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Research Paper

Effects of four short-chain fatty acids or salts on dynamics of fermentation and microbial characteristics of alfalfa silage

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ABSTRACT

The effect of four chemical compounds with antimicrobial properties on the fermentation and microbial dynamics of alfalfa silages were studied under laboratory conditions. Fresh alfalfa was treated with (1) no additive (control), (2) formic acid (FA, 4 g/kg fresh weight), (3) potassium diformate (PD, 5.5 g/kg fresh weight), (4) sodium diacetate (SD, 7 g/kg fresh weight), and (5) calcium propionate (CAP, 10 g/kg fresh weight). First cut alfalfa was harvested at about 10% bloom stage, chopped to approximately 1.5 cm particle length, and ensiled into plastic laboratory silos (1-L capacity, 9.5 cm diameter × 18.7 cm height). Six silos for each treatment were opened after 0.5, 1, 1.5, 2, 3, 5, 7, 14 and 30 days of ensiling, respectively. The pH readings for FA and PD silages rapidly decreased during the first 5 days of ensiling and remained the lowest two values among the treatments through the entire ensiling. The lactic acid fermentation in FA and PD silages were retarded from the d 0.5 of ensiling, indicated by consistently lower lactic acid concentrations than control over the entire ensiling process. During the first 3 days of ensiling, SD and CAP silages showed lower ($P < 0.05$) lactic acid concentrations than untreated silages, however, this trend was reversed after 14 days of ensiling. Three short-chain fatty salts decreased the butyric acid content and dry matter loss, preserved more WSC in alfalfa silage. Of the chemical additives, potassium diformate was as effective as formic acid in immediately reducing pH, while the addition of sodium diacetate and calcium propionate did not reduce silage pH to the level that ensure good conservation of alfalfa silage after 30 days of ensiling.

1. Introduction

With the development of animal husbandry, alfalfa (*Medicago sativa* L.) has been widely cultivated in southern China because of its high-protein content and high yield potential. Due to the warm and humid climate in these regions, ensiling is the preferred method of conserving alfalfa. However, alfalfa has some limitations for silage production including low water soluble carbohydrate concentration and high buffering capacity, which retard the lactic acid production and delay the reduction in pH value.

Abbreviations: ADF, acid detergent fibre; aNDF, neutral detergent fibre; AN, ammonia nitrogen; BA, butyric acid; CAP, calcium propionate; cfu, colony-forming units; DM, dry matter; FA, formic acid; FW, fresh weight; LAB, lactic acid bacteria; PA, propionic acid; PD, potassium diformate; SD, sodium diacetate; TN, total nitrogen; WSC, water soluble carbohydrates; VFAs, volatile fatty acids.

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The direct acidification through organic acid additive in silages is associated with immediate drop in pH and inhibition in activity of undesirable bacteria, and ultimately a reduction in crop nutrients loss (McDonald et al., 1991; Cazzato et al., 2011). However, organic acids are difficult to handle because they have pungent and offensive odor and can cause severe burns, safer salts of organic acid have been proposed as alternative silage additives (Mayne and O'Kiely, 2005). Calcium propionate had been widely used as main components of preservatives in food and forage because of its ability to inhibit the growth of molds and other microorganisms (Mills and Kung, 2002). Calcium propionate is not toxic to organisms, but does prevent them from reproduction and posing a health risk to animals and humans (Draughon et al., 1982). Sodium diacetate, an acetate derivative, consisting of acetic acid and sodium acetate, is widely used as food preservative, and pH buffer. Previous studies also suggested that sodium diacetate was an effective microbial inhibitor and could be used as an antibacterial agent to prolong the shelf life of silages (Shockey et al., 1990; Yitbarek and Tamir, 2014). The undissociated form of these salts pass through the cell membranes of yeasts and molds and release their protons into the cytoplasm, thereby acidifying the intracellular region (Kleinschmit et al., 2005). Potassium diformate has been approved in the European Union as 'growth promoter', mainly based on their strong antimicrobial effect against several bacteria (Øverland et al., 2009).

Production of wilted silages is often difficult due to wet weather during summer, when growth of plant is maximum and surplus material is available for conservation. And fresh alfalfa silage is more prone to clostridium spoilage and higher levels of butyric acid due to the low DM content beyond low sugar content and high buffering capacity (Knicky and Sporndly, 2009). It is necessary to explore novel silage additives for low DM silages under wet condition, we hypothesized that short-chain fatty salts could be used as additives based on their antimicrobial and growth-promoting attributers. Therefore, in this study we evaluated the effects of three short-chain fatty salts on dynamics of fermentation and microbial characteristics of fresh alfalfa silage as compared to silage treated with or without formic acid.

2. Materials and methods

2.1. Silage preparation and treatments

Alfalfa was grown in experimental fields at College of Animal Science, FengYang Campus, Anhui Science and Technology University (32°52′ latitude N, 117°33′ longitude E, Anhui, China). First cut alfalfa was harvested by hand at about 10% bloom stage from six plots in two experimental fields, and chopped to approximately 1.5 cm particle length. The unwilted alfalfa was ensiled with various organic acid or salts: (1) no additive (control), (2) formic acid (FA; 88%, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) applied at 4 g/kg fresh weight (FW), (3) potassium diformate (PD; ≥98%, Hubei Ju Sheng Technology Co., Ltd., Hubei, China) applied at 5.5 g/kg FW, (4) sodium diacetate (SD; ≥98%, Hubei Ju Sheng Technology Co., Ltd., Hubei, China) applied at 7 g/kg FW, (5) calcium propionate (CAP; ≥98%, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) applied at 10 g/kg FW, these dosages was based on our previous study, which evaluated the effect of different rates of organic salts on pH of alfalfa silage after 30 days of ensiling (non-published data). Additives were prepared on the day of ensiling and applied, in solution (i.e. diluted with water), at a rate of 20 mL/kg of herbage. An equal volume of distilled water was added to the control. Additives were added and thoroughly mixed with the chopped forage before being packed into silos. About 780 g of chopped fresh alfalfa was immediately packed into plastic laboratory silos (plastic drum, 1-L capacity, 9.5 cm diameter × 18.7 cm height, Lantian biological experimental instrument Co., Ltd., Jiangsu, China), followed by sealing with two screw tops (internal and external). A total of 270 laboratory silos (five treatments × nine ensiling days × six replicate for each ensiling days) were made and stored at ambient temperature (29–36 °C).

2.2. Chemical and microbiological analysis

Six silos for each treatment were opened after 0.5, 1, 1.5, 2, 3, 5, 7, 14 and 30 days of ensiling, respectively. The entire content of each silo was emptied and placed into an ethanol-disinfected plastic container and mixed to uniformity, a sub-sample of silages (35 g) were homogenized for 1 min in 70 mL of distilled water and then filtered with two layers of cheesecloth. The extract pH was measured immediately with a pH meter (HANNA pH 211, Hanna Instruments Italia Srl, Villafranca Padovana, Italy). The extracts was centrifuged for 10 min at 10000*g*, and the supernatant was reserved for organic acid (including lactic, acetic, propionic and butyric acid) analysis, which were carried out using Agilent 1260 HPLC system equipped with a refractive index detector (Carbomix® H-NP5 column, 2.5 mM H_2SO_4, 0.5 mL/min).

In addition, each sample (100 g) of fresh material or silage was freeze dried to determine dry matter (DM). The dried material was ground to pass a 1-mm screen with a mill (FW100, Taisite Instrument Co., Ltd., Tianjin, China) and used for chemical compositions analysis. The DM losses were calculated from weight losses and differences in DM content. Total nitrogen (TN) was determined by Kjeldahl nitrogen analyzer (Kjeltec 8400, FOSS, Sweden), crude protein content was calculated by multiplying TN by 6.25. The content of WSC was analyzed by colorimetry after reaction with anthrone reagent (Thomas, 1977). Neutral detergent fibre (aNDF) and acid detergent fibre (ADF) were analyzed according to the procedures of Van Soest et al. (1991), heat stable amylase and sodium sulphite were used in the aNDF procedure and the results of aNDF and ADF were expressed on DM basis including residual ash.

The microorganism numbers in the fresh materials and silages were determined by the plate count method. Samples (10 g) were blended with 90 mL of sterilized water, and the extract serially diluted in sterilized water. The LAB were counted on deMan, Rogosa and Sharp (MRS) agar medium (Shanghai Bio-way Technology Co., Ltd.) after incubation in an anaerobic