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Effects of lactic acid bacteria silage inoculation on methane emission and productivity of Holstein Friesian dairy cattle

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ABSTRACT

Inoculants of lactic acid bacteria (LAB) are used to improve silage quality and prevent spoilage via increased production of lactic acid and other organic acids and a rapid decline in silage pH. The addition of LAB inoculants to silage has been associated with increases in silage digestibility, dry matter intake (DMI), and milk yield. Given the potential change in silage and rumen fermentation conditions accompanying these silage additives, the aim of this study was to investigate the effect of LAB silage inoculants on DMI, digestibility, milk yield, milk composition, and methane (CH_4) production from dairy cows in vivo. Eight mid-lactation Holstein-Friesian dairy cows were grouped into 2 blocks of 4 cows (multiparous and primiparous) and used in a 4 × 4 double Latin square design with 21-d periods. Methane emissions were measured by indirect calorimetry. Treatments were grass silage (mainly ryegrass) with no inoculant (GS), with a long-term inoculant (applied at harvest; GS+L), with a short-term inoculant (applied 16 h before feeding; GS+S), or with both long and short-term inoculants (GS+L+S). All diets consisted of grass silage and concentrate (75:25 on a dry matter basis). The long-term inoculant consisted of a 10:20:70 mixture of *Lactobacillus plantarum*, *Lactococcus lactis*, and *Lactobacillus buchneri*, and the short-term inoculant was a preparation of *Lc. lactis*. Dry matter intake was not affected by long-term or short-term silage inoculation, nor was dietary neutral detergent fiber or fat digestibility, or N or energy balance. Milk composition (except milk urea) and fat and protein-corrected milk yield were not affected by long- or short-term silage inoculation, nor was milk microbial count. However, milk yield tended to be greater with long-term silage inoculation. Methane expressed in units of grams per day, grams per kilogram of DMI, grams per kilogram of milk, or grams per kilogram of fat and protein-corrected milk yield was not affected by long- or short-term silage inoculation. However, CH_4 expressed in units of kilojoules per kilogram of metabolic body weight per day tended to be greater with long-term silage inoculation. Results of this study indicate minimal responses in animal performance to both long- and short-term inoculation of grass silage with LAB. Strain and dose differences as well as different basal silages and ensiling conditions are likely responsible for the lack of significant effects observed here, although positive effects have been observed in other studies.
Key words: lactating cows, lactic acid bacteria, silage inoculant, methane

INTRODUCTION

Microbial silage inoculants such as lactic acid bacteria (**LAB**) are used to improve silage fermentation and prevent spoilage of ryegrass and maize silages through increased organic acid production, mainly lactic acid (**LA**) and acetic acid, and a more rapid pH decline (Muck, 2013). Although plants contain native LAB, the number of viable LAB on forage can be insufficient and delay the decline in pH during ensiling, allowing greater loss of nutrients, other microbes to dominate fermentation, or both. Common LAB used for silage inoculation include homofermentative species such as *Lactobacillus plantarum*, *Enterococcus faecium*, and *Pediococcus* spp. Ensiling with these LAB results in conversion of water-soluble carbohydrate (**WSC**) to LA and a rapid decline in silage pH under anaerobic conditions (McDonald et al., 1991), which helps to prevent the development of clostridia, yeasts, molds, and fungi (Muck, 2013). Nutritionally, because of a more rapid pH decline, LAB inoculants tend to reduce the ammonia N content of silage via reduced fermentation of AA and reduced protein breakdown, as well as improved DM recovery (McDonald et al., 1991; Spoelstra, 1991; Henderson, 1993). Other heterofermentative LAB

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(e.g., *Lactobacillus buchneri*) that grow more slowly and produce a greater concentration of acetic acid are also often included and are typically inhibitory to fungi and preserve silage susceptible to spoilage upon exposure to air (e.g., Driehuis et al., 1999).

The use of LAB silage inoculants has been associated with increases in DMI, digestibility, and milk yield, although the mechanism remains convoluted (Weinberg and Muck, 1996; Weinberg et al., 2003; Ando et al., 2006). In addition, LAB have also been postulated as a potential probiotic with mastitis mitigation potential (e.g., Fang et al., 1996). Across all effects, results appear to be strain, dose, and silage specific. For example, an early meta-analysis (Muck and Kung, 1997) demonstrated positive responses in DMI to microbial inoculants 28% of the time, in body weight gain 53% of the time, and in milk production 47% of the time, based on literature from 1990 to 1995. Several hypotheses exist on the cause of improved animal performance, when observed, including (1) changes in the chemical composition of the silage, (2) inhibition of detrimental microbes and the production of toxins (e.g., bacteriocins; Gollop et al., 2005), and (3) interaction of LAB with rumen microbes and alteration of rumen fermentation (Fellner et al., 2001; Weinberg et al., 2003). The hypothesis for mastitis mitigation generally revolves around reduction of milk pH and competition for nutrients with detrimental bacteria (e.g., Fang et al., 1996), but the pathway by which oral LAB probiotics may reach the mammary gland is uncertain and at times convoluted (Fernández et al., 2013).

Nutritional or microbial changes within the rumen may also affect enteric methane (CH_4) production. Methane is a greenhouse gas produced as the result of enteric fermentation in the ruminant digestive tract, representing an energetic loss of 2 to 12% (Ellis et al., 2008). Globally, enteric fermentation from domestic ruminants accounts for ~6.0% of the global anthropogenic CO_2 equivalent production (FAOSTAT, 2013). Improving forage (either fresh or conserved) quality and digestibility is among the most effective mitigation strategies to decrease CH_4 emission intensity (CH_4 per unit product; e.g., Hristov et al., 2013), which makes examining the potential impact of silage inoculants relevant. So far, several in vitro studies have suggested reductions in total gas production [Muck et al. (2007) using alfalfa silage and considering 14 individual and mixed LAB treatments] or CH_4 production [Cao et al. (2010), using whole-crop rice silage with *Lb. plantarum*; Cao et al. (2011), using vegetable residue silage with *Lb. plantarum*] without changes in digestibility. However, not all in vitro studies have reported reductions in CH_4 [Contreras-Govea et al. (2011), using alfalfa and corn silage, with individual and mixed LAB treatments], likely pointing to strain and inoculant differences, substrate differences, or in vitro methodology differences. Indeed, Ellis et al. (2016) found that LAB silage inoculants in vitro were effective on grass silage and not corn silage, and that some strains increased but others decreased (or did not change) total gas production, CH_4 production, or DM digestibility.

Based on these in vitro results, the purpose of this study was to examine in vivo the long-term use (applied at harvesting, a 10:20:70 cfu/g product ratio of *Lb. plantarum*, *Lc. lactis*, and *Lb. buchneri*) and short-term use (applied 16 h before feeding, *Lc. lactis* as a probiotic) of LAB inoculants applied to grass silage for its effects on CH_4 emission, DMI, diet digestibility, milk yield, milk composition, and milk microbial changes. The long-term inoculant was applied to examine potential effects due to changes in silage fermentation and silage composition, and the short-term inoculant was applied to examine any direct probiotic effects. We hypothesized that LAB may have direct or indirect CH_4 mitigation potential in vivo when applied to grass silage fed to dairy cattle.

MATERIALS AND METHODS

This study was conducted at the Carus animal facility at Wageningen University, Wageningen, the Netherlands, in accordance with Dutch law and approved by the Institutional Animal Care and Use Committee of Wageningen University (Wageningen, the Netherlands).

Cows, Experimental Design, and Diets

Eight mid-lactation Holstein-Friesian dairy cows were grouped into 2 blocks of 4 cows and used in a 4 × 4 double Latin square design (21-d periods). Cows within blocks were matched for age, parity, stage of lactation, and milk production. The cows in block 1 (multiparous, average 4.5 ± 0.25 lactations) averaged 95 ± 18.0 DIM at the start of the experiment with an average BW of 676 ± 20.2 kg, and the cows in block 2 (primiparous) averaged 112 ± 2.0 DIM at the start of the experiment with an average BW of 521 ± 9.4 kg. Prior to the onset of the experiment, animals were group-housed for 7 d and fed a regular dairy ration while acclimatizing to the experimental location. Periods for blocks 1 and 2 were staggered by 4 d to facilitate measurements with the 4 individual climate respiration chambers available.

The experimental diet consisted of 75% grass silage (mainly *Lolium perenne*) and 25% concentrate mixture [concentrate, g/kg product basis: 242 maize, 64 wheat, 10 linseed, 84 palm kernel expeller, 34 formaldehyde-treated rapeseed meal, 72 rapeseed meal, 259 formaldehyde-treated soybean meal, 100 beet pulp, 14.4 lime, 2.8