (aNDF) concentration. However, many studies have reported a lack of effect or a negative effect of fibrolytic enzyme use on the dry matter degradability (DMD) and aNDF degradability (NDFD) of alfalfa silage, despite improvements in the silage fermentation (Kung et al., 1991; Nadeau et al., 1996, 2000). This is possibly due to the enzyme-action being limited to the more digestible component of NDF because of the relatively high lignin concentration of alfalfa, which reduces the opportunity for potential improvements in fibre degradability (Broderick et al., 1997; Nadeau et al., 2000).

Silage inoculants that produce ferulic acid esterase in addition to fermentation products commonly associated with forage preservation have recently become commercially available (Muck, 2010). Ferulic acid esterase can break the linkages between lignin and the cell wall carbohydrates of forages, and subsequently can increase its degradability and nutritive value (Krueger et al., 2008; Addah et al., 2012). Nsereko et al. (2008) reported improvements in the preservation and NDFD of grass silages when ensiled with ferulic acid esterase-producing inoculants; however, studies that have investigated this technology on alfalfa silage are rare. In addition the action of ferulic acid esterase may reduce the influence of lignin on the cell wall carbohydrates and thus, increase the opportunity for fibrolytic enzymes to improve the fibre degradability of alfalfa silage, and subsequently enable an improved forage preservation and nutritive value.

The objective of this study was to determine the effect of three fibrolytic enzyme products produced for ruminants, applied at ensiling either alone or in combination with a ferulic acid esterase-producing bacterial additive, on the chemical composition, conservation characteristics and degradability of alfalfa silage.

2. Materials and methods

2.1. Crop and harvesting

Second-cut alfalfa was mowed at early bloom, wilted for 9 h to 340 g DM/kg fresh crop and chopped (10 mm theoretical chop length) with a forage harvester (John Deere 6610; Moline, IL, USA) on August 19th 2012 at the Lethbridge Research Centre, Lethbridge, AB. The chemical composition of the herbage prior to ensiling is detailed in Table 1. Herbage (approx. 700 kg) was harvested into one trailer and transported to the site of ensilage, where it was placed onto a sheet of black polyethylene. Total forage was then manually mixed and twenty-four 20 kg subsamples were randomly taken, placed into pales, and stored in a cool shaded area until ensiling.

Table 1
Chemical composition of alfalfa at ensiling ($n = 3$).

Item	Mean ± standard deviation
Dry matter (g/kg)	356 ± 5.7
pH	5.9 ± 0.03
Neutral detergent fibre (g/kg DM)	341 ± 5.6
Crude protein (g/kg DM)	236 ± 5.2
Buffering capacity (mEq/kg DM)	348 ± 5.4
Lactic acid bacteria ($\log_{10}$ cfu/g)	5.19 ± 0.092
Total culturable bacteria ($\log_{10}$ cfu/g)	6.92 ± 0.158
Yeast ($\log_{10}$ cfu/g)	5.59 ± 0.045
Moulds ($\log_{10}$ cfu/g)	5.39 ± 0.207