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Effects of addition of malic or citric acids on fermentation quality and chemical characteristics of alfalfa silage

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ABSTRACT

We studied the effects on alfalfa preservation and chemical composition of the addition of different levels of malic acid and citric acid at ensiling as well as the utilization efficiency of these 2 organic acids after fermentation. Alfalfa was harvested at early bloom stage. After wilting to a dry matter content of approximately 40%, the alfalfa was chopped into 1- to 2-cm pieces for ensiling. Four levels (0, 0.1, 0.5, and 1% of fresh weight) of malic acid or citric acid were applied to chopped alfalfa at ensiling with 4 replicates for each treatment, and the treated alfalfa forages were ensiled for 60 d in vacuum-sealed polyethylene bags (dimensions: 200 mm × 300 mm) packed with 200 to 230 g of fresh alfalfa per mini silo and an initial density of 0.534 g/cm^3. The application of malic or citric acids at ensiling for 60 d led to lower silage pH than was observed in the control silage (0% of malic or citric acids). Application of the 2 organic acids led to higher lactic acid concentration in alfalfa silage than in the control silage except with the application rate of 1% of fresh weight. Silages treated with both organic acids had lower nonprotein nitrogen concentrations than the control silages, and the nonprotein nitrogen concentrations in ensiled forages decreased with the increase in malic or citric acid application rates. The application of the 2 organic acid additives led to lower saturated fatty acid proportions and higher polyunsaturated fatty acid proportions in ensiled alfalfa than in the control silage. The amount of malic and citric acids degraded during ensiling of alfalfa was 1.45 and 0.63 g, respectively. At the application rate of 0.5% of fresh weight, residues of malic acid and citric acid in alfalfa silage were 11.1 and 13.6 g/kg of dry matter. These results indicate that including malic or citric acids at the ensiling of alfalfa effectively improved silage fermentation quality, limited proteolysis, improved fatty acid composition of the ensiled forage, and could provide animals with additional feed additives proven to promote animal performance. However, when the application rate of both organic acids reached 1%, the concentration of lactic acid in silages decreased notably. Additionally, 0.5 and 1% application rates also increased the yeast count in ensiled alfalfa.
Key words: malic acid, citric acid, alfalfa silage, organic acid

INTRODUCTION

Extensive research over recent years has explored the potential of using organic acids (e.g., fumaric, malic, citric, and succinic acids) as alternatives to feed antibiotics to improve performance and feed efficiency. As a key intermediate in the citric acid cycle of biological tissues and in the succinate-propionate pathway of ruminal bacteria (Castillo et al., 2004), malic acid has been introduced as a feed additive for ruminants because of its numerous beneficial effects. Many in vitro studies have shown that malic acid could stimulate rumen fermentation, increase rumen pH (Martin and Streeter, 1995; Carro and Ranilla, 2003), improve microbial N production and microbial efficiency (Sniffen et al., 2006), decrease methane production (Carro and Ranilla, 2003), and increase feed digestibility (Carro et al., 1999). Feeding trials also confirmed that malic acid had a positive effect on increasing milk production in dairy cows (Sniffen et al., 2006; Devant et al., 2007; Wang et al., 2009b) and improving beef cattle performance (Martin et al., 1999; Castillo et al., 2007). Citric acid is another essential intermediate in the citric acid cycle, which has a similar function in stimulating rumen fermentation and improving animal performance (Packett and Butcher, 1963; Sun et al., 2008; Wang et al., 2009a).

To date, little information is available on the application of malic acid or citric acid in ensiled forage, and

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whether these organic acids have positive effects on promoting the fermentation quality of ensiled alfalfa is not clear. Even so, the existing literature shows that malic acid or citric acid could accelerate the growth of lactic acid bacteria (**LAB**; Branen and Keenan, 1970; Salou et al., 1991; Passos et al., 2003). Therefore, it is plausible that the application of malic acid or citric acid could possibly improve the fermentation of ensiled forage and consequently decrease proteolysis by promoting pH decline. Based on the positive effects of the abovementioned organic acids in stimulating microbial fermentation and their use as feed additives for improving animal performance, we propose that the application of these organic acids at ensiling may not only improve fermentation quality and nutritive value of ensiled forage but could also indirectly provide animals with feed additives after ingestion of organic acid–treated silages. However, whether these 2 acids have different effects on fermentation of ensiled forage is not clear. It is also necessary to clarify the degradation of the added organic acids after fermentation to see whether the residues in silages are enough to meet the amount of organic acids for improving animal performance as suggested by previous studies. In addition, the fatty acid (**FA**) composition in ensiled forages has been of interest. It has been reported that a high intake of PUFA could increase the concentration of PUFA in ruminant products (Wood and Enser, 1997; Kalač and Samková, 2010) and consequently be beneficial to human health (Simopoulos, 2001). It was reported that ensiling led to lower PUFA but higher SFA compared with fresh forage due to lipolysis and biohydrogenation (Alves et al., 2011). Because malic and citric acids are well-known antioxidants, they might inhibit the lipolysis caused by lipoxygenases during ensiling. Thus, the objectives of this study were to investigate the effects of different levels of malic or citric acids on fermentation quality, proteolysis, and lipolysis of alfalfa silage and to evaluate the utilization efficiency of these 2 organic acids in ensiled alfalfa after fermentation.

MATERIALS AND METHODS

Experimental Treatments and Mini Silo Preparation

Alfalfa (*Medicago sativa* L.) was mowed in the late bud to early bloom stage and wilted to a DM content of approximately 400 g/kg of fresh weight (**FW**). A total of 8 kg of the wilted forage was then chopped into 1- to 2-cm pieces using a paper cutter within 40 min. The chopped forage was then treated with malic acid or citric acid at application rates of 0, 0.1, 0.5, and 1% of FW. To apply the organic acids to the chopped alfalfa forage, malic acid or citric acid was dissolved in 12 mL of distilled water and mixed thoroughly with the forages after uniform spraying onto the piles prepared for each treatment. For the untreated forage, the same amount of distilled water alone was applied. The pH values in alfalfa treated with malic acid at 0.1, 0.5, and 1% at ensiling were 6.02 ± 0.01, 5.21 ± 0.02, and 4.95 ± 0.06, respectively, and the pH values in alfalfa treated with citric acid at 0.1, 0.5, and 1% at ensiling were 6.0 ± 0.02, 5.3 ± 0.01, and 4.79 ± 0.02, respectively. To accurately trace the degradation of the organic acids before and after fermentation, laboratory vacuum packed mini silos were used (Johnson et al., 2005; Hoedtke and Zeyner, 2011). All the treated forages were packed into polyethylene plastic bags with initial density of 0.534 g/cm^3 (dimensions: 200 mm × 300 mm; Embossed Food saver bag; Taizou Wenbwu Soft-Packing Color-Printing Co. Ltd., Zhejiang, China) and vacuum sealed tightly. Each polyethylene bag was packed with 200 to 230 g of wilted forage, and there were 4 replicates for each treatment. The silos were then stored at an ambient temperature of 25 ± 2°C for 60 d.

Chemical and FA Analyses

After 60 d of ensiling, the bags were opened and a portion of silage immediately was frozen (−20°C) for further analysis. Initial fresh forage samples were taken before samples were ensiled. A 20-g FW sample from each bag was placed in a juice extractor (BA-828, Mannengda Plasthetics Co. Ltd., Guangzhou, China), diluted with 180 mL of distilled water, squeezed for 30 s at a high speed, and filtered through 4 layers of medical gauze. The filtrate was divided into 2 parts. The pH was measured immediately; then, one part of the filtrate was acidulated with 7.14 *M* H_2SO_4 and filtered with a 0.45-μm dialyzer. Lactic acid, acetic acid, propionic acid, and butyric acid were analyzed by HPLC (Shodex KC-811 column; Shimadzu, Kyoto, Japan; oven temperature: 50°C; flow rate: 1 mL/min; SPD-10Avp, Shimadzu: 210 nm) as described previously (Ding et al., 2013). A total of 1 mL of 250 g/L (wt/vol) trichloroacetic acid was added to 4 mL of the second part of the filtrate from each bag. This solution was held overnight at 4°C to precipitate the protein and was centrifuged at 18,000 × *g* for 15 min at 4°C. Next, the supernatant fluid was analyzed for ammonia nitrogen (**NH_3-N**) and AA nitrogen (**AA-N**) according to Broderick and Kang (1980) and for water-soluble carbohydrates (**WSC**) using the method of Thomas (1977). The NPN content of forage samples was analyzed after precipitating protein by using trichloroacetic acid as described by Licitra et al. (1996). Enumeration of LAB, yeasts, and molds in fresh alfalfa