The Professional Animal Scientist 32 (2016):598–604; http://dx.doi.org/10.15232/pas.2015-01494


# Effect of homofermentative lactic acid bacteria and exogenous hydrolytic enzymes on the ensiling characteristics and rumen degradability of alfalfa and corn silages

**Jeffery M. Chilson,* Pedram Rezamand,*[1] Mary E. Drewnoski,*[2] W. Price,† and Carl W. Hunt,* PAS**

*Department of Animal and Veterinary Science, and †College of Agriculture and Life Sciences, University of Idaho, Moscow 83844

## ABSTRACT

*Homofermentative lactic acid bacteria increase lactic acid concentrations and, therefore, decrease pH of silages. Fibrolytic enzymes hydrolyze structural carbohydrates and increase fiber degradation. The goal of this research was to examine the effects of a combination of 4 homofermentative lactic acid bacteria and 4 hydrolytic, predominately fibrolytic, enzymes on the ensiling characteristics of alfalfa and corn silages in a laboratory. Alfalfa and corn were treated with water (control: CON) or the lactic acid bacteria/fibrolytic enzymes (TRT) and ensiled in tube mini silos (volume of 1,206 cm$^3$) and bucket mini silos (volume of 21,504 cm$^3$) for 59 d. The pH was measured on d 1, 2, 3, 7, and 13 for alfalfa and d 1, 2, 3, 7, and 17 for corn. The additive increased the rate of pH decline (P = 0.005), DM degradation (P = 0.02), and concentration of lactic acid (P = 0.01) in the TRT alfalfa silage compared with CON. Additionally, pH on d 59 (P = 0.07) tended to be decreased in the TRT alfalfa silage compared with CON. In contrast, no difference was detected in rate of pH decline (P = 0.91) or DM degradation (P = 0.71) between treatments for the corn silages. Lactic acid (P = 0.08), however, tended to be greater on d 59 in the TRT corn silage compared with the CON. These findings indicate that the additive may improve silage characteristics and fiber degradation in alfalfa, while having no detectable effect on corn silage.*

**Key words:** inoculant, fibrolytic enzyme, homofermentative, silage, haylage

## INTRODUCTION

Over the past 40 yr, silage has become a popular method of forage preservation used in dairies and in finishing lamb rations (Brassley, 1996; Burke et al., 2007). During ensiling, water soluble carbohydrates (**WSC**) are converted to organic acids (**OA**; Bergen et al., 1991; Seglar, 2003). Lactic acid (**LA**) is one of the OA most responsible for the pH decline of the silage, although it is not considered a strong antimycotic (Woolford, 1975). To increase OA production, the addition of inoculants containing lactic-acid bacteria (**LAB**) is one of the most common methods used (Filya et al., 2007). These inoculants may contain homo- or heterofermentative, or both, LAB. Silages treated with homofermentative LAB decrease pH to a greater extent and produce greater amounts of LA than those treated with heterofermentative LAB

[1] Corresponding author: rezamand@uidaho.edu

[2] Current address: Department of Animal Science, University of Nebraska, Lincoln 68583.

(Filya et al., 2006, 2007). However, homofermentative LAB inoculants do not always increase LA production and decrease pH compared with untreated silages, especially in corn silages (Filya et al., 2006; Tabacco et al., 2011). Additionally, homofermentative LAB have not shown consistent improvement in nutrient retention, DM loss, rumen degradation, or aerobic stability (Muck and Kung, 1997; Filya et al., 2006, 2007).

Fibrolytic enzymes (**FE**) increase LA production and decreases pH, especially in forages high in structural carbohydrates (Sun et al., 2012). The use of FE has been shown to increase WSC, while decreasing NDF, ADF, and cellulose (Thomas et al., 2013; Lynch et al., 2015). Additionally, FE have also been reported to improve digestibility of structural carbohydrates, especially NDF, in the first 24 h of incubation (Colombatto et al., 2003a; Romero et al., 2015).

The objective of this research was to evaluate the effects of a silage treatment, which contained 4 homofermentative LAB in combination with 4 hydrolytic enzymes, with predominately fibrolytic activity within a small, laboratory scale using alfalfa and corn silage.

## MATERIALS AND METHODS

### *Experimental Design*

Mature alfalfa (*Medicago sativa* L.) was harvested and chopped to an average length of 2.5 cm at the University of Idaho Dairy Center in Moscow, Idaho. Fresh cut, whole plant corn (*Zea mays* L.) was obtained from a private farm near Colfax, Washington, in late summer 2013. Each forage type was ensiled on the same day as it was harvested in mini silos made from PVC pipes (volume of 1,206 $cm^3$) and buckets (volume of 21,804 $cm^3$). At chopping, the alfalfa was 57% DM and was hydrated to approximately 40% DM before ensiling. The treatment (**TRT**) consisted of a LAB + FE mixture that consisted of 4 homofermentative LAB (*Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus*) plus 4 hydrolytic, predominately fibrolytic, enzymes (cellulase, xylanase, β-glucanase, and α-amylase) reconstituted per manufacturer's directions of 5.3 g of additive (Sil-All 4 × 4, Lallemand Animal Nutrition, Milwaukee, WI) per liter of water and applied at the concentration of $2 \times 10^5$ cfu of LAB/g of fresh forage for both corn and alfalfa using a common compression garden sprayer. The control (**CON**) was treated with an equal amount of water. Alfalfa and corn was packed into preweighed mini silos and bucket silos for a density of 168 kg (DM)/$m^3$ or 175 kg (DM)/$m^3$, respectively. Bucket silos were hand packed until no further forage could be placed in the silos, and PVC mini silos were packed with forage so that PVC mini silos and bucket silos had equivalent packing densities.

The PVC mini silos were opened on d 1, 2, 3, 7, and 13 for alfalfa and on d 1, 2, 3, 7, and 17 for the corn silages. Samples were collected to measure pH. Bucket silos were opened for both corn and alfalfa silages on d 59, and samples were obtained to measure pH, titratable acidity, DM, VFA, NDF, ADF, cellulose, ash, CP, $NH_3$-N, WSC (alfalfa silage), starch (corn silage), in situ degradation, LAB cultures, and yeast-mold cultures. Weights were obtained on d 59 for the bucket silos to determine DM loss. The pH and titratable acidity of the silage were determined using an Oakton pH 11 series pH/mV/°C meter (Cole-Parmer, Court Vernon Hills, IL). Titratable acidity was determined by adding 1 *N* NaOH to 70 mL of a slurry prepared by blending silage and distilled water, per method described in Kung et al. (2000), until pH was raised to 6.80. Additionally, samples were prepared for pH as described by Kung et al. (2000). Lactic acid bacteria were cultured as described by Reich and Kung (2010) with a slight modification: samples were prepared in physiological saline instead of Ringer's solution. Yeast cultures were applied to 3M Petri-film yeast-mold plates (3M, St. Paul, MN) per manufacturer's instructions and incubated at room temperature for 5 d. Only plates with 30 to 200 cfu were counted for LAB, and 10 to 200 cfu were counted for yeast.

Dried samples (60°C) of alfalfa and corn silage were ground using a Wiley mill (Thermo Fisher, Waltham, MA), through a 1-mm screen, until completely ground. Laboratory DM (105°C) and ash were performed as described by the AOAC (1999). Neutral detergent fiber, ADF, and cellulose analyses were performed as described by Van Soest et al. (1991) in an Ankom 200 (ANKOM Technologies, Macedon, NY) fiber digester. Frozen samples were sent to Dairyland Laboratories (Arcadia, WI) and analyzed for CP (AOAC, 1999), ammonia (as part of CP; AOAC, 1999), and WSC (alfalfa; Derias, 1961) or starch (corn; Vidal et al., 2009).

Volatile fatty acids were determined using GLC (Hewlett-Packard 6890 series GLC using an Agilent DB-FFAP column, Hewlett-Packard, Avondale, PA). Samples were prepared by mixing 100 g of silage with 400 mL of water. The blended slurry was then filtered through 2 layers of cheesecloth and centrifuged at 2,400 × *g* at 4°C for 20 min on a Sorvall ST 16R centrifuge (Thermo Scientific, Sunnyvale, CA). After centrifugation, the supernatant was frozen at −20°C. Samples were then thawed at room temperature and centrifuged at 2,400 × *g* at 4°C for 30 min. Five milliliters of supernatant was then transferred into a 15-mL conical tube, 1 mL of 25% metaphosphoric acid was added to remove any remaining proteins, and the supernatant was placed in the −20°C freezer overnight. After thawing at room temperature, the alfalfa samples were centrifuged on a Sorvall Evolution RC centrifuge (Thermo Scientific) at 1,000 × *g* for 10 min at 23°C, and the corn samples were centrifuged on the same machine at 750 × *g* for 10 min at 23°C. Oven temperature was initially 100°C, increased to 150°C for 10 min, and then 175°C for 1.5 min with a constant pressure of 13.79 KPa, using hydrogen gas as the carrier gas, and a 1:10 split.