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Chitosan improves the chemical composition, microbiological quality, and aerobic stability of sugarcane silage

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ABSTRACT

The purpose of the current study was to evaluate the effects of inoculants on chemical composition, dry matter (DM) and neutral detergent fiber (aNDF) *in vitro* degradation, fermentative and effluent losses, microbiology, fermentative profile, and aerobic stability of sugarcane mini-silos. Treatments were randomly distributed to the mini-silos, in which: (1) Control (CON); (2) *Lactobacillus buchneri* (Lb), addition of Lb at 2.6×10^{10} cfu/g; (3) *Lactobacillus buchneri and Bacillus subtilis* (Lb + Bs), addition of Lb at 2.6×10^{10} cfu/g and Bs at 1×10^{9} cfu/g; and (4) Chitosan (CHI), addition of 1% of CHI on wet basis of sugarcane ensiled. Treatments 2 and 3 were incorporated to the silage at 2 g/t of natural matter ensiled. Lb and Lb + Bs did not alter the *in vitro* degradation of DM and NDF. Chitosan incorporation increased the DM content ($P = 0.013$, 18.7 g/kg DM) and improved ($P = 0.029$, 45.6 g/kg DM) the NDF *in vitro* degradation of sugarcane silage. In addition, CHI incorporation showed higher ($P = 0.002$) DM content in silage than Lb and Lb + Bs. Microbial inoculants (Lb and Lb + Bs) reduced the total losses ($P = 0.009$) of sugarcane silage. Moreover, CHI incorporation showed lower ($P = 0.001$, 84.9 g/kg DM) total losses and higher ($P = 0.031$, 84.8 g/kg DM) dry matter recovery than Lb and Lb + Bs. Lactic acid bacteria concentration was increased ($P = 0.001$) with additives, and CHI incorporation showed higher ($P = 0.001$) lactic acid bacteria concentration than silages treated Lb and Lb + Bs. All additives decreased the ethanol concentration in sugarcane silage, but CHI showed lower ($P = 0.002$) ethanol concentration compared to Lb and Lb + Bs. Inoculants improved the aerobic stability of sugarcane silage. In general, the incorporation of CHI to sugarcane silage showed better results of NDF *in vitro* degradation and gas and effluent losses than Lb and Lb + Bs. Moreover,

Abbreviations: aADF, acid detergent fiber; aNDF, neutral detergent fiber; Bs, *Bacillus subtilis*; CHI, chitosan; CON, control; CP, crude protein; DM, dry matter; DMR, dry matter recovery; Lb, *Lactobacillus buchneri*; TDN, total digestible nutrient.

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CHI incorporation showed higher concentration of lactic acid bacteria and lower concentration of ethanol compared to silages treated Lb and Lb + Bs. Chitosan may be an alternative additive to microbial inoculants used in sugarcane ensiling.

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1. Introduction

Sugarcane (*Saccharum officinarum*) is often used in Brazil as a forage source for dairy and beef cattle, since the harvesting phase coincides with the winter which is a period of shortage of feed. Ensiling sugarcane may be a strategy to decrease daily manpower and theoretically maintain similar nutrient composition from the beginning until the end of the silo. Sugarcane crop has a high DM production per hectare (25–40 t; Ávila et al., 2009), high water-soluble carbohydrates content, and a low buffering capacity that enables rapid decrease of pH (Freitas et al., 2006). However, its fermentation can produce high amounts of ethanol, increasing DM losses (Kung and Stanley, 1982), and accumulating fiber components causing a decrease of DM digestibility (Santos et al., 2009); thus, the advantages of ensiling sugarcane may be limited by these factors.

Microbial inoculants have been used to shift alcoholic fermentation and improve sugarcane silage digestibility. Several studies have evaluated *Lactobacillus buchneri* as a silage additive during the last decade (Santos et al., 2015; Carvalho et al., 2012; Pedroso et al., 2010). This heterolactic bacteria has been shown to improve silage fermentation due to a reduction of ethanol production and pH values (Pedroso et al., 2008). Kleinschmidt and Kung (2006) evaluated forty-three experiments and reported the effectiveness of *L. buchneri* to reduce the pH and yeast population, and its effectiveness to increase the acetic acid concentration and aerobic stability of silages from several plants species (corn, sorghum, wheat, barley, and grass forages). However, studies related to *Bacillus subtilis* treatment during the ensiling process are scarce in literature. Todovora and Kozhuharova (2010) reported that *B. subtilis* produces metabolites with antifungal and antibacterial activity. Phillip and Fellner (1992) evaluated the addition of *B. subtilis* in corn silage and reported improvements of the aerobic stability.

Chitosan is a biopolymer obtained by the partially deacetylation of chitin, the second most abundant biopolymer in nature, and the major component of crustaceans and insects exoskeleton (Senel and McClure, 2004). The antimicrobial activity of CHI is well known against bacteria and fungi (Senel and McClure, 2004), and have been used as rumen modulator. Chitosan was able to completely inhibit the growth of dimorphic fungus (Olicón-Hernández et al., 2015). Araújo et al. (2015) reported that CHI quadratically affected the ruminal ammonia nitrogen concentration and the molar proportions of propionate in beef steers. In addition, the same authors found that CHI increased the digestibility of DM, NDF and crude protein (CP; Araújo et al., 2015).

Our hypothesis was that inoculants would positively affect the fermentation pattern and aerobic stability, decreasing the DM losses of sugarcane silage. Furthermore, CHI would alter microbiology and reduce fungi amounts in the silage. The objective of the current study was to evaluate the effects of three inoculants on chemical composition, DM and NDF *in vitro* degradation, fermentative and effluent losses, microbiology, fermentative profile, and aerobic stability of sugarcane mini-silos.

2. Material and methods

The experiment was conducted between May and September of 2013 at the Department of Animal Science, School of Agrarian Sciences, Federal University of Grande Dourados, Dourados, Brazil; 22°14′S latitude, 54°49′W longitude and 450 m altitude.

2.1. Treatments and ensiling

Sugarcane variety RB 84-5257 was manually harvested from 10 batches within one 0.35-ha plot after 10 months of regrowth (second cut). Approximately 50.0 kg of sugarcane tillers from each location was separately chopped in a stationary cutter to a theoretical cut length of 10 mm. A randomized experimental design was used, and contained 4 treatments distributed into 40 mini-silos. Mini-silos were produced in plastic bucket (30 cm of height and 30 cm of diameter) containing *Bunsen* valves to avoid the gas scape. Two kilograms of sand were placed at the bottom of mini-silos, separated from the forage by a nylon screen to determine the effluent production. Silos were packed (650 kg/m^3, on wet basis), sealed, weighed, and stored at room temperature (28.5 ± 2.3 °C) for 60 days. Mini-silos were weighed immediately after the opening to record DM and gas losses.

Treatments were randomly distributed to the mini-silos, in which: (1) Control (CON); (2) *L. buchneri* (Lb), addition of Lb at 2.6×10^{10} cfu/g; (3) *L. and B. subtilis* (Lb + Bs), addition of Lb at 2.6×10^{10} cfu/g and Bs at 1×10^{9} cfu/g; and (4) Chitosan (CHI), addition of 1% of CHI on wet basis of sugarcane ensiled. Chitosan used during all experiment had the technical specifications: apparent density of 0.64 g/mL, 20.0 g/kg of ash, 7.0–9.0 of pH, viscosity <200 cPs and deacetylation level of 95% (Polymar Industria, Ceara, Brazil). In addition, the CHI had 873 g/kg of DM and 316 g/kg of CP. The treatments 2 (Lb) and 3 (Lb + Bs) were added at 2 g/t of natural matter ensiled. Microbial inoculants were diluted in water (2 g/L) and sprayed onto the forage,