

# ASE STUDY: Microbial inoculant and ensiling time effects on fermentation profile, nitrogen fractions, and ruminal in vitro and in situ starch digestibility in corn shredlage and latematurity corn silage

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### **ABSTRACT**

Two experiments were conducted to evaluate the effects of ensiling time and microbial inoculation on N fractions and starch digestibility in either well-processed corn shredlage (SHRD: Exp. 1; 76% of starch passing through a 4.75-mm screen, 39.3% DM, and 32.2% amylase-treated NDF) or late-maturity corn silage (Exp. 2; 48.0% DM and 22.9% amylase-treated NDF). For Exp. 1, unfermented SHRD was allocated into 24 samples of 600 g each and randomly assigned to 6 treatments in quadruplicate. Treatments were a combination of SHRD noninoculated (CON) or inoculated at the recommended inoculation rate (1X;  $5 \times 10^4$  cfu of Lactobacillus

plantarum, Lactobacillus casei, Streptococcus faecium, and Pediococcus per gram of fresh whole-plant corn) or twice the recommended inoculation rate (2X; $10 \times 10^4$  cfu/g of fresh whole-plant corn) of a microbial inoculant and ensiled for 30 or 120 d. Exp. 2 used the same experimental methodology except for evaluating treatments within latematurity corn silage rather than SHRD. In Exp. 1, DM and starch concentrations were unaffected by treatments. Although not affected by inoculation, content of CP increased from 30 to 120 d of ensiling. Measurements of pH were reduced from 3.96 at 30 d to 3.88 after 120 d. Concentrations of lactate and ethanol were similar but acetate and total acids were greater after 120 d. Ammonia-N concentration and starch digestibility increased from 30 to 120 d. Fermentation profile, including ammonia-N, and starch digestibility of SHRD were unaffected by inoculation. In Exp. 2, ensiling time

did not affect concentrations of DM, CP, and starch. However, DM and starch contents were 2.5 and 3.4 percentage units greater for 2X than other treatments. Concentrations of lactate and total acids were greater for CON and 1X than 2X. Propionate and ethanol concentrations tended to be greater for CON than other treatments. Despite the lower ammonia-N concentration for 2X, starch digestibility was unaffected by microbial inoculation. Greater lactate, acetate, and total acid concentrations after 120 d of ensiling were observed. Reductions in pH and ethanol concentration were also observed for 120 d compared with 30 d. Late-maturity corn silage fermented for 120 d had greater ammonia-N (5.4 vs. 4.0% of CP) and starch digestibility (66.7 vs. 61.7% of starch) compared with 30 d. Ammonia-N concentration and starch digestibility were greater after 120 d of fermentation in both experiments, suggesting that extended ensiling time is

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**862** Ferraretto et al.

advantageous in both scenarios. Inoculation with lactate-producing bacteria, however, did not improve starch digestibility in either experiment.

**Key words:** storage length, microbial inoculant, starch digestibility, corn shredlage

### INTRODUCTION

Benefits of prolonged ensiling time on ruminal in vitro starch digestibility have been observed in whole-plant corn silage (WPCS; Der Bedrosian et al., 2012; Ferraretto et al., 2015b). Moreover, prolonged ensiling time increased concentrations of ammonia-N and soluble CP in these trials, implying proteolysis or solubilization of zein proteins (Hoffman et al., 2011). Although the overall benefits of prolonged ensiling time are well established, its effect on starch digestibility in specific scenarios, such as when WPCS is harvested with excellent kernel processing or at late maturity, remains unknown.

Corn shredlage (SHRD), a new method of harvesting WPCS, increased kernel breakage at harvest compared with conventionally processed WPCS (Ferraretto and Shaver, 2012a; Vanderwerff et al., 2015). Hence, greater total-tract starch digestibility and corresponding lactation performance by high-producing dairy cows were observed in these trials. Overall, reduction in kernel particle size attenuates the effects of zein proteins on starch digestibility of WPCS and dry or high-moisture corn grain (Johnson et al., 2002; Ferraretto et al., 2013). Effects of fermentation, measured as concentration of ammonia-N (% CP), on starch digestibility in high-moisture corn are also lower when mean particle size is reduced, as underscored in the model of Hoffman et al. (2012). Thus, the potential for additive effects of prolonged ensiling time when excellent kernel processing is achieved at harvest remains uncertain.

Delayed harvest and the corresponding increase in DM content of WPCS reduced apparent total-tract starch

digestibility and impaired lactation performance by dairy cows in a recent meta-analysis of published studies (Ferraretto and Shaver, 2012b). This has been attributed to an increase in the proportion of vitreous endosperm in the kernel associated with greater maturity (Correa et al., 2002; Ngonyamo-Majee et al., 2009). Furthermore, pH decline is slower and accumulation of organic acids reduced for late-maturity WPCS (Der Bedrosian et al., 2012), presumably due to decreased bacterial growth related to lower water availability (Muck, 1988). Reduced bacterial growth may lessen both proteolysis and solubilization of zein proteins (Simpson, 2001; Lawton, 2002; Hoffman et al., 2011). Perhaps reduced pH and greater concentrations of organic acids associated with the addition of microbial inoculants at ensiling (Muck, 2010) may increase zein-protein solubilization and thereby starch digestibility (Simpson, 2001; Lawton, 2002).

Therefore, the objective of this study was to evaluate the effect of a microbial inoculant and ensiling time on fermentation profile, N fractions, and starch digestibility of (1) SHRD or (2) late-maturity WPCS. We hypothesized that extended ensiling time will increase soluble CP, ammonia-N, and starch digestibility in SHRD and late-maturity WPCS, and microbial inoculation will improve fermentation profile and starch digestibility in SHRD and late-maturity WPCS.

### **MATERIALS AND METHODS**

Two experiments were conducted simultaneously to evaluate either well-processed SHRD (Exp. 1) or latematurity WPCS (Exp. 2).

# Silage Production and Treatments

For Exp. 1, 20 kg of unfermented SHRD was obtained at harvest from the University of Wisconsin–Madison Agricultural Research Station (Arlington, WI) on September 6, 2012. A self-propelled forage harvester (Claas Jaguar, Claas of America Inc.,

Omaha, NE) equipped with SHRD processing rolls (Shredlage LLC; http://www.shredlage.com/) set for 26-mm theoretical length of cut and 2-mm roll gap was used to harvest a conventional corn hybrid as shredlage. Samples were homogenized and allocated into 24 samples of 600 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 24 subsamples of approximately 600 g were prepared. The remainder was frozen at  $-20^{\circ}$ C until it was processed for analysis to characterize the material (Table 1). Samples were randomly assigned to 6 treatments with 4 replications per treatment. The 6 treatments were a combination of corn shredlage noninoculated (CON) or inoculated at the recommended inoculation rate (1X;  $5 \times$ 10<sup>4</sup> cfu/g of fresh whole-plant corn) or twice the recommended inoculation rate (2X;  $10 \times 10^4$  cfu/g of fresh whole-plant corn) with a microbial inoculant and ensiled for either 30 or 120 d. The microbial inoculant contained Lactobacillus plantarum, Lactobacillus casei, Streptococcus faecium, and Pediococcus sp. (Silo Charger "D," NU-AG Bosko Inc., Oskaloosa, IA). All 24 samples, including the noninoculated corn shredlage samples, received the same amount of double distilled water to ensure protocol similarity among all samples. Immediately after microbial inoculant treatment application, samples were placed in nylon-polyethylene standard barrier vacuum pouches (0.09-mm thickness,  $25.4 \times 35.6$  cm; Doug Care Equipment Inc., Springville, CA) and vacuum heat sealed using an external clamp vacuum machine (Bestvac; distributed by Doug Care Equipment Inc.) and stored at room temperature (approximately 20°C) in the dark. After reaching the targeted ensiling time (30 or 120 d), samples were immediately frozen at  $-20^{\circ}$ C to stop fermentation and stored until being processed for analysis.

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