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Effects of lactic acid bacteria with bacteriocinogenic potential on the fermentation profile and chemical composition of alfalfa silage in tropical conditions

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ABSTRACT

The fermentation profile, chemical composition, and microbial populations of alfalfa silages treated with microbial inoculants (MI) at different fermentation periods (T) were evaluated in tropical conditions. A 4 × 6 factorial arrangement was used in a randomized design with 3 replicates. Fresh alfalfa was treated with (1) no treatment (CTRL), (2) commercial inoculant (CIN), (3) *Pediococcus acidilactici* (strain 10.6, S1), and (4) *Pediococcus pentosaceus* (strain 6.16, S2). An inoculant application rate of 10^6 cfu/g of fresh forage was used. The fermentation periods were 1, 3, 7, 14, 28, and 56 d. Alfalfa was harvested 82 d after sowing at the early flowering stage, chopped into 1.5-cm particle size, and ensiled in 25 × 35 cm vacuum-sealed plastic bags. The numbers of lactic acid bacteria, enterobacteria, mold, and yeast in alfalfa before ensiling were 5.42, 5.58, 4.82, and 4.8 log cfu/g, respectively. Silage chemical composition was evaluated only at 56 d. All parameters were affected by the interaction MI × T, except the concentrations of lactic and propionic acids. Alfalfa silage treated with S1 or S2 had lower pH values than CTRL from the first day until 28 d. However, the inoculants resulted in similar pH after 56 d, and these values were lower than the CTRL. The highest concentration of lactic acid was observed in the silage treated with S1 and S2 at 7 and 14 d of ensiling. The concentration of acetic acid was lower in the silages treated with S1 and S2 than the CTRL and CIN at 3 and 28 d of fermentation. There was no effect of MI or MI × T interaction on the microbial populations. However, the number of enterobacteria decreased over the fermentation period until 14 d and increased slightly after this time point. The chemical composition of alfalfa silage was not affected by MI at 56 d of ensiling. The strain *P. pentosaceus* 6.16 was the most efficient in dominating the fermentation process by decreasing the pH more quickly and increasing the concentration of lactic acid, suggesting its potential use as a silage inoculant.
Key words: inoculant, lactic acid bacteria, organic acid, alfalfa silage

INTRODUCTION

Alfalfa (*Medicago sativa*) is the main legume used for silage production in the world because of its nutritional quality (Kung et al., 2003; Coblentz et al., 2014). However, the fermentation process and chemical composition of alfalfa silage have not been studied in tropical regions (Rangrab et al., 2000; Magalhães and Rodrigues, 2003).

Alfalfa has shown the same limitations for silage production under tropical conditions as in other regions, including low concentration of DM and water-soluble carbohydrates (Dewhurst et al., 2003) as well high buffering capacity (McDonald and Henderson, 1962). Wilting is usually performed after harvesting to increase DM content and decrease the growth of clostridial bacteria. Clostridia growth increases butyric acid and proteolysis in legume silages (Muck and Kung, 2007).

Homofermentative lactic acid bacteria (**LAB**) are widely used as inoculants, and their effects on increasing the concentration of lactic acid while lowering the pH and the concentration of ammonia nitrogen have been reported in the literature (Bolsen et al., 1992; Filya et al., 2007; Contreras-Govea et al., 2011). However, studies conducted in Brazil have not shown that LAB inoculants improve alfalfa fermentation (Rangrab et al., 2000; Rodrigues et al., 2004). This suggests that environmental conditions encountered in the tropics can affect the physiology and metabolism of the inoculated strains and may influence their effects on the fermentation process. In addition, the success of an inoculant

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in the silage depends on its ability to grow fast, the presence of substrate, and the relationship between the application rate and the epiphytic population in the forage (Muck, 1988). Recently, strains of LAB that produce bacteriocins have been proposed to control the growth of spoilage microorganisms in silages because of their antimicrobial activity (Mantovani and Russell, 2003; Amado et al., 2012). The results have shown better efficiency in improving the fermentation profile of grass silages (Mantovani and Russell, 2003; Amado et al., 2012; Ferreira et al., 2013). Based on those results, Silva et al. (2012) performed a screening of LAB with bacteriocinogenic activity isolated from silages of a tropical legume and found a few strains with antimicrobial activity from a bacteriocin-like substance. However, the effect of these strains on the fermentation of legume silages in tropical conditions has not yet been evaluated.

We hypothesize that using LAB with bacteriocinogenic potential isolated from a tropical-legume silage enhances the fermentation profile of alfalfa silage under tropical conditions. Therefore, this study aimed to quantify the microbial populations and to evaluate changes in the fermentation profile and chemical composition of alfalfa silages treated with microbial inoculants.

MATERIALS AND METHODS

Location and Silage Preparation

The experiment was conducted at the Animal Science Department of the Federal University of Viçosa (Universidade Federal de Viçosa; **UFV**) located in Viçosa, Minas Gerais, Brazil, between May and July 2013. Viçosa is located at 20°45′ S, 42°51′ W, and 657 m above sea level with a mean annual rainfall of 1,341 mm.

Alfalfa cv. 'Crioula' was grown in a 1,000-m^2 area at the Forage Crops Sector of the Animal Science Department of UFV. Alfalfa was harvested 82 d after sowing, at the early flowering stage, using a backpack mower. After 6 h wilting, the alfalfa (313 g/kg of average DM) was chopped into 1.5-cm particle size in a stationary forage chopper (model PN Plus 2000, Nogueira S.A., São João da Boa Vista, Brazil). Three replicated piles (each pile treated individually) containing approximately 12 kg of fresh alfalfa were prepared for each treatment (for a total of 12 piles). A total of 500 g of fresh alfalfa was packed into nylon-polyethylene bags (25 × 35 cm; Doug Care Equipment Inc., Springville, CA), and the air was evacuated from the bags using a vacuum sealer (Eco vacuum 1040, Orved, Italy). The bags were stored in the laboratory at room temperature (range, 23–27°C).

Experimental Design and Inoculant Application

A 4 × 6 factorial scheme (4 inoculants × 6 fermentation periods) was used in the randomized design with 3 replicates. The fermentation periods were 1, 3, 7, 14, 28, and 56 d. The periods were defined to describe with more details the alterations in the early fermentation (e.g., pH, organic acids), because the major changes occur during that time, according to previous study (Santos et al., 2014). The inoculants evaluated were (1) control (**CTRL**), (2) commercial inoculant (**CIN**), (3) *Pediococcus acidilactici* (strain 10.6, **S1**), and (4) *Pediococcus pentosaceus* (strain 6.16, **S2**). The commercial inoculant used was Sil-All 4 × 4 W.S. (Alltech, Sao Paulo, Brazil), which contains sucrose, *Lactobacillus plantarum*, *P. acidilactici*, *Enterococcus faecium*, *Lactobacillus salivarus* ssp. *salivarus*, silicon dioxide, amylase, cellulase, hemicellulase, and xylanase. An inoculant application rate of 10^6 cfu/g of fresh forage was used, and the inoculants were diluted in distilled water. The same quantity of water used to dilute them was applied to the CTRL. The S1 and S2 strains were isolated from *Stylosanthes* silage (*Stylosanthes macrocephala* × *Stylosanthes capitata* cv. Campo Grande), and were provided by the Anaerobic Microbiology Laboratory of the Department of Agricultural Microbiology of UFV (Silva et al., 2012). The screening process aimed to find strains with bacteriocinogenic activity, and among 256 isolates, the S1 and S2 strains showed antimicrobial activity against 6 different microorganisms (*Listeria monocytogenes* ATCC 7644, *Alicyclobacillus acidoterrestris* DSMZ 2498, *Lactococcus lactis* ATCC 19435, *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* ATCC 10240, and *Streptococcus bovis* JB1). The S1 and S2 strains also exhibited high growth rate, and their acid-free extracts were sensitive to proteinase, indicating its proteinaceous nature (Silva et al., 2012).

Chemical, Microbiological, Fermentation Profile, and Statistical Analyses

Chemical Analysis. Samples of fresh alfalfa and its silages were dried in a forced-air oven at 55°C for 72 h, and then ground in a Willey mill with a 1-mm sieve. The concentrations of DM (method 934.01), CP (method 984.13), ash (method 942.05), ether extract (method 920.39), and ADF (method 973.18) were analyzed as described by the Association of Official Analytical Chemists (AOAC, 1990). For the NDF analysis, samples were treated with heat-stable α-amylase without use of sodium sulfite and were corrected for residual ash (Mertens, 2002). Correction of the NDF and ADF for nitrogen compounds and estimation of NDIN and ADIN were performed according to Licitra et al. (1996).