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Lactobacillus plantarum effects on silage fermentation and *in vitro* microbial yield☆

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ABSTRACT

In four parallel experiments, herbage [three harvests of alfalfa (308 to 379 g dry matter (DM)/kg), one of whole-plant corn (331 g DM/kg)] was ensiled with three different treatments: no inoculant (control), *Lactobacillus plantarum* (LP) or formic acid (FA), in 1-L mini-silos and fermented for 60 d at room temperature (22 °C). Mini-silos were opened and analyzed for fermentation characteristics and soluble N fractions. A subsample of wet silage from each mini-silo was ground to 4 mm and stored at −20 °C. Silages were thawed and subjected to 9 h ruminal *in vitro* incubations to measure gas production and volatile fatty acid (VFA) production as well as microbial biomass yield (MBY) and microbial non-ammonia N (MNAN) formation using ^{15}N as a marker. In all four experiments, silage fermentation products and pH indicated good preservation across all treatments. Analysis of data showed that FA- and LP-treated silages had lower concentrations of ammonia-N and free amino acids N than control. The FA treatment was lower in soluble N, but higher in peptide-N, than control. Silage pH was lowest in FA (4.25), followed by LP (4.28), and control (4.38). Ruminal *in vitro* gas production and VFA concentrations were not different among treatments (P>0.05). Compared to control, FA- and LP-treated silage yielded greater MNAN and MBY. These findings suggested that *L. plantarum* preserved more true protein during silage fermentation than control, which in turn increased *in vitro* ruminal microbial growth.

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1. Introduction

The probiotic effect of lactic acid bacteria (LAB) has been documented in humans and animals. Probiotic *Lactobacillus* species stimulate indigenous lactic acid bacteria and the production of short-chain fatty acids, and may modulate the intestinal immune response through the promotion of secretion of certain cytokines and immunoglobulin A in the intestinal mucosa (Ohashi and Ushida, 2009). In the ensiling of forage crops, LAB have been used as additives to increase the likelihood of getting a good preservation of crop nutritive value by reducing plant respiration and enzyme activity and by inhibiting deleterious epiphytic microbial populations. There is little doubt that adding LAB to the ensiling crop frequently

Abbreviations: A:P, acetate:propionate molar ratio; ADF, acid detergent fiber expressed inclusive of residual ash; cfu, colony-forming units; CP, crude protein; DM, dry matter; FAA-N, free amino acid N; LAB, lactic acid bacteria; L:A, lactate:acetate weight ratio; MBY, microbial biomass yield; MDM, microbial dry matter; MNAN, microbial non-ammonia N; aNDF, amylase-treated neutral detergent fiber expressed inclusive of residual ash; NPN, soluble nonprotein N; TDM, truly digested dry matter; TS, total solids dry matter; VFA, volatile fatty acids; WSC, water-soluble carbohydrates.

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accomplishes these goals (Muck and Kung, 1997). Moreover, Nsereko et al. (2008) reported that some LAB can produce ferulate esterase enzymes during fermentation and increase neutral detergent fiber degradation of the inoculated crop during ensiling. Beyond effects in the silo, there is evidence that LAB inoculation of crops at ensiling may enhance animal performance when treated silage is fed to ruminants, even in cases where inoculation had little or no apparent effect on silage fermentation (Weinberg and Muck, 1996; Kung and Muck, 1997; Kung et al., 2003; Weinberg et al., 2003). These results suggest that perhaps some inoculants may have a probiotic effect on the cow.

The effect of LAB on the rumen environment has been studied in a number of experiments. Lactic acid bacteria can survive during *in vitro* ruminal incubation and potentially affect volatile fatty acids (VFA) composition (Weinberg et al., 2003, 2004). Muck et al. (2007) demonstrated that microbial silage inoculants had an effect on *in vitro* ruminal gas and VFA production, but effects differed by inoculant. In addition, Gollop et al. (2005) reported that many strains of LAB silage inoculants have antibacterial activity, and that this activity is often, but not always, present in the inoculated silages. It is possible that antibacterial compounds in silages may alter rumen fermentation although that has not been examined.

One specific LAB strain that has shown recurring positive effects on animal performance is *Lactobacillus plantarum* (Ecosyl strain MTD/1, Ecosyl Products Ltd., Stokesley, North Yorkshire, UK). Kung et al. (2003) summarized 12 published studies in which this inoculant strain was used and found that this *L. plantarum* was reported to have positive effects on milk production in nine of these publications, giving an average increase of 4.6% above untreated silage. Recently Contreras-Govea et al. (2011) studied the effects of four inoculants, including *L. plantarum* MTD/1, on silage fermentation and *in vitro* ruminal production of gas, microbial biomass, and VFA. Some of the inoculants affected mainly the soluble nonprotein N (NPN) fractions in silage, which was also reported previously by Jones et al. (1992). In ruminal *in vitro* fermentations, Contreras-Govea et al. (2011) found that gas and VFA production were not different between uninoculated and inoculated silage, but three of the four inoculated silages, one of which was *L. plantarum* MTD/1, produced greater microbial biomass than the uninoculated silage. These workers concluded that some microbial inoculants are capable of altering ruminal microbial biomass formation, even though the effects in silage composition were small. Moreover, they suggested that improved protein preservation during ensiling by inoculant treatment could be one of the mechanisms affecting ruminal fermentation.

Therefore, the objectives of this study were to: a) determine if *Lactobacillus plantarum* MTD/1 alters the soluble NPN fractions during fermentation, and b) determine if silage treated with this *L. plantarum* strain improves microbial biomass production when the silage is incubated in a ruminal *in vitro* system.

2. Material and methods

2.1. Ensiling process

During summer of 2007, three harvests of alfalfa and one corn were ensiled without treatment or treated with one of two additives and fermented for 60 d. The alfalfa was mown and field-wilted prior to chopping, and the corn was harvested directly. The four experimental crops were second cutting alfalfa harvested on 28 June at 25% bloom (Alfalfa1, 350 g dry matter (DM)/kg), third cutting alfalfa harvested on 2 August at late bud stage (Alfalfa2, 379 g DM/kg) and 17 August at 50% bloom (Alfalfa3, 308 g DM/kg); and whole-crop corn (Mycogen TMF2Q716, Mycogen, Indianapolis, IN, USA) harvested at ½ milk line (CS716, 331 g DM/kg) on 6 September. All crops were chopped at a theoretical length of cut of 10 mm with a conventional forage harvester and treated with: 1) No inoculant (control), 2) *Lactobacillus plantarum* (LP, Ecosyl MTD/1, Ecosyl Products Ltd., Stokesley, North Yorkshire, UK), or 3) formic acid (FA). Formic acid was selected because it is a widely applied silage additive known to rapidly reduce pH and protein degradation in alfalfa silage (Nagel and Broderick, 1992). All forages were collected from independent fields at the time they were chopped for ensiling in conventional farm silos. Approximately 30 kg of each forage was randomly collected from a wagonload, placed in a plastic bag and transported to the laboratory for ensiling. At the laboratory, each crop was ensiled in 1-L glass jars (Weck, Wher-Oftlingen, Germany) at a density of 500 g/L of fresh material, four jars per treatment. Treatments were individually applied to the forage ensiled in each jar; therefore, jar was considered the experimental unit (Robinson et al., 2006). Control treatment was sprayed with 10.0 g distilled water/kg fresh forage; LP treatment was applied at a 10^6 cfu/g fresh weight, 10.0 g of solution/kg fresh forage; and FA treatment was applied at a rate of 8.8 g/kg fresh forage along with distilled water (1.2 g/kg). Three samples of each crop were taken over the course of filling for analysis of pre-ensiling characteristics. Glass jar mini-silos were stored at room temperature (~22 °C) during the 60-day fermentation.

2.2. Sample processing

Both the forages prior to ensiling (pre-ensiled) and the resulting silages were analyzed similarly with two exceptions. Only the pre-ensiled forages were analyzed for LAB, and *in vitro* ruminal incubations were performed only on the silages. At sampling, each pre-ensiled crop or silage was mixed, a 20 g subsample was taken, diluted 10-fold on a mass basis with distilled water, and macerated for 30 s in a high-speed blender. For the pre-ensiled samples, numbers of epiphytic LAB were measured on the sample extract using Rogosa SL agar (Difco 0480, Becton Dickinson, Sparks, MD) and pour-plate technique. The extract was filtered through 4 layers of cheesecloth, and pH was measured immediately with a pH meter (Thermo Orion Model 525, Thermo Fisher Scientific, Waltham, MA, USA). Filtrates were either centrifuged (25,100 × *g*, 4 °C, 20 min) and the supernatants frozen for later analysis of fermentation products and water soluble carbohydrates (WSC; Dubois et al., 1956),