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Impact of days post-burning and lime as an additive to reduce fermentative losses of burned sugarcane silages

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ABSTRACT

The objectives of our study were: 1) to investigate the effects of burning and the time elapsed between burning and ensiling on characteristics of sugarcane silages, and 2) to evaluate the effects of lime on fermentation and aerobic stability of *in natura* and burned sugarcane silages. In trial I, silages were prepared from burned sugarcane that remained in the field for varying number of days post-burning (1, 5, 10, 15, 20, 25, and 30 d). In trial II, the characteristics of burned and *in natura* sugarcane silages treated with various concentrations of lime (0, 5, 10, 15, and 20 g/kg of sugarcane, on an as-is fresh matter basis) were compared. In trial I, 10-d post-burning, sugarcane crop displayed great degrees brix (18.3°Bx) and sucrose (677.3 g/kg of sugarcane broth) values. The yeast population in sugarcane crop 1-d post-burning (4.47 cfu/g of fresh forage) was lesser than that 25-d post-burning (7.11 cfu/g of fresh forage). After the silos were opened, all silages showed low pH. The silage from 1-d post-burning had the least dry matter (DM) recovery (637.5 g/kg of DM). The greatest DM recovery was found in the silage prepared 15-d post-burning (740.0 g/kg). Silage from 20-d post-burning displayed the greatest aerobic stability (36.7 h); however, in general, all silages had low aerobic stability (<40 h). In trial II, both the *in natura* and burned silages had reduced fiber content due to lime addition. Considering the overall mean, burned silages produced 47 g acetic acid/kg of DM against 25.6 g/kg of DM in *in natura* silages. Lime was more effective in increasing the production of acetic acid in *in natura* silages, but only when applied at great concentrations (15 and 20 g/kg). DM recovery of *in natura* silages decreased with increased addition of lime, whereas the opposite effect was observed for burned silages. In natura and burned silages treated with lime at 15 and 20 g/kg had greater aerobic stability (>8 d) than those treated with lesser quantities of lime. Considering the approach in which this study was carried out, a period of 10–15 days is ideal for ensilage of burned sugarcane prior to the silage quality significantly drops. Lime may be used as an additive for both *in natura* and burned silages since in greater levels (15 and 20 g/kg).

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1. Introduction

Sugarcane crops display great herbage production (25–40 t/ha of dry matter [DM]) and great energy values at maturity compared to other crops (Ávila et al., 2009); it is widely used as silage in diets for beef cattle and dairy cows in Brazil (Bernardes and Rêgo, 2014; Millen et al., 2009) because the difficult to perform daily cuts of fresh sugarcane on the field. Sugarcane is often harvested during the dry season. In this period, there is a great risk of accidental burning, which prevents the use of sugarcane in a direct cut system. Therefore, ensiling may be an effective strategy to avoid greater losses of quality and DM in this situation, but the maximum time that sugarcane can remain in the field post-burning before quality declines significantly is not conclusive (Roth et al., 2010).

Conversely, sugarcane ensiling may result in large DM losses (>300 g/kg) during fermentation due to the conversion of sucrose to ethanol by yeast, leading to the reduction in silage nutritive value, aerobic stability, and feed intake by livestock (Kung and Stanley, 1982; Pedroso et al., 2005). Thus, the addition, of an alkaline agent (e.g., lime) at the farm level has been recommended for reducing the fungi population and increasing the growth of the lactic-acid bacteria (LAB) population (Cavali et al., 2010), as well as to increase DM recovery (Rabelo et al., 2014), and improve the sugarcane silage digestibility (Siqueira et al., 2007, 2011a). These treatment effects are relatively well known for *in natura* sugarcane silage, but few studies have evaluated the process of ensiling burned sugarcane or the effect of chemical additives on this process. In that few studies, lime addition increased the *in vitro* digestibility (Roth et al., 2010) and DM recovery of burned sugarcane silages (Siqueira et al., 2011a).

Therefore, our objectives were to investigate the effects of the time between burning and ensiling on sugarcane silage characteristics and the effect of lime on fermentation and aerobic stability of *in natura* and burned sugarcane silages.

2. Material and methods

2.1. Ensiling process

2.1.1. Trial I

In trial I, we used a crop of the IAC 86-2480 sugarcane cultivar after 12 months of growth (third cut). The sugarcane field was burned at sunset on the day before cutting. The stems were not severed from their roots until harvest taking into consideration any incidence of accidental fire.

On days 1, 5, 10, 15, 20, 25, and 30 post-burning, sugarcane was mechanically harvested using an ensilager (Menta Mit, ColhiFlex model, Cajuru, SP, Brazil). Mini-silos (plastic buckets of 7 L capacity) were used in quadruplicate for ensiling, which were closed with plastic lids and sealed with adhesive tape; a density of 676 kg fresh matter/m^3 was obtained.

2.1.2. Trial II

The sugarcane used in trial II was similar to that used in trial I. The sugarcane field was burned at sunset the day before cutting, and 1-d post-burning sugarcane was used to ensilage. After harvest, the burned and *in natura* sugarcane either remained untreated or was treated with lime (micro-pulverized calcium oxide) at 5, 10, 15, and 20 g/kg (on an as-is fresh matter basis). Lime was diluted in water at a ratio of 1.0 kg lime per 4 L of water and applied at 20, 40, 60, and 80 mL/kg of fresh sugarcane to obtain the concentrations described earlier. Lime was then well-mixed with the sugarcane multiple times to ensure good homogenization.

Mini-silos (plastic buckets of 20 L capacity) were used in quadruplicate for ensiling, and were closed with a plastic lid and sealed using adhesive tape; a density of 872 and 562 kg fresh matter/m^3 was obtained for burned and *in natura* sugarcane, respectively.

2.2. Fermentative losses

In both trials, the mini-silos were weighed immediately upon ensiling and after opening at the end of the ensiling period (56 d) to determine gas and DM losses, which were calculated as:

$$\text{Gaslosses(g/kgofDM)} = (((F_{en} \times DM_{en}) - (F_{op} \times DM_{op}))/(F_{en} \times DM_{en})) \times 100 \quad (1)$$

where F_{op} and DM_{op} = forage mass and DM content of the silos at opening, respectively; F_{en} and DM_{en} = forage mass and DM content at ensiling, respectively.

$$\text{DMrecovery(g/kg)} = (F_{op} \times DM_{op})/(F_{en} \times DM_{en}) \times 100 \quad (2)$$

where F_{op} and DM_{op} = forage mass and DM content of the silos at opening, respectively; F_{en} and DM_{en} = forage mass and DM content at ensiling, respectively.

2.3. Aerobic stability

To determine aerobic stability, a silage sample from each mini-silo was placed in a plastic bucket of 7 L capacity and kept at ambient temperature. A data logger was placed in the silage and the silage temperature was measured every hour for 9

d. The ambient temperature was measured using data loggers placed close to the mini-silos. Aerobic stability was defined as the number of hours that the silage temperature remains stable before increasing more than 2 °C above the ambient temperature (Moran et al., 1996). Additionally, the sum of accumulated daily temperatures was calculated as the sum of the difference between the silage and ambient temperatures after 5 and 9 d (SUM 5 and SUM 9) of aerobic exposure (O'Kiely, 1999). The heating rate was calculated as the maximum recorded temperature divided by the time required reaching the maximum temperature (Ruppel et al., 1995). The pH values were recorded at the initial time (day 0), and after 3, 6, and 9 d of aerobic exposure.

2.4. In vitro gas production and digestibility

Animal care and handling procedures used in this study were approved by the Sao Paulo State University's Animal Care Committee, in agreement with the guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA).

Dried silage samples (0.2 g) were placed in 115-mL serum bottles and incubated in a water bath at 39 °C (Mauricio et al., 1999) with a rumen inoculum and buffer solution in a ratio of 4:1 (30 mL for each bottle) for 144 h. Prior to incubation, CO_2 was continuously purged for 30 min, and the reducing solution was added to the ruminal fluid. The bottles (4 for each treatment) were then sealed with a rubber stopper plus an aluminum crimp cap and stored in a water bath (39 °C). For the buffer solution, we used the Kansas State "synthetic saliva" (Marten and Barnes, 1979), which was adapted from the two-stage technique of Tilley and Terry (1963). The ruminal fluid was collected from 2 rumen-cannulated sheep in the morning before feeding. The animals were fed 600 g/kg sugarcane silage treated with lime at 10 g/kg and 400 g/kg concentrate composed of ground corn, soybean meal and urea, on a DM basis. The rumen fluid was filtered through 4 layers of cheesecloth into pre-warmed thermos flasks, homogenized, and mixed with the buffer solution.

The accumulated headspace gas pressure was measured using a needle attached to a pressure transducer connected to a visual display (Datalogger pressure- pressDATA 800, MPL, Piracicaba, SP, Brazil). Readings were taken at regular intervals throughout the incubation period and at an increased frequency during the initial lag and rapid fermentation phases (i.e., 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 48, 52, 56, 60, 72, 78, 84, 96, 108, 120, 132, and 144 h). To correct changes in the atmospheric pressure, two flasks were incubated without samples (blank) (Pell and Schofield, 1993). The volume of gas in the blanks was subtracted from the reading of gas volume to obtain the true sample gas volume.

Since *in vitro* gas production is mostly due to digestible carbohydrate content, rather than that of protein and fat, we used a multiple regression analysis (Menke and Steingass, 1988) to calculate the digestible organic matter (IVOMD):

$$\text{IVOMD(g/kg)} = 14.88 + (0.889 \times \text{gas24}) + (0.045 \times \text{CP}) + (0.065 \times \text{Ash}) \quad (3)$$

where gas24 = gas production after 24 h (mL/0.2 g of DM) and crude protein (CP) and ash contents are expressed as g/kg of DM.

2.5. Chemical analyses

One sample of forage and silage from each mini-silo was used for the chemical analyses. Each sample was divided into three sub-samples. The first sub-sample was weighed and placed in a forced air chamber at 55 °C for 72 h. After this period, these sub-samples were again weighed, ground in a knife grinder until the particle size reduced to less than 1 mm, and stored in plastic pots for determination of DM (105 °C for 12 h) and ash (500 °C for 5 h). CP was determined following the methodology recommended by AOAC (1996), method no. 960.52). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the sequential method (Robertson and Van Soest, 1981). The NDF was assayed without a heat stable amylase, and NDF and ADF were expressed inclusive of residual ash. Cellulose was measured after hydrolysis in ADF residues in 72% H_2SO_4 (Van Soest, 1994), and lignin (sa) content was calculated as the difference between ADF and cellulose.

The second sub-sample was used to prepare a water extract (Kung et al., 1984) and pH was measured using an electrode (model MA522, Marconi Laboratory Equipment, Piracicaba, Sao Paulo, Brazil). Acetic acid was measured using a gas chromatograph (Shimadzu model GC2014, Shimadzu Corp., Kyoto, Japan) equipped with a HP-INNOWax capillary column (30 m × 0.32 mm; Agilent Technologies, Colorado, USA) at an initial temperature of 80 °C for 3 min followed by a heating rate of 20 °C/min until a final temperature of 240 °C was achieved. The amount of total soluble solids (Brix), sucrose content, redox sugars (glucose + fructose), and purity of sugarcane broth also were measured (Bovi and Serra, 1999).

The third sub-sample was used for microbiological analyses performed according to Kurtman and Fell (1998). Briefly, a 25 g silage sample from each replicate was homogenized in 225 mL of saline solution (0.85% NaCl) for 1 min. Then 1 mL of this solution was transferred into tubes with 9 mL of saline solution, from which 0.1-mL samples were transferred to Petri plates at dilutions from 10^{-1} to 10^{-5}. Potato dextrose agar (PDA) was used as a medium to cultivate yeasts. The plates with PDA were kept at 28 °C for 72 h. Microbiological data were log-transformed for statistical analysis.

2.6. Statistical analyses

Both experiments were performed using a completely randomized design with four replicates. Data from fermentation and aerobic stability were analyzed with a mixed model using the MIXED procedure of SAS (v 9.4, SAS Inst. Inc., Cary),

Table 1
Chemical composition (g/kg of DM, unless otherwise stated) of sugarcane at different days post-burning and before ensiling.

Item[a]	Days post burning							SEM	P-value	Contrast[b]
	1	5	10	15	20	25	30			
DM, g/kg as fed	270.4	268.4	241.2	256.7	242.9	242.0	255.3	3.94	< 0.0001	Q**
Ash	15.3	20.3	14.3	16.3	33.1	28.4	16.0	1.16	<0.0001	C**
CP	29.9	29.0	27.3	28.8	30.0	29.0	30.6	0.50	0.0031	Q*
NDF	413.7	430.0	405.6	433.8	491.8	464.2	469.2	8.41	<0.0001	Q*
ADF	248.7	262.5	255.4	272.3	318.3	294.9	294.5	7.31	<0.0001	Q*
Lignin (sa)	38.5	40.5	38.5	44.7	78.3	53.3	47.4	3.39	<0.0001	Q**
IVOMD	545.7	539.1	540.1	554.1	555.7	538.9	559.6	8.48	0.4371	NS
Brix, °	17.1	18.2	18.3	16.8	17.1	18.1	17.9	0.11	<0.0001	C**
Sucrose, g/kg of sugarcane broth	602.2	661.7	677.3	565.7	570.8	562.4	549.4	6.41	<0.0001	C**
Pooled sugars, g/kg of sugarcane broth	147.7	161.6	165.2	139.2	140.1	137.4	134.4	1.54	<0.0001	C**
Redox sugar, g/kg of sugarcane broth	6.8	5.9	5.7	8.0	8.3	10.4	10.6	0.29	<0.0001	C**
Purity, g/kg of total sugars	863.0	888.4	894.8	826.2	821.2	758.7	751.4	8.33	<0.0001	C**
Yeasts, log cfu/g	4.47	5.20	5.36	5.28	5.79	7.11	6.14	0.09	<0.0001	C*

[a] DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; IVOMD = *in vitro* organic matter digestibility.
[b] NS = no significant; Q = quadratic effect; C = cubic effect.
* $P < 0.05$.
** $P < 0.01$.

factoring in the number of days that sugarcane remained in the field post-burning (trial I), silage type (*in natura* or burned) and lime levels (trial II) as fixed effects, and the residual error as a random effect. The following general models were used:

Trial I

$$Y_{ij} = \mu + D_i + e_{ij}$$

where Y_{ij} = response variable; μ = overall mean; D = effect of ensiling in different post-burning days i; and e_{ij} = error term.

Trial II

$$Y_{ijk} = \mu + S_i + L_j + SL_{ij} + e_{ijk}$$

where Y_{ijk} = response variable; μ = overall mean; S = effect of silage type (*in natura* or burned) i; L = effect of lime levels j; SL = effect of the interaction between silage type i and lime levels j; and e_{ijk} = error term.

Differences between means were determined using the DIFF option of the LSMEANS statement, which differentiates means based on Fisher's F-protected least significant difference test. Contrasts were constructed, and the single degree-of-freedom orthogonal comparisons included the linear, quadratic, and cubic effects of ensiling on different numbers of post-burning days (trial I) and lime levels (trial II). For both trials, quartic effects were studied; however, we did not use this effect even when was it significant because a biological explanation would be difficult and likely would not make much sense. When there was significant interaction between the factors studied in trial II, contrasts were constructed based on that interaction. Differences were declared significant at $P \leq 0.05$.

The pH values over aerobic exposure time were analyzed as a completely randomized design with repeated measures over time. The covariance matrix that best fit the data according to the Bayesian information criterion (BIC) was selected to perform the analysis. The following general models were used:

Trial I

$$Y_{ijk} = \mu + D_i + T_j + DT_{ij} + e_{ijk}$$

where Y_{ijk} = response variable; μ = overall mean; D = effect of ensiling on post-burning day i; T = effect of aerobic exposure time j; DT = effect of interaction between ensiling on post-burning day i and aerobic exposure time j; and e_{ijk} = error term.

Trial II

$$Y_{ijkl} = \mu + S_i + L_j + SL_{ij} + T_k + ST_{ik} + LT_{jk} + SLT_{ijk} + e_{ijkl}$$

where Y_{ijkl} = response variable; μ = overall mean; S = effect of silage type (*in natura* or burned) i; L = effect of lime levels j; SL = effect of interaction between silage type i and lime levels j; T = effect of aerobic exposure time k; ST = effect of interaction between silage type i and aerobic exposure time k; LT = effect of interaction between lime levels j and aerobic exposure time k; SLT = effect of interaction among silage type i, lime levels j and aerobic exposure time k; and e_{ijkl} = error term.

Table 2
Chemical composition, fermentation, and aerobic stability of sugarcane silages prepared 1–20 days post burning.

Item[a]	Days post burning							SEM	P-value	Contrast[b]
	1	5	10	15	20	25	30			
Chemical composition, g/kg of DM										
DM, g/kg as fed	162.5	168.5	165.2	179.7	168.7	180.8	171.2	2.50	0.0008	Q*
Ash	25.3	24.2	21.3	20.5	35.4	32.0	24.4	1.29	<0.0001	C**
CP	45.2	40.4	41.8	41.1	44.7	42.9	49.2	0.77	<0.0001	Q**
NDF	704.4	679.7	633.5	605.9	728.3	704.7	742.3	13.41	<0.0001	C*
ADF	434.1	425.8	412.3	392.6	477.1	469.5	473.8	11.21	0.0002	C*
Lignin (sa)	70.5	79.0	73.2	64.6	96.5	96.4	86.8	5.35	0.0021	L*
IVOMD	485.2	488.2	490.4	478.0	472.5	458.4	437.7	5.39	<0.0001	Q*
Fermentation process, g/kg of DM										
Acetic acid	133.7	39.0	31.9	35.4	71.8	90.1	127.3	21.60	0.0034	Q*
pH	3.26	3.26	3.33	3.41	3.27	3.29	3.16	0.03	0.0003	Q**
Gas losses	361.4	328.2	289.5	259.1	338.9	323.9	329.9	6.59	0.0047	C*
DM recovery	637.5	670.6	709.4	740.0	660.2	675.4	669.2	15.88	0.0047	C*
Aerobic exposure										
Initial T, °C	28.3	28.2	27.9	28.1	28.0	28.1	28.1	0.18	0.8191	NS
Maximum T, °C	44.8	42.4	44.1	46.0	42.1	43.1	43.3	0.39	<0.0001	L*
Heating rate, °C/h	0.60	0.30	0.43	0.56	0.15	0.52	0.28	0.07	0.0009	L*
SUM 5, °C[c]	24.9	26.3	24.1	27.9	21.0	26.0	24.0	1.78	0.1846	NS
SUM 9, °C[c]	38.2	40.5	43.1	42.6	42.4	37.0	32.0	3.52	0.4207	NS
Aerobic stability, h	14.7	33.3	34.8	29.5	36.7	21.0	33.0	2.29	<0.0001	C**

[a] DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; IVOMD = *in vitro* organic matter digestibility.
[b] NS = no significant; L = linear effect; Q = quadratic effect; C = cubic effect.
[c] Sum of the difference of temperatures between silages and ambient after 5 and 9 d of aerobic exposure.
* $P < 0.05$.
** $P < 0.01$.

3. Results

3.1. Trial I

3.1.1. Characteristics of sugarcane post-burning

All variables were affected by the number of days post-burning, except the coefficients of IVOMD ($P > 0.05$; Table 1). The DM content had a quadratic response, and the greatest ($P < 0.01$) value was observed 1-d post-burning. Ash content had a large range during the evaluated times, and the least and greatest values ($P < 0.01$) were found 10- and 20-d post-burning, respectively. A quadratic response was observed for CP, and the greatest content ($P < 0.01$) was observed 30-d post-burning. The NDF, ADF, and lignin contents showed a quadratic response; the least values ($P < 0.01$) were found between 1- and 10-d post-burning.

All characteristics associated with sugars showed cubic responses (Table 1); the values of brix, sucrose, pooled (total) sugars, and purity were greatest ($P < 0.01$) for 10-d post-burning, whereas at this time the redox sugar had its least value. There was a large range (cubic response) in yeast populations, and the least value ($P < 0.01$) was observed 1-d post-burning (4.47 cfu/g of burned sugarcane), whereas the greatest value (7.11 cfu/g of burned sugarcane) was observed 25-d post-burning.

3.1.2. Chemical composition and fermentation profile of burned sugarcane silages

All variables were affected by the time between burning and ensiling (Table 2). DM content exhibited a quadratic response, where the greatest value ($P < 0.01$) was obtained 25-d post-burning. Ash also showed a large range, and the greatest value ($P < 0.01$) was found 20-d post-burning. CP showed a quadratic response, and the silage prepared 30-d post-burning exhibited the greatest content ($P < 0.01$). Considering overall means, burned sugarcane silages showed an increase in CP (43.6 g/kg of DM) compared with the material prior to ensiling (29.2 g/kg of DM). Lesser quantities of NDF, ADF, and lignin were found 15-d post-burning ($P < 0.01$). Despite the quadratic response, the coefficients of IVOMD were greater ($P < 0.01$) on 1-, 5-, and 10-d post-burning.

In the fermentation profile, acetic acid showed a quadratic response, and the greatest values ($P < 0.01$) were found in 1- and 30-d post-burning silages (Table 2). Comparison of the means of the 1- and 30-d silages with the means of the other silages revealed that the production of acetic acid was greater in the 1- and 30-d silages (130.5 g/kg of DM) than in other silages (53.6 g/kg of DM). The least pH value was obtained in the 30-d post-burning silage ($P < 0.01$); however, this value was remarkably low for all silages regardless of the number of post-burning days. Silage prepared 1-d post-burning had the greatest ($P < 0.01$) gas losses and least ($P < 0.01$) DM recovery (361.4 and 637.5 g/kg of DM). The least gas losses and greatest DM recovery was observed in 15-d post-burning silage (reduction of 29.5 g/kg of DM on gas losses and increment of 102.5 g/kg of DM on DM recovery compared to 1-d silage).

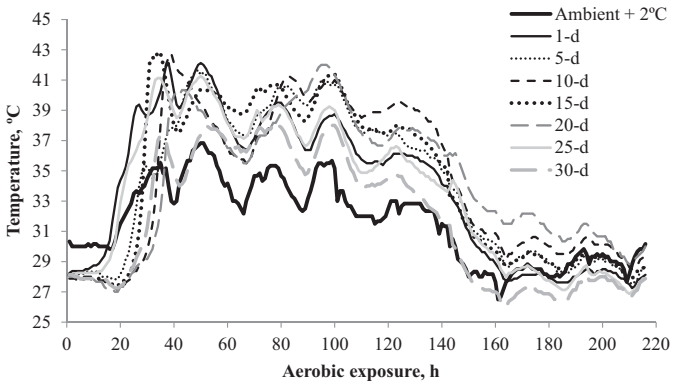

Fig. 1. Temperature of burned sugarcane silages prepared in different days post burning during aerobic exposure.

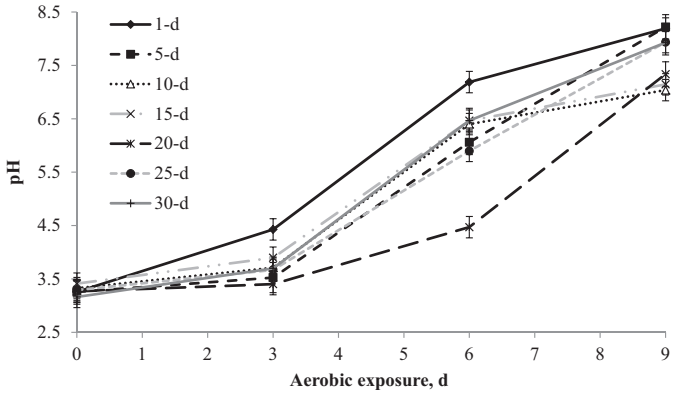

Fig. 2. pH values of burned sugarcane silages during aerobic exposure (effect of days post burning: $P < 0.01$; aerobic exposure days: $P < 0.01$; interaction between the factors: $P < 0.01$).

3.1.3. *Aerobic stability of burned sugarcane silages*

The initial temperature and sum of the differences between silage and ambient temperatures after 5 and 10 d of aerobic exposure were unaffected ($P > 0.05$) by treatments (Table 2). However, the 20-d post-burning silage had the least maximum temperature and heating rate ($P < 0.01$), as well as the greatest ($P < 0.01$) aerobic stability. All silages lost aerobic stability quickly and had temperatures at least 2 °C above the ambient temperature after 35 h of aerobic exposure (Fig. 1).

An interaction ($P < 0.01$) between the time before ensiling and the number of aerobic exposure days was observed for pH values (Fig. 2). Until 6 d after the silos were opened, the 20-d post-burning silage had the least pH values, which is consistent with the greater aerobic stability observed in this silage. However, after 9 d of aerobic exposure, the least pH value was found in 10-d post-burning silage.

3.2. *Trial II*

3.2.1. *In natura and burned sugarcane treated with different lime levels prior to ensiling*

Interactions ($P < 0.01$) between forage type (*in natura* and burned) and lime levels for DM, ash, CP, NDF, and ADF contents were observed (Table 3). As expected, *in natura* sugarcane had greater ($P < 0.01$) DM content than burned sugarcane. However, lime decreased the DM when applied to *in natura* sugarcane. For burned sugarcane, silages treated with 15 and 20 g/kg of lime had greater DM content than those treated with 5 and 10 g/kg. Conversely, the ash content quadratically increased with increased lime addition, and the greatest value was found in *in natura* sugarcane treated with 20 g/kg of lime.

Overall, CP content quadratically decreased due to the addition of lime (Table 3). For *in natura* sugarcane, the reduction in CP was 4.1 g/kg of DM compared to untreated sugarcane and that treated with 20 g/kg of lime (29.2 g/kg of DM). A greater CP reduction (5.8 g/kg of DM) was observed for burned sugarcane compared to sugarcane untreated and treated with 10 g/kg of lime.

Lime quadratically reduced the NDF content in both sugarcanes (Table 3). A reduction of 136.2 g NDF/kg of DM was found for *in natura* sugarcane when treated with 20 g/kg of lime compared to untreated forage. The burned sugarcane treated with 10 g/kg had 42.2 g NDF/kg of DM less compared to untreated burned sugarcane. Application of 20 g/kg lime quadratically

Table 3
Chemical composition (g/kg of DM) of *in natura* and burned sugarcane either untreated or treated with lime (g/kg of sugarcane, on an as-is fresh matter basis) before ensiling.

Item[1]	In natura					Burned					SEM	P-value[2]			Contrast[3]	
	0	5	10	15	20	0	5	10	15	20		S	L	S × L	Main	Interaction
DM, g/kg as fed	327.8^{a}	321.0^{ab}	313.9^{bc}	309.9^{cd}	303.4^{d}	260.8^{ef}	256.1^{f}	256.2^{f}	265.7^{e}	266.4^{e}	3.04	<0.0001	0.0417	<0.0001	L**	
Ash	28.2^{f}	42.9^{e}	64.8^{d}	96.3^{b}	117.6^{a}	17.5^{g}	46.5^{e}	73.9^{c}	93.2^{b}	97.2^{b}	3.17	0.0938	<0.0001	<0.0001	Q**	
CP	33.3^{ab}	34.5^{a}	34.0^{ab}	31.7^{b}	29.2^{c}	29.2^{c}	26.1^{d}	23.4^{e}	25.3^{de}	26.2^{d}	0.76	<0.0001	0.0007	0.0002	Q**	
NDF	607.0^{a}	614.2^{a}	573.0^{b}	531.8^{c}	470.8^{d}	407.4^{e}	379.5^{fg}	365.2^{g}	392.7^{ef}	397.2^{ef}	9.27	<0.0001	<0.0001	<0.0001	Q**	
ADF	329.2^{ab}	335.8^{a}	316.8^{bc}	299.4^{c}	267.8^{d}	245.5^{e}	232.9^{e}	228.2^{e}	240.3^{e}	233.6^{e}	6.25	<0.0001	<0.0001	<0.0001	Q**	
Lignin (sa)	47.0	54.5	46.1	47.4	35.9	27.2	26.0	25.8	27.6	19.3	2.86	<0.0001	0.0060	0.3685	Q*	

(a–g) Means in the same row with different superscripts differed (P < 0.05).
[1] DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.
[2] S = silage; L = level of lime; S × L = interaction between factors.
[3] L = linear effect; Q = quadratic effect.
* P < 0.05.
** P < 0.01.

reduced ADF content by 61.4 g/kg of DM in *in natura* sugarcane. However, lime had no effect on ADF content in burned sugarcane where values remained constant.

Although there was no interaction (P > 0.05) between silage type and lime levels observed for lignin content, the *in natura* sugarcane exhibited greater (P < 0.01) lignin content than burned sugarcane (Table 3). Overall, the greatest lime level (20 g/kg) reduced the lignin content by 11.1 and 7.9 g/kg of DM when applied on *in natura* and burned sugarcane compared to the respective untreated forages (P < 0.05).

3.2.2. Chemical composition and fermentation profile of in natura and burned sugarcane silages treated with different lime levels

All variables were affected by the interaction (P < 0.05) between silage type and lime levels, as shown in Table 4. *In natura* silages had greater (P < 0.01) DM content than burned silages. However, *in natura* silages showed no change in DM content due to the lime application, whereas there was a quadratic increase in DM content in burned silages following lime application. Ash content exhibited a similar response; in other words, a quadratic increase (P < 0.01) in ash content following lime application was observed in both types of silage; in general, *in natura* silages had greater ash contents than burned silages.

A quadratic reduction (P < 0.01) in CP content was observed following lime application, regardless of silage type (Table 4). The greatest values of CP were observed in untreated *in natura* sugarcane or that treated with 10 g/kg of lime and also in untreated burned silage.

Overall, *in natura* silages exhibited greater (P < 0.01) NDF contents than burned silages (Table 4). There was a quadratic reduction in NDF content with increased lime addition for both *in natura* and burned silages. If untreated silages are used as a baseline, lime reduced the NDF content by 143.2 and 265.1 g/kg of DM when *in natura* silage was treated with 20 g/kg lime and burned silage was treated with 10 g/kg, respectively. Likewise, these also were the most effective treatments for reducing (P < 0.01) the ADF content. Addition of lime led to a marginal and quadratic decrease in lignin content (P < 0.05) of *in natura* silage, and the application of 10 and 20 g/kg of lime led to also a quadratic decrease in lignin content of burned silages.

All burned silages (except those treated with 5 g/kg of lime) produced more (P < 0.01) acetic acid than *in natura* silages revealing overall means of 47.0 vs. 25.6 g/kg of DM, respectively (Table 4). Lime was more effective in increasing the production of acetic acid in *in natura* silages, but only when applied at great concentrations (15 and 20 g/kg).

There was a difference (P < 0.01) in pH values between silage types only when great concentrations of lime (15 and 20 g/kg) were used in the treatment of silages; *in natura* silages had greater pH than burned silage (Table 4). However, as expected, pH cubically increased with an increase in the concentration of lime applied.

The gas losses of *in natura* silages quadratically increased (P < 0.01) with lime addition; comparing untreated silage with those treated with 15 and 20 g/kg of the additive, the increase was 44.8 g/kg of DM (Table 4). Conversely, the use of lime quadratically reduced the gas losses in burned silages, especially when used at 10 g/kg (103.5 g/kg of DM less than untreated silage). Overall, lime addition quadratically decreased (P < 0.01) DM recovery in *in natura* silages (except the level of 5 g/kg, which increased this variable), whereas the opposite effect was observed for burned silages.

3.2.3. Aerobic stability of in natura and burned sugarcane silages treated with different lime levels

All variables related to aerobic stability were changed (P < 0.05) by the interaction between silage type and lime levels, as shown in Table 4. Overall, lime had the desired effect on initial temperature, decreasing (P < 0.05) this variable for both silage types, especially when great levels were used (15 and 20 g/kg). Similarly, the addition of lime reduced (P < 0.05) the maximum temperature, particularly at 15 g/kg for both silage types. Lesser (P < 0.05) heating rates were observed when *in natura* and burned silages were treated with 15 and 20 g/kg of lime, as well as for *in natura* silage treated with 10 g/kg. Similar results were found for SUM 5 and SUM 9 (P < 0.01). Consequently, aerobic stability increased (P < 0.01) when silages

Table 4
Chemical composition, fermentation, and aerobic stability of *in natura* and burned sugarcane silages untreated or treated with lime (g/kg of sugarcane, on an as-is fresh matter basis).

Item[1]	In natura					Burned					SEM	P-value[2]			Contrast[3]	
	0	5	10	15	20	0	5	10	15	20		S	L	S × L	Main	Interaction
Chemical composition, g/kg of DM																
DM, g/kg as fed	272.7^{a}	269.8^{a}	259.0^{a}	254.4^{a}	265.1^{a}	170.8^{d}	202.6^{c}	214.9^{bc}	226.3^{b}	212.2^{bc}	7.43	<0.0001	0.2014	0.0007		Q*
Ash	34.2^{f}	48.5^{e}	67.7^{d}	108.8^{b}	140.8^{a}	23.4^{g}	46.3^{e}	65.7^{d}	96.1^{c}	112.5^{b}	2.70	<0.0001	<0.0001	0.0007		Q**
CP	42.5^{a}	38.6^{b}	43.6^{a}	38.1^{b}	37.3^{b}	43.2^{a}	34.5^{c}	29.7^{e}	30.8^{de}	32.5^{d}	0.95	<0.0001	<0.0001	<0.0001		Q**
NDF	755.5^{a}	689.0^{bc}	708.7^{b}	683.6^{c}	612.3^{d}	681.1^{c}	506.7^{e}	416.0^{g}	440.9^{f}	443.8^{f}	10.04	<0.0001	<0.0001	<0.0001		Q**
ADF	426.2^{a}	386.7^{b}	402.4^{b}	416.3^{a}	392.9^{b}	428.3^{a}	310.1^{c}	277.0^{d}	302.5^{c}	310.0^{c}	6.03	<0.0001	<0.0001	<0.0001		Q**
Lignin (sa)	53.0^{ab}	46.7^{b}	48.7^{b}	56.4^{ab}	44.6^{b}	67.0^{a}	35.5^{bc}	24.6^{c}	39.6^{bc}	29.2^{c}	5.71	0.0077	0.0029	0.0393		Q*
Fermentation process, g/kg of DM																
Acetic acid	21.6^{c}	18.0^{c}	13.4^{d}	36.0^{b}	38.9^{b}	50.7^{a}	39.9^{b}	50.4^{a}	48.7^{a}	45.2^{ab}	2.63	<0.0001	<0.0001	<0.0001		Q*
pH	3.42^{f}	3.66^{e}	4.18^{d}	5.52^{b}	5.98^{a}	3.31^{f}	3.65^{e}	4.11^{d}	4.48^{c}	4.58^{c}	0.05	<0.0001	<0.0001	<0.0001		C**
Gas losses	149.2^{cd}	83.2^{f}	156.6^{c}	227.1^{b}	238.4^{b}	272.2^{a}	122.1^{de}	111.9^{e}	149.4^{de}	145.8^{de}	10.80	0.1028	<0.0001	<0.0001		Q**
DM recovery	849.5^{cd}	912.0^{a}	842.1^{d}	771.6^{e}	760.3^{e}	727.0^{f}	877.4^{bc}	887.8^{b}	850.1^{bc}	853.6^{bc}	10.96	0.1028	<0.0001	<0.0001		Q**
Aerobic exposure																
Initial T, °C	25.3^{a}	24.5^{bc}	25.5^{a}	24.8^{b}	24.8^{b}	24.8^{b}	24.5^{bc}	24.3^{c}	24.2^{c}	24.3^{c}	0.14	<0.0001	0.0016	0.0220		C***
Maximum T, °C	42.2^{a}	40.8^{a}	30.8^{bc}	29.5^{c}	30.2^{c}	42.2^{a}	41.0^{a}	37.7^{b}	29.8^{c}	34.5^{b}	1.05	0.0033	<0.0001	0.0196		C*
Heating rate, °C/h	0.18^{bc}	0.16^{c}	0.05^{d}	0.05^{d}	0.06^{d}	0.22^{ab}	0.27^{a}	0.13^{c}	0.04^{d}	0.07^{d}	0.02	0.0008	<0.0001	0.0252		C*
SUM 5, °C[4]	15.0^{c}	11.4^{d}	−0.8^{e}	−5.1^{ef}	−0.2^{e}	29.5^{a}	24.1^{b}	−0.2^{e}	−7.8^{f}	−6.6^{f}	1.67	0.0033	<0.0001	<0.0001		L**
SUM 9, °C[4]	21.9^{bc}	20.8^{bc}	10.3^{c}	−8.4^{d}	0.1^{c}	58.7^{a}	38.7^{b}	20.8^{bc}	−10.0^{d}	4.4^{c}	4.01	0.0003	<0.0001	<0.0001		L**
Aerobic stability, h	75.0^{c}	83.0^{c}	194.5^{a}	214.0^{a}	214.0^{a}	36.3^{d}	49.0^{d}	95.0^{c}	204.3^{a}	153.0^{b}	8.12	<0.0001	<0.0001	0.0009		C***

(a–g) Means in the same row with different superscripts differed (P < 0.05).

[1] DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.

[2] S = silage; L = level of lime; S × L = interaction between factors.

[3] L = linear effect; Q = quadratic effect; C = cubic effect.

[4] Sum of the difference of temperatures between silages and ambient after 5 and 9 d of aerobic exposure.

* P < 0.05.

** P < 0.01.

*** P < 0.10.

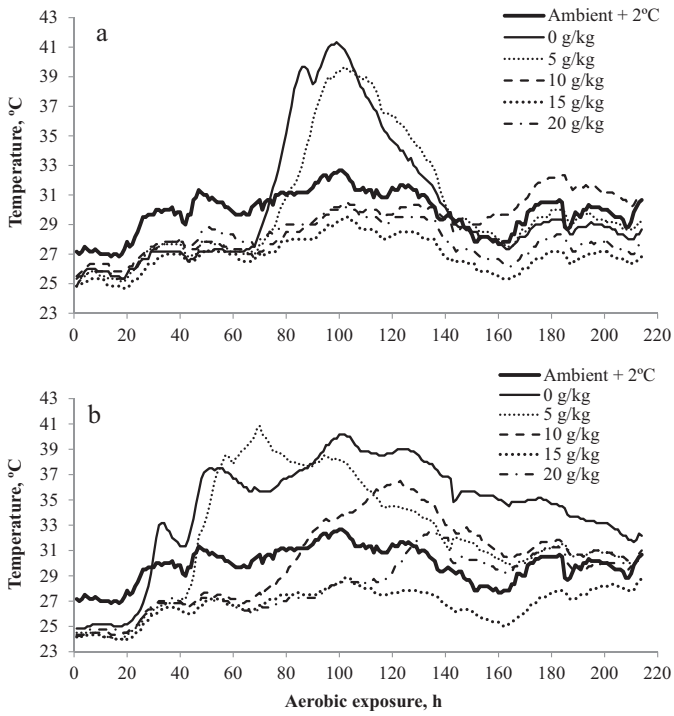

Fig. 3. Temperature of *in natura* (a) and burned (b) silages either untreated or treated with lime (g/kg of sugarcane, on an as-is fresh matter basis) during aerobic exposure.

were treated with great levels of lime. *In natura* silages treated with 10, 15, and 20 g/kg lime and burned silage treated with 15 g/kg lime exhibited great aerobic stability (>8 d). Conversely, untreated silages or those treated with less levels of lime exhibited large peaks of temperature during aerobic exposure (Fig. 3).

During aerobic exposure, pH was affected by interactions ($P < 0.01$) among silage type, lime levels, and time exposed to oxygen (Fig. 4). For the first 3 days of aerobic exposure, both silages exhibited greater pH values when treated with 15 and 20 g/kg of lime. However, after day 3, the most effective treatments were the application of 10 and 15 g/kg of lime for *in natura* and burned silages, respectively.

4. Discussion

4.1. Trial I

Prior to ensiling, the burned sugarcane had a reduction in sucrose content with increases in redox sugars over time while remained in the field likely by invertase activity. Invertase activity can convert sucrose into glucose and fructose during the accumulation of sucrose in tissues of sugarcane (Whittaker and Botha, 1997), and its activity may increase in great temperatures (e.g., sugarcane burning). This hypothesis could explain why sugarcane purity also decreased, taking into consideration the quantity of sucrose in relation to total solids.

Even with cubic effect, we observed that delay in post-burning ensiling increased yeast population in sugarcane likely by exudation or escape of sugars or both, caused by burning, which destroy wax layer that enfolds the cell-wall and cause cracks in the stalk (Bernardes et al., 2007). A previous study also reported increases of yeast population in burned sugarcane throughout the 14-d post-burning (Roth et al., 2010). In our study, increases in yeast population may be the cause of the increased fiber fraction in sugarcane that remained in the field for many days post-burning. Yeasts use water-soluble carbohydrates (WSC) as the main substrate for their growth; in turn, the fiber fraction increases via the concentration effect (Rabelo et al., 2014). However, the increases in NDF content unaffected IVOMD of burned sugarcane for unknown reasons.

DM content also increased according to the length of time the sugarcane remained in the field post-burning likely because the increased yeasts activity, and water loss caused by burning. CP decreased up to 10-d post-burning and increased after this time. Yeasts and other microorganisms are composed of protein, and certainly their overgrowth after 10-d post-burning contributed to increase the CP content of sugarcane.

When the silos were opened, great amounts of acetic acid was observed in the 1- and 30-d post-burning silages likely arising the better conditions found by LAB population to growth. Acetic acid is a powerful antifungal agent (Danner et al., 2003) capable of inhibiting yeast and reducing ethanol formation and DM loss during silage fermentation (Ávila et al., 2009).

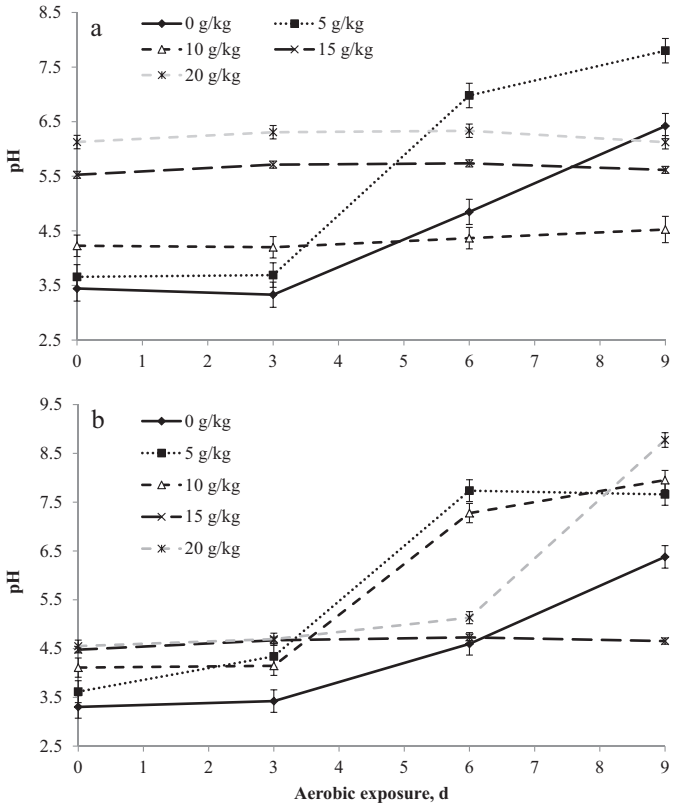

Fig. 4. pH values of *in natura* (a) and burned (b) silages either untreated or treated with lime (g/kg of sugarcane, on an as-is fresh matter basis) during aerobic exposure (effect of silage type: $P > 0.10$; lime: $P < 0.01$; aerobic exposure days: $P < 0.01$; interaction among the factors: $P < 0.01$).

There was not a clear effect of post-burning days on DM recovery (cubic effect); however, despite of the greater acetic acid concentration, 1- and 30-d post-burning silages did not have greater DM recovery. The 15-d post-burning silage had the greatest DM recovery, possibly due to decreased yeast activity because this silage had less sugar contents compared to those that remained in the field for fewer days post-burning. Conversely, all silages had low DM recovery (<750 g/kg of DM). Although all silages exhibited low pH (<3.45), this does not ensure a desirable fermentation, as yeasts are able to growth in a wide range of pH, from 2.5 to 8.5 (Orij et al., 2011). The main metabolic pathway of yeast uses pyruvate decarboxylase acetaldehyde and the posterior reduction of acetaldehyde into ethanol occurs (Rooke and Hatfield, 2003), resulting in DM losses of 489 g/kg as fed during fermentation (McDonald et al., 1991). Indeed, a low DM recovery has been reported in burned silages because the intense yeasts activity (Roth et al., 2010; Siqueira et al., 2010).

The great DM losses also explain the reduction in DM content of silages compared to sugarcane before ensiling in our study, a response previously reported by Siqueira et al. (2007). Similarly, ash, CP, NDF, and ADF increased in silages compared to forage. Fermentation process occurs by converting of soluble sugars to organic acids, and an increase in other compounds is expected due to the concentration effect (Rabelo et al., 2014).

Furthermore, lignin content of burned silages linearly increased with increased post-burning days likely as a response for the yeasts overgrowth. The 1-, 5-, and 10-d burned silages had lesser lignin content when compared with those produced with sugarcanes that remained a long time in the field post-burning. Thus, the lesser lignin contents probably explain the quadratic response observed for the coefficients of IVOMD, where burned silages up to 10-d post-burning had a greater digestibility. The negative effect of lignin on digestibility has been previously documented (Jung and Allen, 1995). However, all silages had low digestibility (435–490 g/kg of organic matter), and our data are in accordance with previous studies (Roth et al., 2010; Siqueira et al., 2011b), which also reported a low digestibility of burned sugarcane silages.

The silage produced from sugarcane that remained in the field 15-d post-burning had the greatest maximum temperature likely by the greater DM recovery. Silages with great DM recovery often exhibit a desirable fermentation pattern (i.e., greater production of lactic acid and preservation of residual WSC) and can have lesser aerobic stability (McDonald et al., 1991), as confirmed in this study. Aerobic exposure of silage leads to extensive oxidization of WSC driven by the growth of epiphytic microorganisms, especially yeasts, with increases in temperature and decreases in the nutritive value of feed (Kung and Stanley, 1982; Wilkinson and Davies, 2012).

The 1-d post-burning silage exhibited the greatest production of acetic acid; however, this silage had low aerobic stability (<15 h). Although acetic acid has been strongly related to enhance aerobic stability (Danner et al., 2003), yeasts are able to

growth in a wide range of conditions (Orij et al., 2011; Pahlow et al., 2003). Conversely, the 20-d post-burning silage showed the greatest aerobic stability, as well as the least pH after 6 d of aerobic exposure. This is probably due to lesser sugar content during ensiling compared to those silages prepared with sugarcane that remained in the field for a shorter time post-burning. The lesser pH found in the 20-d post-burning silage during aerobic exposure is likely associated with the lesser yeasts growth. Yeasts are able to use lactic acid as a substrate, causing an increase in pH values, especially when the pH is greater than 4.5, favoring the growth of other spoilage microorganisms (Wilkinson and Davies, 2012). Consequently, there is an important reduction in the nutritive value of silage.

Overall, although ensiling of burned sugarcane between 10- to 15-d post-burning revealed a greater DM recovery, in our study there was not a clear effect of post-burning days on the fermentation patterns and aerobic stability of silages, since a cubic response occurred for many variables. Likewise, Roth et al. (2010) found inconsistent results in silages produced with burned sugarcane throughout the 14-d post burning. Brazil is the largest producer of sugarcane in the world (FAO, 2015) and accidental fire still occurs in the field, but the quality of burned sugarcane silage has been few investigated, and further studies are necessary to understand the impact of burning on it.

4.2. Trial II

In natura sugarcane had greater DM, CP, and fiber content than burned sugarcane, both before and after ensiling. During burning, straw is the primary part of the plant affected; straw has great DM and fiber content and a less amount of CP. The loss of straw, which comprises mostly senescent leaves that contain great NDF content, during burning, explains our results. A previous study also reported similar results in *in natura* and burned sugarcane (Siqueira et al., 2010).

Ash content quadratically increased in *in natura* and burned sugarcane before and after ensiling by lime addition, once this additive has great ash content; however, this response was not translated in marked increases in DM content likely by water loss caused by heating of sugarcane when lime was added. Calcium oxide in contact with water produces an alkaline compound ($CaO + H_2O \rightarrow Ca(OH)_2 + heat$), and considerable quantity of heat (15,300 cal/mol) may be produced. In addition, CP quadratically reduced with increasing additions of lime, which is probably due to a dilution effect.

Overall, the fiber fraction quadratically decreased by lime addition, and the greatest levels (i.e., 15 and 20 g/kg) reflected in lesser values. Alkaline additives have been used in order to reduce the fiber fraction and increase the fiber digestibility, or both (Santos et al., 2009). Lime often has the capacity to expand the fiber (hydrolysis), causing a rupture in the ester linkages between lignin and hemicellulose, and breaking the hydrogen linkages between cellulose and hemicellulose (Klopfenstein, 1980). Thus, our results are in accordance with the chemical role played by lime.

Despite of cubic effect, lime addition increased the pH values for both silages; however, pH increased more strongly in *in natura* silages than burned silages. Increases in pH values are expected because the application of lime to sugarcane causes hydration of calcium oxide and production of alkali (Daniel et al., 2013).

Acetic acid concentration increased in *in natura* silages due to the application of lime. If lime is able to break linkages between lignin and hemicellulose (Klopfenstein, 1980), an increase in the production of organic acids is expected (verified for acetic acid in the present study) because LAB metabolize nonstructural carbohydrates into organic acids (Rooke and Hatfield, 2003). Moreover, LAB (mainly heterofermentative LAB) probably had greater activity in elevated-pH conditions, since these microorganisms growth slowly when pH values are below 3.5 (McDonald et al., 1991). A quadratic response was observed for gas losses, and great levels of lime (15 and 20 g/kg) led to an increase in gas losses in *in natura* silages, resulting in lesser DM recovery (except for silage treated at 5 g/kg). This may be explained by an increase in acetic acid production because there is production of CO_2 in heterolactic pathway caused by the fermentation of WSC, and this via is less efficient than the homolactic pathway in DM recovery (Muck, 2010). Additionally, the reduction in DM recovery in *in natura* silages with increased lime application likely suggest greater ethanol production (not measured in our study), which is the major problem in sugarcane silages (Kung and Stanley, 1982). Greater DM recovery is not always observed when using great doses of alkaline additives (Pedroso et al., 2007), which is consistent with our study.

An opposite response in DM recovery due to the lime addition was observed in burned silages. Lime quadratically increased DM recovery in burned silages due to the lesser gas losses. Yeasts use WSC producing ethanol and CO_2 (McDonald et al., 1991). A great and positive correlation ($R^2 = 0.89$) between ethanol and gas losses has been observed (Pedroso et al., 2005). In this case, the great production of CO_2 is likely due to the low efficient metabolic pathway of yeasts, once acetic acid level is no longer increased by the application of lime. Our results are consistent with the greater DM recovery observed in other cultivars of sugarcane ensiled with lime (Santos et al., 2008).

Application of lime (>10 g/kg) reduced the heating rate in silages, thereby these silages exhibited a long period of stability when aerobically exposed. Lime possesses antifungal properties, and the inhibition of spoilage microorganisms must be related to changes in the osmotic potential of silage that reduce the growth of yeast (Pahlow et al., 2003). The positive effect of lime to enhance aerobic stability has been reported in several studies (Amaral et al., 2009; Balieiro Neto et al., 2009; Rezende et al., 2011).

Succession of yeast species occurs from the anaerobic phase to the aerobic phase when silos are opened (Pahlow et al., 2003), and this process continues during the aerobic exposure phase (Pitt et al., 1991). A majority of yeast species that are active during oxygen exposure are the same epiphytic species found in the forage before ensiling (Pahlow et al., 2003). Increased aerobic stability should be expected in burned sugarcane because heat could interfere with the succession process (Siqueira et al., 2010). However, taking into consideration the overall mean, the *in natura* silages had great stability compared

to burned silages (156.1 vs. 107.5 h, respectively). Our hypothesis is that burned sugarcane probably had greater production of lactic acid (which was not measured in this study) suggested by the great DM recovery. Lactic acid no has an antifungal effect *per se* (Moon, 1983), and can be largely used as a substrate for the growth of yeast (Wilkinson and Davies, 2012). Thus, better fermentation usually results in low aerobic stability of silages (McDonald et al., 1991).

Linear decreases in accumulated temperature of *in natura* and burned silages were observed; however, lime addition had a cubic effect on aerobic stability. Different lime levels led to inconsistent results, mainly when considered the different types of sugarcane (*in natura* and burned). Conversely, the greater levels of lime (15 and 20 g/kg) seems to be consistent to enhance aerobic stability of silages, regardless of sugarcane type.

5. Conclusions

The impact of post-burning days on the characteristics of burned silage was not clear, and further studies should be considered. However, considering the approach in which this study was carried out, if a sugarcane field is burned accidentally, the farmers have 10–15 d time period to ensile the forage so that the digestibility and conservation of nutrients may be maximized.

Lime consistently improved DM recovery in burned silages. The greater levels of lime (15 and 20 g/kg) reduced fiber fraction and enhanced aerobic stability for both *in natura* and burned silages. In summary, lime may be used as an additive for both *in natura* and burned silages since in greater levels.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.anifeedsci.2016.03.010.

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