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Effects of an exogenous protease on the fermentation and nutritive value of corn silage harvested at different dry matter contents and ensiled for various lengths of time

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ABSTRACT

The objective of this experiment was to evaluate the effects of adding an experimental protease to corn plants harvested at different maturities on silage fermentation and in vitro ruminal starch digestibility (IVSD). Corn plants were harvested at maturities resulting in plants with 31 or 40% dry matter (DM). Plants were chopped, kernel processed, and treated with (1) only a 0.1 *M* phosphate buffer (pH 5.5, 5% vol/wt of fresh forage), (2) buffer with protease to obtain a final concentration of 20 mg of protease/kg of wet forage, and (3) buffer with protease to obtain a final concentration of 2,000 mg of protease/kg of wet forage. Treated forages (about 500 g) were ensiled in nylon-polyethylene pouches and stored between 21 and 23°C for 0, 45, 90, and 150 d. Data were analyzed as a $2 \times 3 \times 4$ factorial arrangement of treatments, with the main effects of harvest DM, dose of protease, days of ensiling, and their interactions. The treatment with the highest dose of protease resulted in more robust fermentations across harvest DM with higher concentrations of lactic and acetic acids compared with untreated silage. Concentrations of soluble protein (% of crude protein) increased with time of ensiling, regardless of DM content at harvest. However, averaged over both harvest DM contents, it increased by 37% for silages treated with the high dose of protease compared with an average 11% increase for untreated silages and silage treated with the low dose of protease, between d 0 and 45. Averaged over both harvest DM contents, the concentration of soluble protein peaked in silages treated with the high dose of protease after 45 d of ensiling, whereas it peaked at d 90 in untreated silages and silage treated with the low dose of protease. Similar changes occurred in the concentration of NH_3-N due to length of ensiling and treatment with protease. In fresh forages, the concentration of starch for early- and late-harvested forages was similar, but IVSD was lower in the latter. After 45 d of ensiling, IVSD was highest in both early- and late-harvested silages that were treated with the high level of protease. After 150 d of ensiling, IVSD was similar among silages treated with protease, regardless of DM at harvest. Treating corn plants with a high dose of an experimental protease at harvest accelerated proteolysis during ensiling, resulting in corn silages with levels of IVSD after 45 d of ensiling that were only obtained in untreated corn silages after 150 d of ensiling.
Key words: rumen, starch, protease, silage

INTRODUCTION

The optimum time for harvesting plants to make corn silage for horizontal silos occurs when the whole plant reaches a DM of about 32 to 35% (Shaver et al., 1999; Johnson et al., 2002). However, in practice, corn is often harvested when plants are lower (less mature) and higher in DM (more mature), for a variety of reasons. In some instances, harvest begins early because inadequate capacity exists to harvest large amounts of forage in a short period of time. In contrast, harvesting of mature plants often occurs because equipment often cannot keep pace with increasing plant maturity or because custom harvest equipment is not available at the optimal harvest time. Lack of monitoring whole-plant DM is also a common reason for harvest to occur outside of the recommended range of DM. The common characteristics for corn plants harvested with low DM contents are low yields of DM and starch, whereas the opposite is true for plants harvested when they are overly mature (Johnson et al., 2002). Although it would appear advantageous to harvest corn at higher concentrations of DM because of the higher yields and concentrations of starch, this material is more difficult to pack and the resulting silage often spoils rapidly when it is exposed to air (Montgomery et al., 1974). Another difference between early- and late-harvested corn plants is that in the latter, an increased complexity of the prolamin-starch matrix yields plants with relatively low ruminal starch digestibility compared

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with more immature plants (Hoffman et al., 2011). To compensate for this finding, it is suggested that mature corn plants undergo mechanical processing, which increases the amount of readily accessible starch by breaking corn kernels into small pieces (Johnson et al., 2003). The result of this practice is an improvement in ruminal starch digestibility in well- versus poorly processed corn plants (Philippeau and Michalet-Doreau, 1998; Ebling and Kung, 2004). However, even overly mature corn that is well processed is often still lower in ruminal starch digestion than corn that is harvested at optimum maturity and relatively poorly processed (Schwab et al., 2003).

Protease enzymes have the potential to make starch more available for general fermentations and digestion. For example, proteases have been used in the biofuels industry to stimulate the production of ethanol by yeasts (Vidal et al., 2009). In ruminant applications, Colombatto et al. (2003) treated feeds with enzymes containing protease activity just before ruminal in vitro digestibility experiments and reported an increase in fiber digestibility in some feeds. Eun and Beauchemin (2005) treated a pellet supplement with a protease formulation and incorporated that into TMR for lactating cows. They reported an increase in total-tract digestion of fiber and starch when cows were fed the treated feed. Using proteases as silage additives has not been studied because proteolysis has usually been considered to be undesirable during silage fermentations (Rooke and Hatfield, 2003). However, we (Young et al., 2012) recently reported that the addition of exogenous proteases to corn plants at harvest accelerated the increase in ruminal in vitro starch digestibility (**IVSD**) that naturally occurs with time of ensiling. Because the starch in mature corn is more difficult to digest than starch in immature corn, we hypothesized that the effectiveness of adding a protease to corn plants differing in stages of maturity may vary. Thus, the objective of the current experiment was to evaluate the effects of adding a protease enzyme to corn plants harvested at 2 maturities on the resulting silage fermentation and potential ruminal IVSD of corn silage.

MATERIALS AND METHODS

Forage Preparation and Treatments

Corn plants (Mycogen hybrid A6867; Mycogen Seeds, Indianapolis, IN) were grown at the University of Delaware (Newark) and harvested by hand from 5 random locations within a field at 2 stages of maturity: 30.69% DM (early, **ER**) or 40.33% DM (late, **LT**). The LT corn was harvested from the same field and ensiled 13 d after ER corn. Plants were chopped to a theoretical length of 19 mm using a pull-type chopper equipped with a kernel processer and a roller gap setting of 1.35 mm (John Deere 3975; John Deere, Moline, IL). Each of the 5 piles of chopped forage at each harvest were further divided into 3 additional piles and treated with 0.1 *M* phosphate buffer (pH 5.5, 5% vol/wt of fresh forage, **CT**), the same phosphate buffer with a protease formulation resulting in 20 mg of protease/kg (**LO**) of forage (AB Vista, Wiltshire, UK), or the phosphate buffer with a protease formulation resulting in 2,000 mg of protease/kg of wet forage (**HI**). The protease was a preparation from *Aspergillus niger* with a low pH (3) optimum for activity and was the same preparation (E85 formulation) used in the study by Young et al. (2012). Young et al. (2012) measured protease activity to be 1,865 U of activity/mg of solids, where 1 U produced a change in absorbency at 280 nm (ΔA_{280nm}) of 0.001/min at pH 3.0 and 50°C, measured as TCA-soluble products using hemoglobin as a substrate. There were no detectable carbohydrase activities. Treatments were applied with separate spray bottles to the piles of forage while mixing. After application, approximately 500 g of chopped forage from each pile was packed into triplicate nylon-polyethylene standard barrier microlayered pouches (3.5-mil thickness, 15.2 × 30.5 cm; Doug Care Equipment Inc., Springville, CA) for each treatment and replicated pile, vacuumed to remove air, and heat sealed using an external clamp vacuum machine (Fast-Vac; distributed by Doug Care Equipment Inc.). Bags were stored in a temperature-controlled laboratory between 21 and 23°C and ensiled for 45, 90, and 150 d. At each ensiling point, 5 replicate bag silos were opened for each treatment for sample workup. Freshly treated samples from each replicated pile were collected and immediately frozen (−20°C) and represented d-0 samples.

Chemical Analysis

Water extracts were prepared by combining 25 g of fresh forage or silage with 225 mL of sterile quarter-strength Ringer solution (Oxoid BR0052G; Oxoid Ltd., Cambridge, UK) and homogenizing the mixture for 1 min on a medium setting in a Proctor-Silex 57171 blender (Hamilton Beach/Proctor-Silex Inc., Washington, NC). A portion of the homogenate was filtered through Whatman 54 filter paper (Whatman Ltd., Florham, NJ) and 10 mL was acidified with 3 drops of 50% H_2SO_4 to reduce the pH of the extract to <2.0, and the water extract was frozen (−20°C) until further analysis. Water extracts were analyzed for NH_3-N (Okuda et al., 1965) and water-soluble carbohydrates (**WSC**; Nelson, 1944). Water extracts from silages were also analyzed for lactic and acetic acids and ethanol by HPLC (Muck