J. Dairy Sci. 98:1–10
http://dx.doi.org/10.3168/jds.2015-9891


# Influence of ensiling, exogenous protease addition, and bacterial inoculation on fermentation profile, nitrogen fractions, and ruminal in vitro starch digestibility in rehydrated and high-moisture corn

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## ABSTRACT

Exogenous protease addition may be an option to increase proteolysis of zein proteins and thus starch digestibility in rehydrated and high-moisture corn (HMC) ensiled for short periods. In addition, microbial inoculation may accelerate fermentation and increase acid production and thus increase solubilization of zein proteins. Four experiments were performed to evaluate the effect on fermentation profile, N fractions, and ruminal in vitro starch digestibility (ivSD) of the following: (1) rehydration and ensiling of dry ground corn; (2) exogenous protease addition to rehydrated un-ensiled and ensiled corn; (3) exogenous protease addition or inoculation in rehydrated ensiled corn; and (4) exogenous protease addition or inoculation in HMC. Experiments 1, 2, and 3 were performed with 7 treatments: dry ground corn (DGC); DGC rehydrated to a targeted dry matter content of 70% (REH); REH treated with exogenous protease (REH+); REH ensiled for 30 d (ENS); ENS treated with exogenous protease (ENS+); ENS treated with a microbial inoculant containing *Lactobacillus plantarum*, *Lactobacillus casei*, *Enterococcus faecium*, and *Pediococcus* sp. (ENSI); and ENS treated with exogenous protease and microbial inoculant (ENSI+). Experiment 1 compared DGC, REH, and ENS with ivSD being greater for ENS (64.9%) than DGC and REH (51.7% on average). Experiment 2 compared REH and ENS without or with exogenous protease addition (REH+ and ENS+, respectively). Ensiling and exogenous protease addition increased ivSD, but exogenous protease addition was more effective in ENS than REH (6.4 vs. 2.6 percentage unit increase). Experiment 3 compared the effects of exogenous protease addition and inoculation in ENS corn (ENS, ENS+, ENSI, and ENSI+). The addition of protease, but not inoculant, increased ivSD. Inoculation reduced pH and acetate, propionate, and ethanol concentrations, and increased lactate and total acid concentrations. In experiment 4, 8 treatments were a combination of HMC noninoculated or inoculated with 1 of 3 microbial inoculants and with or without exogenous protease addition. The inoculant treatments contained (1) *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus*, (2) *L. buchneri* 40788, and (3) a mixture of *P. pentosaceus* and *Propionibacterium freudenreichii*. Protease, but not inoculation, increased ivSD by 7.5 percentage units (44.4 vs. 51.9%). Protease addition increased ivSD in rehydrated corn and HMC. Microbial inoculation improved fermentation profiles but did not affect ivSD.

**Key words:** corn, protease, inoculant, ruminal in vitro starch digestibility

## INTRODUCTION

Corn grain is the predominant feed energy source in the US livestock industry with approximately 122 million tonnes fed during the 2014–2015 season (USDA-ERS, 2015). Starch composes approximately 70% (DM basis) of corn grain and accounts for 75% of its energy value for dairy cattle (calculated from NRC, 2001). Therefore, improvements in starch digestibility may improve lactation performance by dairy cows.

Greater starch digestibility and lactation performance by dairy cows fed high-moisture corn (**HMC**) compared with dry ground shelled corn (**DGC**) are consistently reported in the literature as evidenced by reviews of Firkins et al. (2001) and Ferraretto et al. (2013). This is likely related to the reduction in zein protein subunits that cross-link starch granules during the ensiling process (Hoffman et al., 2011). Digestibility of starch is mainly influenced by this hydrophobic starch-protein matrix surrounding starch granules that impedes microbial attachment and fermentation in the rumen or hydrolytic and enzymatic digestion in the abomasum and small intestine (Giuberti et al., 2014). Furthermore, factors such as mean particle size (**MPS**), harvest maturity and moisture content, and endosperm type may alter the starch-protein matrix, and thus, the

Received June 1, 2015.
Accepted June 24, 2015.
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extent of starch digestibility for HMC (Giuberti et al., 2014).

Recent reports indicate that an extended ensiling period increases ruminal in vitro starch digestibility (**ivSD**; Ferraretto et al., 2014; Kung et al., 2014), underscoring that maximum starch digestibility occurs only after several months of storage. To help overcome this lag, 2 options may merit consideration. First, addition of exogenous protease at ensiling has enhanced the breakdown of zein proteins and increased ivSD (Kung et al., 2014). Second, pH has an inverse relationship with ivSD (Ferraretto et al., 2014) and pH decline is associated with greater concentrations of organic acids (Muck, 2010). Perhaps reduced pH and greater concentrations of organic acids associated with the addition of microbial inoculants at ensiling may increase zein protein solubilization and thereby starch digestibility. In addition, a combination of these factors (exogenous protease plus microbial inoculant) may potentiate the increase in starch digestibility of HMC.

Rehydration and ensiling of DGC became a common practice in Brazil in recent years due to limited machinery availability and excessive rainfall during the HMC harvest period, which can delay harvest (Bitencourt, 2012). Delayed harvest, and the corresponding decrease in DM content, for HMC may impair silo fermentation and reduce ivSD (Goodrich et al., 1975; Ferraretto et al., 2014). Benton et al. (2005b) reported similar in situ DM disappearance for HMC and rehydrated and ensiled DGC after 28-d fermentations. In addition, beef steers were more efficient (measured as feed:gain ratio) when rehydrated and ensiled corn or sorghum were used in comparison with their dry-rolled counterparts (Stock et al., 1987; Benton et al., 2005a). Perhaps rehydration and ensiling of DGC may be an alternative in areas where HMC harvest and fermentation may be compromised by weather conditions (e.g., excessive rainfall, freezing temperatures), limited by machinery availability, or both. Literature, however, is scarce in this area. Furthermore, benefits of microbial inoculation or exogenous protease addition in rehydrated and ensiled DGC are unknown.

Although reports evaluating exogenous protease addition to HMC exist in the literature (Kung et al., 2014), research evaluating its use in combination with varied microbial inoculants commonly used in the industry is warranted. Furthermore, although the benefit of increasing DM digestibility of DGC through rehydration and ensiling has already been reported (Benton et al., 2005a,b), research evaluating potential benefits of microbial inoculation or exogenous protease addition is still warranted. Therefore, experimental objectives were to evaluate the effect on fermentation profile, N fractions, and ivSD of the following: (1) rehydration and ensiling of dry ground corn; (2) exogenous protease addition to rehydrated un-ensiled and ensiled corn; (3) exogenous protease addition or microbial inoculation in rehydrated ensiled corn; and (4) exogenous protease addition or microbial inoculation in HMC. We hypothesized that rehydration will increase ivSD only when done in conjunction with ensiling; addition of exogenous protease will increase ivSD in rehydrated and HMC; and microbial inoculation will improve fermentation profile and ivSD.

## MATERIALS AND METHODS

### *Experiments 1, 2, and 3*

Ten kilograms of DGC was obtained from the University of Wisconsin Feed Mill (Arlington, WI) on March 2013 after fine grinding (616 μm of MPS), homogenized, and allocated into 21 samples of approximately 300 g each using a quartering technique: homogeneous samples were divided into 4 equal subsamples. Two subsamples were saved for later treatment, whereas the 2 other subsamples were rehomogenized and redivided. The process was repeated until 21 subsamples of approximately 300 g were prepared. The remainder was frozen at −20°C until processed for analysis to characterize the DGC. Samples were randomly assigned to 7 treatments with 3 replications per treatment. The treatments were DGC, DGC rehydrated to a targeted DM content of 70% (**REH**), REH treated with exogenous protease (**REH+**), REH ensiled for 30 d (**ENS**), ENS treated with exogenous protease (**ENS+**), ENS treated with a microbial inoculant (**ENSI**), and ENS treated with exogenous protease and microbial inoculant (**ENSI+**). An experimental exogenous bacterial protease produced in *Bacillus licheniformis* (DSM Nutritional Products, Basel, Switzerland/Novozymes, Bagsvaerd, Denmark) was added at a supplier recommended rate of 1,825 mg of protease per kg of corn DM to protease treatments, which is equivalent to 136.9 PROT units per kg of corn DM. One PROT unit is the amount of enzyme that releases 1 μmol of *p*-nitroaniline from 1 μ*M* of substrate (Suc-Ala-Ala-Pro-Phe-pNA) per minute at pH 9.0 and 37°C. The supplier-recommended application rate ($5 \times 10^4$ cfu/g of rehydrated corn) of a microbial inoculant containing *Lactobacillus plantarum*, *Lactobacillus casei*, *Enterococcus faecium*, and *Pediococcus* sp. (Silo Charger "D," NU-AG Bosko Inc., Oskaloosa, IA) was applied to the inoculant treatments. Exogenous protease and microbial inoculant were mixed in the same solution before application of ENSI+ treatment.

Samples of DGC, REH, and REH+ were frozen 12 h after exposure to treatment. Samples of ENS, ENS+, ENSI, and ENSI+ were placed in nylon-polyethylene