CHEMICAL COMPOSITION, FERMENTATION PROFILE AND APPARENT DIGESTIBILITY OF SUGARCANE SILAGE TREATED WITH CHEMICAL ADDITIVES[1]

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ABSTRACT: The aim of this study was the indication of an additive for the ensilage of sugarcane (*Saccharum officinarum* L.). In a laboratory trial, nine treatments were applied (g of additive/kg of fresh forage - FF) to the sugarcane (RB867515), before ensiling in minisilos (15 x 30 cm PVC tubes) during 78 days: untreated; urea (5) + sodium benzoate (0.5); urea (7.5) + benzoate (0.5); urea (5) + benzoate (0.75); urea (7.5) + benzoate (0.75); sodium propionate (1, 2 and 4); calcium hydroxide (10). Urea + benzoate in the lowest doses, propionate in the higher concentration and calcium hydroxide were selected, considering the ethanol content (26.5, 27.2 and 7.4 g/kg DM, respectively), total DM loss (88, 46 and 58 g/kg DM, respectively) and digestibility (541, 496 and 516 g/kg DM, respectively) of the silages. Silages treated with these doses of additives and the untreated silage (80 d of storage) were fed (nine + seven d) to 16 castrated male sheep (Santa Inês) housed in metabolic cages. The silage with calcium hydroxide presented coefficients of apparent digestibility of DM (0.44), of NDF (0.4) and DM intake (20 g/kg live weight) in the higher levels. Calcium hydroxide was superior to propionate and urea + benzoate, considering alcoholic fermentation control and reduction of losses in the silage and the forage's nutritional value.

Keywords: alcoholic fermentation**,** benzoate, **c**alcium hydroxide, propionate, urea.

COMPOSIÇÃO QUÍMICA, PERFIL FERMENTATIVO E DIGESTIBILIDADE APARENTE DE SILAGENS DE CANA-DE-AÇÚCAR TRATADAS COM ADITIVOS QUÍMICOS

RESUMO: O objetivo deste estudo foi indicar um aditivo para a ensilagem da cana-de-açúcar (*Saccharum officinarum* L.). Em um ensaio de laboratório, nove tratamentos foram aplicados (g de aditivo/kg de forragem fresca - FF) à cana-de-açúcar (RB867515) antes da ensilagem em minisilos (tubos de PVC de 15 x 30 cm) durante 78 d: sem tratamento; uréia (5) + benzoato de sódio (0,5); uréia (7,5) + benzoato (0,5); uréia (5) + benzoato (0,75); uréia (7,5) + benzoato (0,75); propionato de sódio (1, 2 e 4); hidróxido de cálcio (10). Uréia + benzoato nas doses mais baixas, propionato na dose mais alta e hidróxido de cálcio foram selecionados, considerando a concentração de etanol (26,5; 27,2 e 7,4 g/kg MS, respectivamente), perda total de MS (88, 46 e 58 g/kg MS, respectivamente) e digestibilidade (541, 496 e 516 g/kg MS, respectivamente) das silagens. Silagens tratadas com estas dosagens dos aditivos e silagem não tratada (80 d estocagem) foram fornecidas (nove + sete d) a 16 carneiros (Santa Inês) machos castrados mantidos em gaiolas metabólicas. A silagem com hidróxido de cálcio apresentou coeficientes de digestibilidade aparente da MS (0,44), da FDN (0,40) e de ingestão da MS (20 g/kg peso vivo) nos níveis mais altos. Hidróxido de cálcio foi superior ao propionato e à uréia + benzoato, considerando-se o controle da fermentação alcoólica, a redução de perdas e o valor nutritivo da silagem.

Palavras-chave: benzoato, fermentação alcoólica, hidróxido de cálcio, propionato, ureia.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an important feed resource in many tropical countries. Its ability to maintain good quality during winter, the dry season, allows farmers to feed it fresh chopped to livestock, mostly small and large ruminants. Nonetheless, daily harvesting impairs field management practices and may demand excessive labor what has led to the increased use of sugarcane as silage. Ensiling may also be useful to avoid total loss of the forage in the case of accidental fires or severe frosts. It has long been established though that fermentation in sugarcane silages is characterized by intense yeast development and alcoholic fermentation, causing extensive dry matter - DM - loss and reduction in the silage's nutritive value (Alli *et al*., 1982). Therefore, the use of additives has been shown to be essential in the ensilage of this crop (Pedroso *et al*., 2007).

Initial attempts to ensile sugarcane have been frequently unsuccessful due to the lack of efficient machinery and additives and the use of poor-quality varieties. Improvements in these aspects have led to the noticeable expansion in the use of the technique but more information on the efficiency of additives is still necessary.

Urea, sodium benzoate, propionate and calcium hydroxide - $Ca(OH)_2$ - (slaked lime) have shown positive effects as additives in the ensilage of sugar-cane in previous evaluations but variation in effectiveness among trials, the indication that some additives may be more efficient when combined and the necessity to evaluate different doses make further studies indispensable.

The application of urea combined with sodium benzoate was first evaluated by Pedroso *et al*. (2011) in an attempt to improve efficiency since previous results with both additives were to variable. Those authors reported the occurrence of a synergistic effect between these additives but the consistency of that effect and the possibility that other levels of combination might be more efficient still need to be evaluated.

Propionic acid is a potent antimycotic and its salts have been effective in reducing yeasts in corn silages (Kung *et al*., 1998). Nonetheless, the application of calcium propionate did not improve the fermentation pattern when applied at several doses to sugarcane silage (Pedroso *et al*., 2007). It was suggested that salts with higher solubility than calcium propionate should be tested. Sodium propionate, which is five times more soluble than calcium propionate, may be proven to be efficient.

The use of calcium hydroxide - $Ca(OH)_2$ - and calcium oxide (CaO - quicklime) as additives on the ensilage of sugarcane has expanded based on the low cost of application and the possibility of improving forage digestibility by means of fiber hydrolysis. Application of this additive in both forms improved fermentation and the silage's digestibility in previous experiments (Santos *et al*., 2008; Cavali *et al*., 2010; Siqueira *et al*., 2011) but information on its effects on animal performance is still scarce.

The aim of this research was the indication of an additive for the ensilage of sugarcane considering the effectiveness in controlling alcoholic fermentation, in reducing losses and in preserving the forage's nutritional value during ensilage. Four combinations of urea with sodium benzoate, three doses of sodium propionate and calcium hydroxide were tested in a laboratory trial and, subsequently, the three most promising treatments were evaluated in a digestibility trial with sheep.

MATERIAL AND METHODS

This research is in agreement with ethical principles of animal experimentation in accordance with the Institutional Animal Care and Use Committee Guidelines of EMBRAPA.

Trial 1: Laboratory evaluation

The experimental silages were produced with mature sugarcane (variety RB867515; 12 months old; 22 °Bx) mechanically harvested with a harvester adjusted for a cut length between 5 and 10 mm. The additives were applied to the forage before ensiling (amount per kg of fresh forage - FF), as follows: 5 g of urea + 0.5 g of sodium benzoate - U5B5; 7.5 g of urea + 0.5 g of benzoate - U7B5; 5 g of urea + 0.75 g of benzoate - U5B7; 7.5 g of urea + 0.75 g benzoate - U7B7; 1, 2 and 4 g of sodium propionate - PROP1, PROP2 and PROP4, respectively; 10 g of calcium hydroxide - CAL. The doses were selected to include the most used and which presented some benefit during the ensilage of sugarcane. The aims was evaluating the synergistic effect in the case of urea plus benzoate, the effectiveness of a more soluble salt of propionate and get more information on the effects of a effective dose of calcium hydroxide. Silage without additive was used as control. The amounts of additives correspondent to each treatment, sufficient to treat 25 kg mounds of the chopped sugarcane were diluted in 988 mL of water and applied onto the forage using manual sprayers.

The same amount of pure water was applied to the control silage. After thoroughly mixing the treated sugarcane, approximately 3,200 g were compacted manually (using wood bars) into 15 cm wide x 30 cm long PVC tubes (minisilos). Four replicates were prepared for each treatment. The minisilos were provided with tight lids equipped with O-ring seals. After filling, the lids were sealed with adhesive tape as an extra precaution against air penetration and the minisilos stored in the laboratory at room temperature.

The minisilos were weighed, and samples were taken for bromatological analysis, at the time of ensilage and on the opening day (78 d after ensiling). During sampling, the material in the top 5 cm of each minisilo was discarded and the remaining silage thoroughly mixed before samples were taken. Total dry matter loss (TDML) was calculated by dry matter weight loss in the silages, according to the formula:

$$TDML\ (g/kg\ DM) = \{[(NWi \times DMi) - (NWf \times DMf)]/(NWi \times DMi)\} \times 1000$$

where, NWi: initial net weight (g) of the forage in the minisilo; DMi: initial DM content (g/kg FF) of the forage in the minisilo; NWf: final net weight (g) of the forage in the minisilo; DMf: final DM content (g/kg FF) of the forage in the minisilo.

For aerobic stability evaluation, silage samples of approximately 2 kg were taken from each minisilo immediately after opening and loosely transferred to polystyrene boxes kept at room temperature. The lid of each box had three diagonally displaced 0.5 cm perforations to allow air penetration and passage of the thermocouple wire. The polystyrene boxes allowed the use of relatively small amounts of silage in the evaluation since the insulating material avoided the loss of heat from the silages to the ambient, during aerobic deterioration. The temperature in the silages exposed to the air was monitored with a thermocouple placed in the geometrical center of the silage mass in each box. Thermocouples were connected to a data logger that recorded temperatures at 15 min intervals. Aerobic stability was defined as the number of hours the temperature in the silages remained stable before rising more than 2°C above room temperature (Kung *et al*., 2000). Temperature in the silages was controlled until the onset of aerobic deterioration in all four replicates of each treatment.

The processing of forage samples, the chemical analysis and the statistical methods which were common to both trials are described at the end of this section.

Trial 2: Apparent digestibility and intake by sheep

This trial aimed at evaluating intake and apparent digestibility of sugarcane silages treated with the additives which presented the best results in Trial 1: urea (5 g/kg FF) + benzoate (0.5 g/kg FF) - U+B; sodium propionate in the higher concentration (4 g/kg FF) - PROP - and calcium hydroxide (10 g/kg FF) - CAL. Silage produced without additives was used as control. Silages were produced with mature sugarcane (RB867515; 12 months old; 22°Bx) mechanically harvested, with a harvester adjusted for a cut length between 5 and 10 mm. The additives correspondent to each treatment were thoroughly mixed to 500 kg mounds of the chopped sugarcane at the following dilutions: 2.5 kg of urea in 8.8 L of water + 250 g of benzoate in 2.3 L of water; 2 kg of propionate in 4 L of water; 5 kg of calcium hydroxide in 20 L of water. Solutions were applied with manual sprayers and thoroughly mixed with the forage. After treatment, the chopped sugarcane was compacted with the feet into 200 L metal drums (three per treatment) lined with thick-walled plastic bags. After filling, the drums' lids were sealed with adhesive tapes and the silos stored in the shade of a open barn at room temperature.

Silos were opened 80 d after ensiling and the silages fed to 16 Santa Ines (woolless Brazilian breed) wethers (20.1 months old castrated male sheep) with uniform initial live weight - LW - of 44.1± 1.6 kg, housed in metabolic cages, with free access to clean water. During the experimental period the animals were fitted with fecal collection bags. Wethers were distributed among four treatments (diets based on the different silages Table 1) in a complete randomized design with four replicates. Soybean meal and a mineral supplement were used to balance, respectively, protein and mineral content in the diets, based on the expected composition of feeds (Table 2), aiming at 125 g of CP, 610 g of TDN, 4.5 g of Ca and 3.2 g of P/kg DM, with the exception of the diet with silage treated with calcium hydroxide which was expected to have 15 g Ca/kg DM. With this concentration, the Ca:P ratio in the diet containing silage treated with calcium hydroxide (4.7:1) was below the upper limit of 7:1, beyond which intake may be reduced (Wise *et al*., 1963). The silages' DM apparent digestibility coefficient was calculated by difference, considering that the digestibility of soybean meal was known (900 g/kg DM). For the calculation, it was considered the DM intake (DM in the offered ration minus DM in orts), the DM in the feces and the estimated amount of soybean meal DM in feces, considering the percentage included in the diets and

Table 1. Composition of experimental diets

Components (% DM)	[1]Diets			
	Control	U+B	PROP	CAL
Sugarcane silage without additive	76.8	-	-	-
[2]Sugarcane silage with urea + benzoate	-	82.5	-	-
[3]Sugarcane silage with propionate	-	-	77.1	-
[4]Sugarcane silage with calcium hydroxide	-	-	-	78.8
Soybean meal	20.8	15.0	20.5	19.2
Minerals	2.4	2.5	2.4	2.0
Total	100	100	100	100

[1]Control: diet with untreated silage; U+B: diet with silage treated with urea (5 g/kg FF) + benzoate (0.5 g/kg FF); PROP: diet with silage treated with sodium propionate in the higher concentration (4 g/kg FF); CAL: diet with silage treated with calcium hydroxide (10 g/kg FF). [2]Urea (5.0 g/kg FF) + sodium benzoate (0.5 g/kg FF). [3]Sodium propionate (4.0 g/kg FF). [4]Calcium hydroxide (10 g/kg FF); FF: fresh forage.

Table 2. Composition of feeds used to balance the experimental diets

Components	[1]Chemical composition				
	DM	CP	TDN	Ca	P
	g/kg FF	g/kg DM			
Sugarcane silage without treatment	279	30	550	2.0	0.6
Sugarcane silage with urea + benzoate	280	65	580	2.0	0.6
Sugarcane silage with propionate	280	30	580	2.0	0.6
Sugarcane silage with calcium hydroxide	280	30	580	16.0	0.6
Soybean meal	900	500	800	2.4	6.5
Minerals	1000	-	-	100	60

[1]Average values based on the literature; DM: dry matter; CP: crude protein; TDN: total digestible nutrients; Ca: calcium; P: phosphorus; FF: fresh forage.

its digestibility. The apparent digestibility coefficient (DC) was calculated by the formula:

DC = (kg of ingested nutrient – kg of nutrient in feces)/kg of ingested nutrient

During the pre-experimental period (nine days), all animals were fed their respective diets on *ad libitum* basis. Silage samples were dried in an air forced dry oven (65°C, 48 h) for DM determination and adjustment of diets. During the experimental period (seven days), 40% of the daily diet was offered around 9 AM and 60% around 4 PM. Grab samples were taken daily from all silages and kept frozen (-10°C) until the time of analysis. Feces and orts (scraps) were removed daily, weighed, sampled, stored at -10°C and combined in a composite sample per animal at the time of analysis. Daily samples of soybean meal were frozen and combined in one composite sample at the time of analysis.

General chemical and statistical analysis

Silage samples for ethanol, pH, volatile fatty acids (VFA) and lactic acid determinations were frozen (-10°C) before analysis. Other forage samples, as well as diets, orts and feces samples, were dried in an air forced dry oven (65°C, 48 h), grounded in a Wiley mill through a 1 mm screen and analyzed as follows: acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin according to Van Soest and Robertson (1985); DM, ash and crude protein (CP) according to AOAC (1997), methods number 934.01; 942.05 and 984.13, respectively. *In vitro* DM digestibility (IVDMD) was determined using the method proposed by Tilley and Terry (1963; the rumen fluid used in the analysis being collected from rumen-fistulated Holstein dry cows receiving a daily ration of corn silage *ad libitum* and 2 kg of a 18% CP concentrate .

Silage extracts for ethanol, pH and organic acids (VFA and lactic acid) determinations were produced from juice obtained by means of a hydraulic press. Approximately 300 g of silage from each sample were used to produce 50 mL of juice. After pH evaluation with a digital potentiometer, juice samples were centrifuged at 3,000 x*g* for 15 min and 5 mL of supernatants transferred to 10 mL test tubes containing 1 mL of formic acid P.A. From these extracts, 1 mL was filtered through a Millex filter (0.45 µm) and stored at -10°C until analysis. Ethanol and VFA were analyzed by gas chromatography according to Sigma-Aldrich, Co (1998) and lactic acid using high performance liquid chromatography (HPLC) according to Wilson (1971).

Data were analyzed as completely randomized designs and subjected to ANOVA by the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Differences among means were tested using the *t* test. Significant differences were declared if P<0.05. To study the association among some variables in Trial 1, the coefficient of simple correlation was determined by the PROC CORR of SAS (SAS Inst., Inc., Cary, NC).

RESULTS AND DISCUSSION

Trial 1

The silage produced without additive had adequate pH (<4.2) despite the low concentration of lactic acid (Table 3). The acetic acid content was high but butyric and propionic acids concentrations were within levels reported in the literature (Pedroso *et al*, 2011; Schmidt *et al*, 2011). Silages treated with additives also had adequate pH and relatively low concentrations of lactic acid, with exception of the silage treated with calcium oxide, which had a high content of that acid (Table 3). It has been observed that the low buffering capacity of sugarcane allows rapid drop in pH even with relatively small amounts of acids in the silage (Alli *et al*., 1983). In the silages treated with additives, contents of acetic, propionic and butyric acids were within adequate levels, with exception for the silages treated with propionate in which levels of propionic acid increased accordingly to the dose applied.

High acetic acid content may occur in extremely wet and/or poorly compacted silages and also in silages resultant of prolonged fermentation due to a high buffering capacity in the forage (Kung and Shaver, 2001). Poor compaction does not explain the high concentration of the acid in the control silage considering that density was within the adequate range for all silages (534 ± 42 kg/m^3). Prolonged

Table 3. Fermentation parameters and aerobic stability of sugarcane silages treated with additives

[1]Silage	pH	Acetic acid	Propionic acid	Butyric acid	Lactic acid	Ethanol	[2]TDML	Aerobic Stability (h)
		g/kg DM						
Control	3.50 e	56.5 a	1.9c	0.10 a	16.0 d	76.2 ab	188 a	40 bc
U5B5	3.64 cd	19.1 c	0.9 d	0.02 c	22.2 cd	26.5 cd	88 cd	41 bc
U5B7	3.73 b	20.3 c	0.6 d	0.02 c	27.9 bc	34.7 c	77 de	34 c
U7B5	3.69 bc	18.8 c	0.7 d	0.02 c	26.9 bc	29.6 c	115 bc	48 ab
U7B7	3.70 b	19.5 c	0.6 d	0.02 c	24.4 bc	28.7 c	65 de	42 bc
PROP1	3.54 e	24.1 bc	1.6 c	0.02 c	21.9 cd	58.2 b	129 b	45 bc
PROP2	3.63 d	25.8 b	3.1 b	0.02 c	28.8 bc	79.1 a	164 a	46 bc
PROP4	3.67 bcd	23.8 bc	5.4 a	0.02 c	30.0 b	27.2 c	46 e	52 ab
CAL	3.94 a	21.7 bc	0.6 d	0.07 b	69.2 a	7.4 d	58 de	60 a
SE	0.04	3.4	0.4	0.02	4.8	11.5	19	7

[abc]Means in the same column with different superscript differ (P<0.05) by the t test. SE = standard error.
[1]Control: without additive; U5B5: urea (5.0 g/kg FF) + sodium benzoate (0.5 g/kg FF); U75B5: urea (7.5 g/kg FF) + benzoate (0.5 g/kg FF); U5B75: urea (5.0 g/kg FF) + benzoate (0.75 g/kg FF); U7B7: urea (7.5 g/kg FF) + benzoate (0.75 g/kg FF); PROP1, PROP2 and PROP4: sodium propionate at the rates of 1.0, 2.0 and 4.0 g/kg FF, respectively; CAL: calcium hydroxide (10 g/kg FF); FF: fresh forage. [2]TDML: total dry matter loss.

fermentation can also be discarded as sugarcane has low buffering capacity (ALLI *et al*., 1983) and pH lower than 4.0 has been observed in the silage as early as three days after ensiling (PEDROSO *et al*., 2005). Dry matter content also does not explain the excessive amount of acetic acid in this experiment considering that the initial DM content (321 g/kg) was within recommended levels for adequate fermentation (MCDONALD *et al*., 1991). It is possible to speculate that enterobacteria population was high in the ensiled sugarcane and were allowed to grow in the initial phase of the ensilage process leading to the high acetic acid content in the silage. Similar initial populations of enterobacteria (5.45 cfu/g of FF) and lactic acid bacteria (5.09 cfu/g of FF) have being identified in sugarcane silages by CAVALI *et al*. (2010). Enterobacteria are facultative anaerobes sensitive to low pH (<4.5 to 5.0) which principal fermentation product is acetic not lactic acid. Bacteria in this group are the principal competitors of the lactic acid bacteria for the sugars in ensiled crops (MUCK, 2010), what may explain the low lactic acid content in the control silage.

An increase in the number of lactic acid bacteria (CAVALI *et al*., 2010) and higher content of lactic acid (PADUA *et al*., 2014) were observed in previous experiments evaluating the effect of calcium oxide in sugarcane silages. Although these results were not explained, it is possible that the effective inhibition of yeasts by the additive, already evidentiated by ethanol reduction in the treated silages (SANTOS *et al*., 2008; CAVALI *et al*., 2010; SIQUEIRA *et al*., 2011), reduces competition for substrate in the silage favoring the development of lactic acid bacteria.

Ethanol content was high in the control silage (Table 3). The intense alcoholic fermentation in the untreated silage resulted in high DM loss and approximately 20% reduction in the forage's DM content relative to the fresh sugarcane (Table 3, 4). Fermentation in the control silage also resulted in higher concentration of fiber components, CP and minerals and 9.6% reduction in digestibility relative to the fresh sugarcane (Table 4). All these aspects are characteristic of sugarcane silages produced without additives and have been well documented (PEDROSO *et al*., 2005; SIQUEIRA *et al*., 2010). Yeasts are

Table 4. Chemical composition and *in vitro* dry matter digestibility (IVDMD) of fresh sugarcane and sugarcane silages treated with additives

[1]Forage	[2]Chemical composition								IVDMD
	DM	CP	NDF	ADF	LIG	Ash	Ca	P	
	g/kg FF	g/kg DM							
Sugarcane	321 a	26.6 f	516 b	307d	51.5 abc	24.5 f	2.13 d	0.31 d	532 ab
Control silage	257 f	32.6 de	615 a	387 a	57.9 ab	31.0 cd	2.61 c	0.39 abc	481 d
U5B5	286 bc	78.4 c	584 ab	368 ab	51.4 abc	28.4 de	2.60 c	0.36 c	541 a
U5B7	280 cd	83.7 b	586 ab	359 abc	45.8 c	28.5 de	2.82 bc	0.37 bc	514 abc
U7B5	287 bc	109 a	555 ab	378 ab	48.8 bc	28.3 e	2.66 bc	0.37 bc	514 abc
U7B7	289 bc	107 a	567 ab	351 abc	55.4 abc	28.0 e	2.95 bc	0.37 bc	511 bc
PROP1	272 ed	34.1 d	529 ab	343 abc	58.5 ab	30.1 cde	2.99 b	0.40 ab	493 d
PROP2	264 ef	32.0 de	589 ab	342 bcd	60.5 a	31.8 c	2.94 bc	0.42 a	469 d
PROP4	282 bcd	29.0 ef	595 a	357 abc	61.3 a	35.4 b	2.90 bc	0.38 bc	496 cd
CAL	292 b	28.8 ef	538 ab	319 cd	52.9 abc	60.6 a	17.58 a	0.38 bc	516 abc
SE	7.9	3.08	53	28	7.97	1.77	0.25	0.02	20

[abc]Means in the same column with different superscript differ (P<0.05) by the *t* test. SE = standard error.
[1]Control: without additive; U5B5: urea (5.0 g/kg FF) + sodium benzoate (0.5 g/kg FF); U75B5: urea (7.5 g/kg FF) + benzoate (0.5 g/kg FF); U5B75: urea (5.0 g/kg FF) + benzoate (0.75 g/kg FF); U7B7: urea (7.5 g/kg FF) + benzoate (0.75 g/kg FF); PROP1, PROP2 and PROP4: sodium propionate at the rates of 1.0, 2.0 and 4.0 g/kg FF, respectively; CAL: calcium hydroxide (10 g/kg FF). [2]DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; LIG: lignin; Ca: calcium; P: phosphorus; FF: fresh forage.

not inhibited by pH levels normally found in silages and fermentation of sugars by this fungi results in proximately 49% loss of substratum as CO_2 and H_2O (McDonald *et al*., 1991). Uncontrolled yeast fermentation may consume up to 70% of sugars in sugarcane silages causing other components to become more concentrated in the silage's DM (Pedroso *et al*., 2005).

In this trial it was necessary to select three additives to be tested in the next phase of the experiment (*in vivo* digestibility trial). That number being defined accordingly to the availability of experimental animals and equipment. Considering the relatively high number of treatments and characteristics analyzed in the silages, some quality and fermentation parameters had to be chosen in order to make the selection of additives easier. Correlation and regression analysis using data from all treatments indicated that ethanol concentration in the silages was directly related to DM loss (TDML) (r=0.81) and inversely related to i*n vitro* DM digestibility (IVDMD) (r=-0.57). As expected, TDML presented inverse relationship with IVDMD (r=-0.51). Similar results, with stronger correlation coefficients, were obtained previously by Pedroso *et al*. (2005) in an experiment designed to evaluate fermentation dynamics in sugarcane silages. Those researchers reported r: 0.89 for ethanol vs. TDML; r= -0.88 for ethanol vs. IVDMD and r=-0.99 for TDML vs. IVDMD. Thus, considering that fermentation losses and silage digestibility are fundamental when analyzing the feasibility of any ensiling process and the fact that both are negatively affected by ethanol content, in the case of sugarcane silages, these parameters were considered the most important in the selection of the three best additives in this trial.

Taking into account the combined effects the additives had on the ethanol content, on DM loss, on digestibility and aerobic stability of the silages (Table 3 and 4), urea and benzoate combined in the lowest doses (U5B5), propionate in the higher concentration (PROP4) and calcium hydroxide (CAL) were considered the most promising and were selected to be further evaluated in the *in vivo* digestibility trial with sheep. These treatments resulted in, respectively, 65%, 64% and 90% reduction in ethanol content in the silages compared to control and, consequently, the lowest levels of DM losses. Treatments U5B5 and CAL preserved the forage's digestibility, resulting in silages with IVDMD similar (P>0.05) to the fresh sugarcane (530 g/kg, in average) and, approximately, 10% superior compared to the silage without additive. Considering the aerobic stability, silages in these treatments were among the most stable.

Effects of urea and benzoate combined in higher doses (U5B7, U7B5, and U7B7) were not worse if compared to the effects of the additives combined in the lowest doses (U5B5). Nonetheless, as higher doses of any of the products incur in higher costs of application, the combination with probably the lowest cost (U5B5) was selected. Concerning the treatments with propionate, although the three different doses resulted in silages with similar DM digestibility and aerobic stability, the higher dose (PROP4) resulted in more effective control of alcoholic fermentation and, consequently, much lower DM loss, indicating its selection to the next phase of evaluation.

The reduction in alcoholic fermentation observed in the silages treated with urea + benzoate may be credited to the toxic effect of ammonia and benzoate on yeasts (Alli *et al*., 1983). Plant cell urease converts urea applied to ensiled forages into ammonia and the additive applied at the dose of 5.0 g/kg FF resulted in reductions in yeast counts, lower ethanol content and DM losses and higher digestibility in sugarcane silages of previous experiments (Pedroso *et al*., 2007; Castro Neto *et al*., 2008; Pedroso *et al*.; 2008). Higher doses of urea may result in inadequate pH and higher DM losses in the silage (Siqueira *et al*., 2010).

Sodium benzoate is a common food preservative, which has long been known as an effective inhibitor of yeast and molds (Woolford, 1975) but the exact mechanism by which cell growth is inhibited by weak organic acids is yet to be defined. It appears to involve cytosol acidification by acid dissociation in the higher pH inside the cell, disruption of membrane homeostasis and mitochondrial physiology, among others (Krebs *et al*., 1983). Data on benzoate effect on sugarcane silages are scarce and inconsistent though. In an early experiment, Pedroso *et al*. (2007) reported unsatisfactory results for benzoate applied at the rate of 1.0 g/kg FF but treatment with this salt reduced alcohol production and DM losses and improved silage digestibility and aerobic stability in a subsequent evaluation. Siqueira *et al*. (2007) and Siqueira *et al*. (2010) observed improved DM recovery and aerobic stability in sugarcane silages treated with this additive. In an experiment by Pedroso *et al*. (2011) benzoate application did not reduce alcoholic fermentation but somehow improved the nutritional value of the silage that presented lower ADF and lignin contents and higher TDN compared to the untreated silage. Higher doses of this additive could prove to be more efficient but elevation in the cost of application would probably make it impractical.

In an attempt to improve efficiency, the combination of urea and sodium benzoate was first evaluated by Pedroso *et al*. (2011) who reported the occurrence of a synergistic effect between these additives when applied together at the ensilage of sugarcane. This combination of additives may bring some benefits to farmers besides the control of alcoholic fermentation in the silages: the use of a low dose of urea allows partial correction of protein content in the silage without the negative aspects on fermentation that may occur when urea is applied in higher doses; applying urea at ensiling poses less risk of intoxication to animals than mixing the product with the forage in the feed bunk, the traditional way to correct protein content in fresh sugarcane. Accordingly, in this experiment, treatment with urea + benzoate in the lowest doses caused elevation of CP, from 27 g/kg DM in the fresh sugarcane to around 80 g/kg DM in the silage, besides significant reductions in the ethanol content and losses in the conserved forages.

Propionic acid has the highest antimycotic activity among the short chain fatty acids (Woolford, 1975). Accordingly to Kung *et al*. (1998), treatment of silages with the concentrated acid is not recommended due to its corrosive and volatile nature. Thus, acid salts, e.g., calcium, sodium and ammonium propionate, have become more widely used. Doses between 2.0 and 3.0 g/kg FF of these salts have been effective in reducing yeasts in corn silages (Kung *et al*., 1998; Kung *et al*., 2000). In the first evaluation of a propionic acid salt as an inhibitor of alcoholic fermentation in sugarcane silages, Pedroso *et al*. (2007) tested the application of three doses of calcium propionate (0.5; 1.0 and 2.0 g/kg FF;) chosen among the most used accordingly to the literature. Ethanol content, DM losses and DM digestibility were not improved and sometimes worsened with treatment and those researchers concluded that even the highest dose tested, equivalent to 2.7 g of propionate per L of water in the forage, was too low, considering that a concentration of 4.7 g/L is necessary to completely inhibit yeast development (Woolford, 1975). It was suggested that salts with higher solubility than calcium propionate (5% solubility in water) should be tested. Results of this trial corroborate those assertions as sodium propionate, which is more soluble (25% solubility) than calcium propionate, was effective only in the dose of 4.0 g/kg FF, equivalent to 5.9 g of propionate per L of water present in the original forage.

The use of calcium hydroxide and calcium oxide (CaO - quicklime) as additives on the ensilage of sugarcane has expanded based on the low cost of application and the possibility of improving forage digestibility by means of fiber hydrolysis (Van Soest, 1994), including the partial solubilization of hemicelluloses. Results of this trial corroborate data from previous experiments in which application of CaO or $Ca(OH)_2$ reduced DM loss and ethanol content in sugarcane silages, simultaneously improving the forage's digestibility and aerobic stability. Santos *et al*. (2008) tested, among other treatments, calcium oxide at the doses of 10 and 15 g/kg FF applied to the sugarcane at ensiling and reported a similar 92% reduction in the ethanol content in the silages treated with both doses compared to the silage without additives (3.7 vs. 47.8 g/kg DM). Dry matter recuperation was similarly and positively improved by both levels of the additive compared to control (836 vs. 657 g/kg DM). The use of the additives resulted in a 48% average increase in the silages' IVDMD relative to the control silage with approximately 5% improvement for the silage treated with the higher compared to the lower dose (742 vs. 705 g/kg DM). Cavali *et al* (2010) evaluated the doses of 0, 5, 10, 15 and 20 g/kg FF, obtaining a linear increase in DM recovery with the increase in the dose and estimating (quadratic model) the highest IVDMD level for a dose of 18 g/kg FF. It was also observed that doses above 10 g/kg FF affected positively the lactic acid bacteria population and reduced the population of yeasts in the sugarcane silages, reducing gas losses and effluents. In an experiment by Siqueira *et al*. (2011), applying 10 g/kg FF of calcium hydroxide at ensiling improved DM recuperation (713 vs. 635 g/kg DM) and IVDMD (526 vs. 326 g/kg DM) of the treated compared to the untreated silage. It must be noticed that, although the additive is referred as calcium oxide in the article, the active product was actually calcium hydroxide due to the fact that, as indicated by the authors, quicklime was dissolved in water before being applied onto the forage, leading to the reaction: $CaO + H_2O = Ca(OH)_2 +$ heat .

The fact that application of calcium hydroxide, in this trial, resulted in silage with IVDMD similar to the fresh sugarcane is positive considering that digestibility may be greatly reduced during ensilage, if inefficient additives or no additives are used. Nonetheless, the increase in calcium content in silages treated with this additive must be taken into account when balancing diets. In this trial the silage treated with calcium hydroxide had Ca content approximately eight times higher than the fresh sugarcane (Table 2).

Trial 2

The chemical composition of the sugarcane used to produce the experimental silages (Table 5) was within the normal spectrum presented by Brazilian varieties (Fernandes *et al*., 2003). As expected, DM concentration was reduced during ensilage and concentrations of CP and cell wall components were increased (P<0.05) for all silages, compared to the fresh sugarcane. As discussed before, yeasts may consume up to 70% of sugars during the ensilage of sugarcane, causing other plant components to become more concentrated in the silage's DM (Pedroso *et al*., 2005). Dry matter and cell wall components (ADF, NDF and LIG) did not differ (P>0.05) between the control and the silages treated with urea + benzoate and calcium hydroxide. The silage treated with propionate presented higher (P<0.05) levels of ADF and LIG, compared to the other silages, indicating more extensive consumption of sugars during fermentation, causing higher concentration of less degradable components in the silage. As a direct effect of the composition of the additives, CP and Ca levels were much higher in the silages treated, respectively, with urea + benzoate and calcium hydroxide. The high ash concentration in the silage treated with calcium hydroxide (more than double, relative to the fresh sugar cane) is explained by the amount of Ca applied (31.9 g/kg DM).

The low buffering capacity (Alli *et al*., 1983) and high level of sugars, normally present in sugarcanes, allowed for adequate final pH in all silages (Table 6), even in the silage treated with calcium hydroxide, despite the strong alkaline characteristic of this additive. All additives resulted in silages with lower (P<0.05) ethanol content relative to the control silage but application of calcium hydroxide was more efficient, causing approximately 88% reduction in alcohol whereas applications of urea + benzoate and propionate were less efficient, causing a 25% reduction on average. The mode of action of each additive was discussed when presenting the results of Trial 1.

Dry matter apparent digestibility coefficients for the silages treated with urea + benzoate and calcium hydroxide were similar (P>0.05) and 17.3% higher, on average, compared to the silage treated with propionate and the control silage (Table 6). Treatment with propionate did not improve (P>0.05) the DM digestibility coefficient compared to control, consonant with the higher levels of ADF and LIG in the silage. The apparent digestibility of NDF was not improved (P>0.05) by any of the additives compared to control. Treatment with calcium hydroxide resulted higher (P<0.05) NDF apparent digestibility compared to the silage treated with urea + benzoate.

Dry matter intake by the sheep fed silages treated with the additives was not different (P>0.05) compared to those fed the untreated silage (Table 6). Dry matter intake was 50.4% higher (P<0.05) by the sheep fed the silage treated with calcium hydroxide compared to those fed the silage treated with propionate. The relatively high DM intake of

Table 5. Chemical composition of the fresh sugarcane and experimental silages

[1]Item	Fresh sugarcane	[2]Sugarcane silage				SE
		Control	U+B	PROP	CAL	
DM (g/kg FF)	314	243 b	259 a	236 b	270 a	13.4
CP	23.2	35.6b	94.1 a	35.1 c	31.3 c	3.7
NDF	487	665 ab	649 ab	718 a	612 b	67.8
ADF	293	446 b	430 b	493 a	414 b	31.6
LIG	48.3	65.4 b	59.8 b	84.1 a	62.0 b	8.7
Ash	31.3	26.4 c	28.3 bc	31.9 b	66,4 a	4.5
Ca	1.8	2.5 b	1.9 bc	1.6 c	13.6 a	0.7
P	0.31	0.41 a	0.28 b	0.28 b	0.26 b	0.4

[abc]Means in the same row with different superscript differ (P<0.05) by the *t* test. SE = standard error.
[1]Composition expressed in g/kg DM unless otherwise stated; DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; LIG: lignin; Ca: calcium; P: phosphorus; FF: fresh forage.
[2]Control: silage without additive; U+B: silage treated with urea (5.0 g/kg FF) + sodium benzoate (0.5 g/kg FF); PROP: silage treated with sodium propionate (4.0 g/kg FF); CAL: silage treated with calcium hydroxide (10 g/kg FF).

Table 6. Fermentation parameters, apparent digestibility coefficient and intake of experimental silages

[2]Item	[1]Sugarcane silage				SE
	Control	U+B	PROP	CAL	
pH	3.5 c	3.8 b	3.7 b	4.1 a	0.04
Ethanol (g/kg DM)	96.6 a	67.3 b	76.8 b	11.6 c	6.9
Apparent digestibility of DM	0.37 b	0.44 a	0.38 b	0.44 a	0.03
Apparent digestibility of NDF	0.32 ab	0.30 b	0.38 ab	0.40 a	0.06
DM intake (g/kg LW)	18.0 ab	17.3 ab	13.3 b	20.0 a	0.4

[abc]Means in the same row with different superscript differ (P<0.05) by the *t* test; SE = standard error.

[1]Control: silage without additive; U+B: silage treated with urea (5.0 g/kg FF) + sodium benzoate (0.5 g/kg FF); PROP: silage treated with sodium propionate (4.0 g/kg FF); CAL: silage treated with calcium hydroxide (10 g/kg FF). [2]LW: live weight; FF = fresh forage.

the animals fed the control diet (18 g/kg LW) and the high coefficient of variation observed for this variable (21.7) may explain why no difference was observed between the other treatments and the control in this experiment.

The same positive effect on DM apparent digestibility of silages treated with urea or benzoate was not observed in an experiment by Schmidt *et al*. (2007). Those researcher used five rumen cannulated Nelore steers (5 x 5 Latin square design) to evaluate silages produced with 5 g of urea/kg FF or 1 g of sodium benzoate/kg FF in diets containing 65% of forage in DM, compared to the diet composed with sugarcane silage without additive. The additives did not promote alterations in the diets' DM apparent digestibility coefficients (0.64 on average). Voluntary DM intake was also not altered by the additives and was lower than observed in the present experiment for the silage treated with urea + benzoate (13.3 vs. 17.3 g/kg LW) despite the use of higher amount of concentrate in the diets, what may increase DM intake (Dias *et al*., 2000).

In an experiment with Santa Inês lambs, Gentil *et al*. (2007) evaluated fresh sugarcane and sugarcane silages treated with 10 or 15 g of urea/kg FF in diets containing a 50:50 forage concentrate ratio. Dry matter intake and apparent digestibility were not affected by the additives.

It is possible that the improvement in DM apparent digestibility obtained for the silage treated with urea + benzoate in this experiment was caused by the increment in CP resultant from the amount of urea applied to the silage, which more than doubled the CP content in that silage relative to control. Considering that the contents of NDF, ADF and lignin were similar in both silages, it is probable that the 45% higher than expected CP content obtained in the silage treated with urea + benzoate (94.1 vs. 65 g/kg DM) resulted in a diet with a higher level o protein and higher digestibility.

Although treatment with calcium hydroxide did not improve the silage's NDF digestibility in this trial, the low ethanol content in the silage indicates that more sugar was spared during fermentation, probably contributing to its higher DM digestibility. Experiments evaluating the apparent digestibility and intake of sugarcane silages treated with calcium oxide/hydroxide are scarce in the literature. Balieiro Neto *et al*. (2009) used sheep to evaluate sugarcane silages treated with 5 g CaO/kg FF compared to the fresh sugarcane. Those researchers reported reduction in the DM apparent digestibility in the silage treated with the alkali compared to the fresh sugarcane (520 vs. 606 g/kg DM) but similar intake for the silage and the fresh forage. Nonetheless, intake of the silage treated with 5 g CaO/kg FF in that experiment was lower than obtained with the silage treated with 10 g $Ca(OH)_2$/kg FF in this trial (16 vs. 20 g/kg LW), despite the close levels of NDF (604 vs. 612 g/kg DM), ADF (420 vs. 414 g/kg DM) and NDF apparent digestibility coefficients (0.38 vs. 0.40) in the silages. Comparing the results of both experiments it is possible to assume that the higher level of intake of the silage treated with calcium hydroxide in this experiment increased digesta flow rate resulting in a lower DM apparent digestibility coefficient compared to the coefficient obtained in the previous experiment (0.44 vs. 0.52).

Chizzoti *et al*. (2015) evaluated diets (50:50 forage concentrate ratio) containing silages treated with four levels of CaO (0, 5, 10 and 15 g/kg FF), in a digestibility trial with four ruminally and abomasally cannulated Nelore steers (4 x 4 Latin Square design), reporting a linear positive effect of the dose of the additive on the apparent digestibility of DM and NDF but no effect over DM intake (19 g/kg LW, in average). In a performance trial involving 35 crossbred steers (Holstein x Nelore) and the same type of diets as in the first trial, those authors obtained a positive liner effect of CaO level on DM and NDF digestibility and a quadratic effect of CaO level on DM intake, with better result at the 5 g CaO/kg FF dose and reduction of intake at the higher doses. The reduction in intake observed for the two higher levels of CaO was considered by the authors to be associated with an increased osmolality of the ruminal content due to an increase in dissolved particles in the ruminal fluid derived from the additive applied to the forage, as postulated by Carter and Grovum (1990). In the present experiment, it is possible that the relatively small amount of concentrate (soybean meal) used to balance the diet compensated for the higher concentration of the alkali in the digesta, resulting in lower osmolality in the rumen compared to the situation in the experiments by Chizzoti *et al*. (2015), in which the diets had a 50:50 forage concentrate ratio, resulting in high DM intake.

Considering that application of the additive at the rate of 5 g/kg FF may improve the silage's DM intake (Chizzoti *et al*., 2015) but may be less efficient with respect to yeast control than at the dose of 10 g/kg FF (Cavali *et al*., 2010), an intermediate dose must be determined with which a balance could be achieved between gains at the silo and in animal performance.

CONCLUSION

Calcium hydroxide and urea combined with sodium benzoate are efficient additives, considering the most important effects expected of additives to be used in the ensilage of sugarcane, such as ethanol control, dry matter loss reduction and improvement in the silages' digestibility during ensilage, being superior to sodium propionate.

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