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Effects of 8 chemical and bacterial additives on the quality of corn silage

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ABSTRACT

This project aimed to evaluate the effects 8 additives on the fermentation, dry matter (DM) losses, nutritive value, and aerobic stability of corn silage. Corn forage harvested at 31% DM was chopped (10 mm) and treated with (1) deionized water (control); (2) Buchneri 500 (BUC; 1×10^5 cfu/g of *Pediococcus pentosaceus* 12455 and 4×10^5 cfu/g of *Lactobacillus buchneri* 40788; Lallemand Animal Nutrition, Milwaukee, WI); (3) sodium benzoate (BEN; 0.1% of fresh forage); (4) Silage Savor acid mixture (SAV: 0.1% of fresh forage; Kemin Industries Inc., Des Moines, IA); (5) 1×10^6 cfu/g of *Acetobacter pasteurianus*-ATCC 9323; (6) 1×10^6 cfu/g of *Gluconobacter oxydans*-ATCC 621; (7) Ecosyl 200T (1×10^5 cfu/g of *Lactobacillus plantarum* MTD/1; Ecosyl Products Inc., Byron, IL); (8) Silo-King WS (1.5×10^5 cfu/g of *L. plantarum, P. pentosaceus* and *Enterococcus faecium*; Agri-King, Fulton, IL); and (9) Biomax 5 (BIO; 1×10^5 cfu/g of *L. plantarum* PA-28 and K-270; Chr. Hansen Animal Health and Nutrition, Milwaukee, WI). Treated forage was ensiled in quadruplicate in mini silos at a density of 172 kg of DM/m^3 for 3 and 120 d. After 3 d of ensiling, the pH of all silages was below 4 but ethanol concentrations were least in BEN silage (2.03 vs. 3.24% DM) and lactic acid was greatest in SAV silage (2.97 vs. 2.51% DM). Among 120-d silages, additives did not affect DM recovery (mean = 89.8% ± 2.27) or in vitro DM digestibility (mean = 71.5% ± 0.63). The SAV silage had greater ammonia-N (0.85 g/kg of DM) and butyric acid (0.22 vs. 0.0% DM) than other treatments. In contrast, BEN and Silo-King silages had the least ammonia-N concentration and had no butyric acid. The BEN and *A. pasteurianus* silages had the lowest pH (3.69) and BEN silage had the least ethanol (1.04% DM) and ammonia nitrogen (0.64 g/kg DM) concentrations, suggesting that fermentation was more extensive and protein degradation was less in BEN silages. The BUC and BIO silages had greater acetic acid concentrations than control silages (3.19 and 3.19 vs. 2.78% DM), but yeast counts did not differ. Aerobic stability was increased by 64% by BUC (44.30 h) and by 35% by BEN (36.49 h), but other silages had similar values (27.0 ± 1.13 h).
Key words: silage inoculant, chemical additive, corn silage, aerobic stability

INTRODUCTION

Additives are added to ensiled forages to prevent or reduce the growth of undesirable microorganisms in silages and thus enhance silage fermentation and aerobic stability (Kleinschmit and Kung, 2006a; Pedroso et al., 2010). Inorganic acids, such as formic or sulfuric acids, have been successfully used to improve silage preservation by direct acidification (Kung et al., 2003), whereas organic acids with strong antifungal properties, such as propionic, benzoic, and sorbic acids, have been used to increase silage aerobic stability (Kleinschmit et al., 2005). The antimicrobial properties of these acids result from their ability to pass across the cell membrane in the undissociated form and release hydrogen in the cytoplasm. The resulting reduction in cytoplasmic pH or the use of ATP to resist the pH decline and maintain homeostasis causes the cell to reduce or stop growing (Lambert and Stratford, 1999).

Homofermentative bacterial inoculants ferment water-soluble carbohydrates into organic acids, particularly lactic acid, which rapidly acidifies the silage and inhibits the growth of undesirable bacteria. Heterolactic inoculant bacteria ferment water-soluble carbohydrates into antifungal acids, such as acetic and propionic acids, which inhibit the growth of spoilage-causing fungi (Huisden et al., 2009; Filya and Sucu, 2010). Commercially available inoculants contain one or both types of lactic acid bacteria (**LAB**), but few studies have simultaneously compared several of the available inoculants with chemical additives.

Recently, Nishino et al. (2009) suggested that acetic acid bacteria (**AAB**) could be used to improve silage aerobic stability after discovering high concentrations of acetic acid in aerobically exposed corn silages contaminated with high populations of *Acetobacter pasteurianus*. This concept requires validation, as it contradicts the notion that AAB can initiate aerobic

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spoilage (Spoelstra et al., 1988). Acetic acid bacteria in the *Gluconobacter* genus can oxidize ethanol to acetic acid by using alcohol and acetaldehyde dehydrogenase, but cannot completely oxidize acetic or lactic acid because they lack the pertinent enzymes (De Ley, 1961; Schweigeret al., 2007). Thus, the proliferation of such bacteria after ensiling may result in greater residual acetic and lactic acid concentrations in aerobically exposed silages (Holt et al., 1994), which could increase aerobic stability. Research is needed to examine the effects of applying *Gluconobacter* inoculants to silages prone to ethanolic fermentations, such as most sugarcane silages (Schmidt, 2009; Nussio et al., 2009) and some corn silages.

Few studies have simultaneously compared several bacterial and chemical additives for improving silage preservation. This project aimed to evaluate the effect of various existing chemical additives and LAB inoculants and 2 novel AAB inoculants on the fermentation, DM losses, nutritive value, and aerobic stability of corn silages.

MATERIALS AND METHODS

Silage, Treatments, and Design

The study was conducted from November 2010 to February 2011 at the Dairy Research Unit, University of Florida, Gainesville. Fresh corn forage (Dekalb 69–70, Monsanto, St. Louis, MO) was harvested at 31% of DM and chopped to lengths of approximately 10 mm. Additives were diluted in 100 mL of deionized water and sprayed in a fine mist on 100-kg forage piles under constant mixing. The treatments applied were (1) water alone (control; **CONT**); (2) Buchneri 500 applied at 1×10^5 cfu/g of *Pediococcus pentosaceus* 12455 and 4×10^5 cfu/g of *Lactobacillus buchneri* 40788 (**BUC**; Lallemand Animal Nutrition, Milwaukee, WI); (3) sodium benzoate applied at 0.1% of fresh forage (**BEN**); (4) Silage Savor (**SAV**; a mixture of propionic, benzoic, lactic, and sorbic acids applied at 0.1% of fresh forage; Kemin Industries Inc., Des Moines, IA); (5) 1×10^6 cfu/g of *Acetobacter pasteurianus*-ATCC 9323 (**PAS**); (6) 1×10^6 cfu/g of *Gluconobacter oxydans*-ATCC 621 (**SUB**); (7) Ecosyl 200T applied at 1×10^5 cfu/g of *Lactobacillus plantarum* MTD/1 (**ECO**; Ecosyl Products Inc., Byron, IL); (8) Silo-King WS inoculant applied at 1.5×10^5 cfu/g of *L. plantarum, Pediococcus pentosaceus*, and *Enterococcus faecium* (**SK**; Agri-King, Fulton, IL); and (9) Biomax 5 inoculant applied at 1×10^5 cfu/g of *L. plantarum* PA-28 and K-270 (**BIO**; Chr. Hansen Animal Health and Nutrition, Milwaukee, WI). Treated forages were packed in quadruplicate at a density of approximately 172 kg of DM/m^3 into separate 3- and 20-L laboratory silos, which were sealed for 3 and 120 d, respectively, and kept at ambient temperature (25°C) in an enclosed barn. The 3-L silos were 6-mm thick plastic bags sealed with plastic cable ties, and the 20-L (30 × 30 cm) silos were plastic buckets sealed with airtight lids. Silage samples collected on d 3 were analyzed for pH, ammonia, ethanol, VFA, and lactate. In addition, samples from d 120 were also analyzed for chemical composition, DM digestibility, DM losses, and aerobic stability.

Laboratory Analysis

Oven DM was measured by drying silage samples at 60°C for 48 h in a forced-air oven. Dried samples were ground to pass the 1-mm screen of a Wiley mill (A. H. Thomas, Philadelphia, PA) and analyzed for ash in a muffle furnace at 512°C for 8 h. Concentrations of NDF, ADF, and ADL were measured in an ANKOM 200 Fiber Analyzer (Ankom Technologies, Macedon, NY) using methods of Van Soest et al. (1991) for NDF and AOAC (1990) for ADF and ADL. Heat-stable α-amylase and sodium sulfite were used in the NDF assay and the results are presented inclusive of residual ash. Nitrogen was determined by rapid combustion using a Macro elemental N analyzer (Vario MAX CN, model ID 25.00–5003; Elementar, Hanau, Germany) and CP was calculated as N × 6.25. Acid-detergent insoluble nitrogen was quantified by a modification of the method described by Licitra et al. (1996), which involved N quantification by rapid combustion using the N analyzer described above instead of using titration. In vitro true DM digestibility was measured with the method of Van Soest et al. (1966). Ammonia-N was measured by distillation (AOAC, 1985). Starch was quantified by measuring free glucose after sample incubation with amyloglucosidase at 60°C for 30 min (Holm et al., 1986).

Silage extract was prepared by mixing 25 g of corn silage with 225 mL of 0.1% peptone water in a stomacher for 3 min. The solution was filtered through 2 layers of cheesecloth and an aliquot was immediately used for yeast, mold, and clostridia counts, as described by FDA (1998). Briefly, the aliquot obtained from the silage extract was diluted 10-fold and inoculated in triplicate on agar plates containing (1) de Man-Rogosa-Shape agar (Thermo Scientific-Remel, Pittsburgh, PA; R01585) and incubated at 48 h at 32°C to enumerate lactobacilli, (2) malt extract agar (Thermo Scientific-Oxoid, OXCM0059B) and incubated at 72 h at 32°C to enumerate yeast and molds, and (3) tryptose sulfite cycloserine agar (Thermo Scientific, CM0587B) and inoculated for 24 h at 35°C to enumerate clostridia and Brilliant Green lactose bile broth, 2% (Fluka, 16025)