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Inoculant Effects on Alfalfa Silage: In Vitro Gas and Volatile Fatty Acid Production

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ABSTRACT

Alfalfa silages from 2 similar trials were analyzed for in vitro ruminal gas production. In both trials, there were 15 treatments: alfalfa treated at ensiling with 1 of 14 lactic acid bacterial inoculants or untreated alfalfa. First-cut (477 g of dry matter/kg) and second-cut (393 g of dry matter/kg) alfalfa were ensiled in glass jars for a minimum of 35 d at room temperature (~22°C). At opening, a portion of each silage was wet-ground with a mixer. Each silage was then assessed for in vitro ruminal gas production in 3 replicate runs with the wet-ground silage, 1 on the fresh silage and 2 on frozen and thawed silage. In vitro gas production was measured in 160-mL sealed serum vials incubated at 39°C. One gram of silage was incubated with 17.1 mL of nutrient solution, 0.9 mL of reducing solution, and 12 mL of ruminal inoculum (1:2 vol/vol mixture of rumen fluid and buffer). Gas production was measured manually by using a pressure gauge at 3, 6, 9, 24, 48, and 96 h. At 96 h, the rumen fluid was analyzed for pH and volatile fatty acids. In the 2 trials, the untreated control silage produced either numerically the highest or one of the highest levels of gas production per unit of dry matter incubated. In first-cut silage, 9 of the inoculant treatments at 9 h and 4 treatments at 96 h had reduced gas production compared with the control. In second-cut silage, 10 inoculant treatments at both 9 and 96 h had reduced gas production compared with the control. Furthermore, in first-cut silage, the fraction of total gas production at 3, 6, and 9 h was numerically the highest for the control, and only 4 treatments were not significantly lower than the control at 9 h. In second-cut silage, 2 of 14 inoculated treatments produced faster fractional rates of gas production than the control, but most inoculated treatments had numerically slower fractional rates (4 significant) in the first 9 h. The in vitro fermented wet-ground control silages had one of the highest acetate:propionate ratios in both trials, significantly higher than 12 and 8 of the inoculated treatments in first- and second-cut silage, respectively. The response in acetate:propionate ratio in both cuts was similar, even though the control silage was highest in lactic acid in one trial and lowest in the other. Overall, inoculation of crops at ensiling appears to affect in vitro ruminal fermentation of wet-ground silages, even in the absence of large effects during silage fermentation.
Key words: alfalfa silage, in vitro fermentation, lactic acid bacteria

INTRODUCTION

The application of lactic acid bacteria (**LAB**) to crops at ensiling to improve silage quality is a common practice in the United States and Europe. Homofermentative LAB such as *Lactobacillus plantarum*, *Enterococcus faecium*, and *Pediococcus* spp. are used, with the goal of providing a faster fermentation, lower final pH values, raised lactate:acetate ratios, lower ethanol and ammonia nitrogen concentrations, and improved DM recovery (Weinberg and Muck, 1996). Recently, a heterofermentative LAB inoculant species, *Lactobacillus buchneri*, has become available commercially and produces high concentrations of acetic acid in silage, which inhibit fungi and thus preserve silages susceptible to spoilage upon exposure to air (Weinberg et al., 2002; Filya, 2003a,b).

Although the primary function of LAB has been to improve the preservation of crops in the silo, homofermentative LAB inoculants in particular have been shown in various studies to improve milk yield, gain, and feed efficiency (Kung et al., 2003). In a summary of 36 studies, Kung and Muck (1997) reported that milk yield was increased in 47% of the studies when inoculated silage was fed, compared with untreated silage. The average increase in milk production for those studies in which the inoculant enhanced milk yield was 1.4 kg/cow per d (Kung and Muck, 1997). Some LAB strains have shown an even more consistent effect on milk yield. Kung et al. (2003) reported that *L. plantarum*

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MTD1 had a positive effect on milk yield in 83% of the 12 studies reviewed.

Although animal trials have shown improved animal performance from feeding inoculated silages, there are still questions regarding how relatively minor shifts in silage fermentation can produce significant changes in animal performance. Weinberg and Muck (1996) suggested that microbial inoculants may produce a probiotic effect in the rumen, the mechanism of which is unknown. This suggestion of a probiotic effect arises from the magnitudes of the effects that have been observed in various studies as well as from multiple reported studies (e.g., Gordon, 1989; Steen et al., 1989; Kung et al., 1993) in which animal performance was improved when an inoculated silage was fed, even though the inoculant failed to significantly alter silage fermentation compared with fermentation in an untreated control silage.

Various in vitro and in sacco techniques have been used to help predict the digestibility of feedstuffs, and these should be helpful in predicting whether there should be an animal response to silage inoculation. Routine in vitro techniques, such as those by Tilley and Terry (1963) and Goering and Van Soest (1970), measure the residue after a given incubation time in rumen fluid. Rates of fermentation with time can be generated, but with considerable effort. Fermentation rates are easier to generate with in sacco techniques. However, various in vitro gas production techniques have been developed (e.g., Theodorou et al., 1994; Schofield and Pell, 1995; Rymer et al., 1998). These studies have shown the value of measuring gas production as a means of estimating the rate and extent of in vitro rumen fermentation. Furthermore, the VFA produced by ruminal microorganisms during in vitro fermentation should be stoichiometrically related to gas production (Blümmel et al., 1997).

If the LAB in silages are having a direct effect on rumen fermentation, then performing an in vitro procedure with a dried sample may mask the effects occurring in an in vivo situation. Calabrò et al. (2001) recently reported that in vitro gas production was higher with fresh silages (228 mL/g) than with silages dried at 65°C (162 mL/g). Total VFA produced in the in vitro incubations tended to be higher in the fresh silages, and the acetate:propionate ratio was higher from incubating fresh silages. Lee et al. (2002) compared the in vitro ruminal fermentation of freeze-dried and 1-mm ground grass with grass frozen, thawed, and cut into 10-mm lengths. Gas production was higher in this case with the dried samples, but the acetate:propionate ratio was higher in the frozen and thawed grass. These results indicate that undried samples ferment differently in in vitro systems than do dried samples. Because we do not know what an inoculant does to alter ruminant digestion and utilization of a silage, it may be important to measure in vitro digestibility on undried silages to more closely mimic conditions in the cow.

Previously, we reported on 2 alfalfa silage trials comparing 14 inoculant treatments with an untreated control (Filya et al., 2007). In first-cut alfalfa, silage fermentation was substantially affected by the inoculant treatments [with pH values for 13 of the 14 treatments below that of the untreated control ($P < 0.05$), and a maximum difference from the control of 0.75 pH units]. However, there were no significant effects on in vitro true DM digestibility (**IVTDMD**) at 48 h with freeze-dried samples. In second-cut alfalfa, the inoculant treatments had considerably more modest effects on silage fermentation. Only 5 inoculant treatments had pH values significantly below that of the untreated control; the maximum difference was 0.13 pH units. Significant treatment effects on IVTDMD were observed but were due to reductions in IVTDMD by some inoculant treatments relative to the control. The objectives of the study reported here were to compare the effects of inoculant treatment on the rate and extent of in vitro gas production by using undried samples from these 2 trials, and to compare these in vitro results with results of the previously reported IVTDMD values.

MATERIALS AND METHODS

Silage Preparation

In 2003, alfalfa was ensiled in 2 trials [first cut (477 g of DM/kg) and second cut (393 g of DM/kg)] on June 9 and July 2, respectively]. In both trials, alfalfa was harvested with standard field equipment (mower-conditioner, forage harvester) without inoculation. Approximately 40 kg was collected from a load of alfalfa after being dumped during the process of filling a field-scale silo. The chopped alfalfa was ensiled in 1.0- and 0.5-L anaerobic glass jars (Weck, Wher-Oftlingen, Germany), respectively, at a density of 500 g/L. Each trial had 15 treatments (uninoculated control and 14 inoculants), with 4 silos per treatment. Eight inoculants were commercial products, and the others were single strains provided by 2 companies (Table 1). All inoculants were applied at a rate of 1.0×10^6 cfu/g of crop as fed (not label rates) to help ensure the domination of fermentation. All inoculants were diluted with distilled water so that they were all applied at the same rate (10 g of solution/kg of crop as fed). The control received 10 g of water/kg of crop as fed. The amount of chopped alfalfa for a given silo was weighed out by taking approximately 6 random grabs from the collected forage, sprayed with the appropriate inoculant solution with a plant sprayer (one sprayer for each treatment), mixed