Evaluation of relative biological efficiency of additives in sugarcane ensiling[1]

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ABSTRACT - The objective of this study was to evaluate the effects of adding alkalis on the fermentative pattern, aerobic stability and nutritive value of the sugarcane silage. A completely randomized design with 6 additives in two concentrations (1 or 2%), plus a control group, totalizing 13 treatments [(6×2)+1] with four replications, was used. The additives were sodium hydroxide (NaOH), limestone ($CaCO_3$), urea ($CO(NH_2)_2$), sodium bicarbonate ($NaHCO_3$), quicklime (CaO) and hydrated lime ($Ca(OH)_2$). The material was ensiled in 52 laboratory silos using plastic buckets with 12 L of capacity. Silos were opened 60 days after ensiling, when organic acids concentration, aerobic stability and chemical composition were determined. The Relative Biological Efficiency (RBE) was calculated by the slope ratio method, using the data obtained from ratio between desirable and undesirable silage products, according to the equation: D/U ratio = [lactic/(ethanol + acetic + butyric)]. All additives affected dry matter, crude protein, acid detergent fiber, neutral detergent fiber contents and buffering capacity. Except for urea and quicklime, all additives increased the *in vitro* dry matter digestibility. In general, these additives altered the fermentative pattern of sugarcane silage, inhibiting alcoholic fermentation and improving lactic acid production. The additive that showed the best RBE in relation to sodium hydroxide (100%) was limestone (89.4%). The RBE values of urea, sodium bicarbonate and hydrated lime were 49.2%, 47.7% and 34.3%, respectively.

Key Words: alkalis, fermentation, silage

Introduction

Silage production is one of the most important processes in the conservation of forage plants to be used as a feed source for livestock, especially in the dry season. The choice for sugarcane as a roughage feed has grown in the last few years and among several advantages that stimulate this forage use, the facility and tradition of this crop and the high yield per area are distinguished.

Ensiled sugarcane has high concentrations of ethanol, due to its soluble carbohydrates content and also the presence of yeast population which converts sugars into ethanol, CO_2 and water, decreasing the content of soluble carbohydrates and increasing the components of cell wall and dry matter losses, which impairs silage quality (Alli et al., 1983).

Ethanol contents of 7.8 up to 17.5% in DM basis have been observed in isolated sugarcane silage, resulting in losses of up to 29% of DM silage (Andrade et al., 2001). Silva et al. (2009) observed, after exposure of sugarcane to aeration for 0.4 or 8 hours, that regardless of aeration time, ethanol production in sugarcane silages was high (22% of DM). Recently, the use of additives, especially the alkalis agents, has been distinguished in sugarcane ensilage. The finality of the additives is to interfere in the fermentative dynamics, altering pH and osmotic pressure of the forage mass inhibiting the development of undesirable microorganisms during the fermentation of the ensiled material (Santos et al., 2008).

With the objective to control losses during ensiling, several additives have been evaluated, including urea, sodium hydroxide and calcium oxide. Balieiro Neto et al. (2007) evaluated the effects of calcium oxide administered at the moment of ensiling at doses of 0.5; 1.0 and 2.0%, on the chemical composition of sugarcane silage during fermentation and post-opening. The authors observed that the use of this additive promoted reduction in fiber content, increase in digestibility and in the preservation of non-fiber carbohydrates after the silo opening. However, scientific research studies with the use of alkalis additives are scarce and their results need to be studied. The objective of the present study was to evaluate the biological efficiency of

six additives through fermentative pattern and nutritive composition of sugarcane silage.

Material and Methods

The trial was carried out at Department of Animal Nutrition and Production of the Faculty of Veterinary Medicine and Animal Science of Universidade de São Paulo (Pirassununga Campus).

The sugarcane (*Saccharum sp* L.) used was from an industrial variety (SP80-1816/CTC) and was harvested at 390 days of growth (Table 1).

The sugarcane was chopped at the moment of ensiling (Chopper Nogueira, model EM-9F3B) in fragments of approximately 0.95 cm, on average. The evaluation of mean theoretical particle size was performed according to the sieves methodology "Penn State Particle Size Separator" proposed by Lammers et al. (1996). A completely randomized design was adopted and the additives tested were sodium hydroxide (NaOH), limestone ($CaCO_3$), urea ($CO(NH_2)_2$), sodium bicarbonate ($NaHCO_3$), quicklime (CaO) and hydrated lime ($Ca(OH)_2$) in two concentrations (1 or 2%), plus the control group (0% of additive). The additives were mixed to chopped sugarcane in their respective proportions and homogenized. Fifty-two experimental silos were prepared in plastic buckets of 252 mm of height and 245 mm of diameter (capacity of 12 liters). Immediately after treatments preparation, the respective masses were placed inside each silo and compacted to the density of 500 kg of sugarcane/m^3. Silos were sealed with lids, weighed and vertically stored in a covered area at room temperature and only opened after 60 days of storage.

Laboratory analyses were conducted at the Laboratory of Animal Nutrition and Production of the Faculty of Veterinary Medicine and Animal Science of USP. Silos were opened, the mass homogenized and a fraction was separated for determination of dry matter (DM) (at 55 and 105 ºC in forced-ventilation oven) and crude protein (CP), according to AOAC (1990); neutral detergent fiber (NDF); acid detergent fiber (ADF) and lignin, according to Van Soest et al. (1991). Soluble carbohydrates (SC) were determined according to methodology proposed by Johnson et al. (1966) and the acid detergent insoluble nitrogen (ADIN), according to

Van Soest & Robertson (1985). Another fraction was immediately frozen for future counterproof and other, still, was placed in hydraulic press for silage juice extraction.

Immediately after material pressing, 50 mL of silage juice was used for pH determination with a portable digital pH meter (Procyon, model 310), calibrated with pH buffer solutions of 4.0 and 7.0. Afterwards, 2 mL of silage juice were collected and added to 0.4 mL of formic acid and frozen at –20 ºC for further determination of organic acids and ethanol concentration.

Determination of organic acids and ethanol concentration was done by gas chromatography, according to the methodology proposed by Erwin et al. (1961), using a gas chromatographer (Finnigan, model 9001), equipped with silica glass column MEGABOR (Ohio Valley, model OV-351) of 30 m × 0.53 mm and stationary phase of 1.0 micron. The determinations were performed injecting 1.0 µL of the sample in the chromatographer, which was integrated to a computer that processed the quantification calculations through the software Borwin (version 1.21) for chromatography, using a standard solution as basis for organic acids concentrations in the sample. The number of repetitions done per sample was the necessary for the difference between readings to be below 5%. The standard solution was injected every ten successive injections aiming to avoid possible deviations of readings due to column contamination. Organic acids concentration calculations were performed in a computer by the comparison of samples with the standard solution.

Still during sampling, fractions of 2 mL of silage juice were added to 1 mL of sulfuric acid 1 N and frozen at –20 ºC until analysis of ammonia nitrogen (NH_3-N) concentration by colorimetric assay, according to methodology proposed by Kulasek (1972) and adapted by Foldager (1977). Readings in absorbance were performed in spectrophotometer (Beijing Rayleigh AIC model VIS-7220) set in 630 nm. Values of absorbance were used to calculate NH_3-N concentrations in mg of NH_3-N/100 mL, by linear regression equation obtained by the calibration of the equipment with standard solution in different concentrations.

The *in vitro* dry matter digestibility (IVDMD) was determined according to Tilley & Terry (1963). Duplicate samples from oven-dried forage (0.5 g) were weighed in test

Table 1 - Chemical composition of sugarcane used for ensiling

	DM	CP	NDF	ADF	Lig.	SC	ADIN	IVDMD	BC
Sugarcane	34.97	2.14	52.97	32.30	5.26	29.46	26.15	61.28	6.55

DM - total dry matter (%); CP - crude protein (%DM); NDF - neutral detergent fiber (%DM); ADF - acid detergent fiber (%DM); Lig. - lignin (%DM); SC - soluble carbohydrates (%DM); ADIN - acid detergent insoluble nitrogen (% of total nitrogen); IVDMD - *in vitro* dry matter digestibility (%DM); BC - buffering capacity (meq HCL/100 g of DM).

tubes, which were previously dried and calibrated. In the test tubes, 40 mL of McDougall solution (artificial saliva) were added to 10 mL of rumen inoculum of animals kept in *Brachiaria decumbens* pasture, supplemented with 3.0 kg of DM of sugarcane with mineral salt *ad libitum*. Tubes were sealed with rubber corks containing a *Bunsen* valve (immediately after flushing out with CO_2) and incubated in oven for 48 h in controlled temperature (39 ºC), where they were agitated at least 3 to 4 times during fermentation. The second phase occurred after centrifugation and discard of supernatant. Pepsin solution (1:10.000) at 0.2% (50 mL) was added to each tube, followed by agitation at 39 ºC for another 48 hours. After washing, drying and weighing the tubes, calculations were performed as the formula below:

$$IVDMD = \frac{100 \times \text{g of DM in sample} - (\text{g of residual DM} - \text{g of DM of inoculum without sample})}{\text{g of DM in sample}}$$

Results were analyzed by SAS (Statistical Analysis System software, version 8.0), after verifying normality residues by Shapiro-Wilk test (PROC UNIVARIATE). Data (dependent variable) that did not attend to this premise was subjected to logarithmic [Log (X+1)] or by square root [RQ (X+1/2)] transformation. Original or transformed data, when this procedure was necessary, were submitted to polynomial regression analysis by GLM procedure, decomposing the effect in linear and linearity deviation. Relative Biological Efficiency (EBR) was calculated by the "slope ratio" method (Ammerman et al., 1995), in which the regression curve slope of the response variable considering the additive levels (0; 1.0 or 2.0%) was divided by the regression coefficient (Slope) of the standard additive (Sodium hydroxide), assuming to have 100% of Relative Biological Efficiency. In this study, the response variable was considered the relation between the desirable and undesirable products (Rel D/U) of silage fermentation, obtained according to the following formula: Rel D/U = [lactic/(ethanol + acetate + butyrate)]. The "slope ratio" method was used to compare the angular coefficients two by two, in a way to generate the comparison between the Relative Biological Efficiencies of the additives.

Results and Discussion

Sugarcane used for ensiling presented DM content (34.97%) similar to those reported by Andrade et al. (2004), who evaluated 60 varieties of sugarcane, harvested at 12 months of growth used in ruminant diets and observed DM contents varying between 24 and 37%. Crude protein content in sugarcane was 2.14%, similarly to those observed by Ferreira et al. (2007). The concentration of ADF was 32.3% of DM, value within the range observed by Freitas et al. (2006), which was between 30.18 and 35.99% of DM, for 13 genotypes of sugarcane. The content of NDF was 52.97% of DM. Rodrigues et al. (2001) evaluated the quality of 18 sugarcane varieties harvested at 12 months of growth and verified that the NDF content ranged between 44.2% and 56.4% of DM. Sugarcane soluble carbohydrates concentration was 29.46 % of DM and the content of lignin was 5.26 % of DM. The IVDMD observed was 61.28% of DM and was within the range observed by Rodrigues et al. (2001), from 58 to 69% of DM in 18 sugarcane varieties.

The use of additives in sugarcane silage provided higher DM concentration when compared to the silage without it (Figure 1A). Linearity deviation was observed for DM when sodium hydroxide ($\hat{Y} = 24.66 + 6.22x - 1.27x^2$; $P = 0.0127$; $R^2 = 0.97$), limestone ($\hat{Y} = 24.66 + 6.89x - 2.17x^2$; $P = 0.0001$; $R^2 = 0.97$), sodium bicarbonate ($\hat{Y} = 24.66 + 7.32x - 2.72x^2$; $P = 0.0001$; $R^2 = 0.94$), quicklime ($\hat{Y} = 24.66 + 6.74x - 2.45x^2$; $P = 0.0001$; $R^2 = 0.97$) or hydrated lime ($\hat{Y} = 24.66 + 5.99x - 2.09x^2$; $P = 0.0001$; $R^2 = 0.94$) were added to the sugarcane. When urea was added to the silage, the DM content increased linearly ($\hat{Y} = 24.97 + 2.38x$; $P = 0.0001$; $R^2 = 0.85$). Amaral et al. (2009) observed that the sugarcane silage treated with limestone had greater DM content (32.1%), followed by the silage treated with 1% of quicklime (30.5%) and control silage (28.3%). However, Castro Neto et al. (2008) did not observe differences in DM contents of silages treated with 0 or 0.5% of urea. According to these authors, urea, which is classified as an absorbent additive and an inhibitor of nutrient deterioration, did not affect the DM content of the ensiled material. The increase in dry matter content attributed to the tested additives, including urea, can be easily explained by two different mechanisms. The first mechanism would result from the effect of addition, of products in the case of additives, with DM content higher than the sugarcane itself. In this way, when an additive with high DM content is mixed with forage with lower DM content, the resulting silage would have greater DM content than the original forage. The second explanation would be by a technique artifact: while ethanol is an alcohol with high volatility, lactic acid is an organic acid with low volatility. As explained below, several tested additives caused drastic decrease in ethanol production and concomitantly increase in lactic acid concentration in these silages. This fact could result in lower loss of volatile compounds during

silage drying in oven, resulting in greater DM contents in silages treated with additives.

There was linearity deviation for CP concentration (Figures 1B and 1C) of silages treated with sodium hydroxide ($\hat{Y} = 2.84 - 1.09x + 0.36x^2$; P = 0.001; $R^2 = 0.92$), limestone ($\hat{Y} = 2.84 - 0.79x + 0.25x^2$; P = 0.0108; $R^2 = 0.86$), sodium bicarbonate ($\hat{Y} = 2.84 - 0.91x + 0.32x^2$; P = 0.0047; $R^2 = 0.84$) and hydrated lime ($\hat{Y} = 2.84 - 0.94x + 0.30x^2$; P = 0.0333; $R^2 = 0.77$). The crude protein content of the silage treated with quicklime decreased linearly ($\hat{Y} = 2.81 - 0.25x$; P = 0.0001; $R^2 = 0.79$), while urea addition linearly increased the content of this nutrient ($\hat{Y} = 2.61 + 8.87x$; P = 0.0001; $R^2 = 0.96$). Amaral et al. (2009) also observed decreased CP content in sugarcane silages treated with 0 or 1% of quicklime or limestone.

The increase in CP content with urea inclusion is a result of non-protein nitrogen addition in increasing doses, which are considered as CP in total nitrogen determination. The decrease in CP content in relation to control silage, observed for the other additives probably occurred due to a dilution effect. Even observing additive effect on CP contents, the values obtained at the moment of the silos opening are within the range cited by Faria (1993) for fresh sugarcane (1.8 to 4.7% DM).

Linearity deviation of ADIN content was observed (Figure 1D) in the silage with the addition urea ($\hat{Y} = 32.51 - 35.82x + 10.90x^2$; P = 0.0001; $R^2 = 0.99$), i.e., all inclusion levels of this compound decreased ADIN content compared with control. This response occurred due to the dilution effect of ADIN content, in which the increase in total N results in proportional drop of N linked to fiber. There was no effect of the other additives on this parameter.

The contents of ADF (Figure 1E) linearly decreased with the addition of sodium hydroxide ($\hat{Y} = 43.18 - 4.06x$; P = 0.0001; $R^2 = 0.90$), urea ($\hat{Y} = 42.89 - 3.54x$; P = 0.0001; $R^2 = 0.82$), sodium bicarbonate ($\hat{Y} = 42.76 - 1.92x$; P = 0.0099; $R^2 = 0.46$) and quicklime ($\hat{Y} = 43.06 - 0.86x$; P = 0.0338; $R^2 = 0.34$). Linearity deviation of ADF content was verified when sugarcane was treated with limestone ($\hat{Y} = 43.40 - 9.83x + 2.74x^2$; P = 0.0043; $R^2 = 0.93$) or hydrated lime ($\hat{Y} = 43.40 - 7.37x + 2.94x^2$; P = 0.0008; $R^2 = 0.83$). It is worth stressing that although the effect of sodium bicarbonate and quicklime on ADF contents were statistically significant, the coefficient of determination was relatively low, indicating that only 46% and 34%, respectively, from all the variability observed for ADF concentrations was explained by the level of additive added to silage. In this way, the remaining 54% and 66%, respectively, of ADF variability was not explained in this experiment.

The contents of NDF (Figure 1F) linearly decreased with the increase in sodium hydroxide inclusion ($\hat{Y} = 66.36 - 9.98x$; P = 0.0001; $R^2 = 0.96$), urea ($\hat{Y} = 65.94 - 5.34x$; P = 0.0001; $R^2 = 0.84$) and quicklime ($\hat{Y} = 66.42 - 3.15x$; P = 0.0001; $R^2 = 0.85$). Linearity deviation was observed with limestone inclusion ($\hat{Y} = 66.83 - 16.18x + 4.52x^2$; P = 0.0004; $R^2 = 0.97$), sodium bicarbonate ($\hat{Y} = 66.83 - 9.33x + 3.00x^2$; P = 0.0224; $R^2 = 0.80$) and hydrated lime ($\hat{Y} = 66.83 - 12.76x + 4.31x^2$; P = 0.0001; $R^2 = 0.96$).

The decrease in ADF content observed was not so intense, but followed the same pattern observed for NDF concentration. The fact that some additives were effective in decreasing the contents of ADF and NDF of silage could be attributed to several factors. Among them, the fact that these additives restricted the undesirable fermentation, leadint to higher recovery of non-fiber carbohydrates, allowing lower concentration in the contents of those components. Still, according to Van Soest (1994), some bindings that occur during cell wall formation are susceptible to alkalis agents action. Besides the hydrolytic action on lignin, carbohydrates and feluric and p-coumaric acids complexes, alkalis agents can still act in the structure of polysaccharides, where these agents promote the expansion of cellulose molecule, causing ruptures of intermolecular links of hydrogen binds. Moreover, they act solubilizing part of the hemicellulose that was linked to cellulose by means of covalent connections. Another explanation for the decrease in ADF and NDF values was the dilution effect, once the additives are not characterized as fiber. Balieiro Neto et al. (2007) evaluated the addition of 0; 0.5; 1 or 2% of quicklime in fresh matter of sugarcane and verified, at the moment of silo opening, that NDF content of treated silages was lower than control silage, with the lowest value observed in silage with 2% of calcium oxide. Pedroso et al. (2007) observed reduction in NDF values of 30.54 percentage units with an addition of 3% of sodium hydroxide and 11.94 percentage units with an addition of 1.5% of urea when compared with the silage without additives.

There was linear decrease in lignin concentration (Figure 1G) of silages treated with limestone ($\hat{Y} = 7.39 - 1.0x$; P = 0.0003; $R^2 = 0.78$) or urea ($\hat{Y} = 7.33 - 1.03x$; P = 0.014; $R^2 = 0.64$), with no effect of other tested additives on this variable. According to Klopfeinstein (1980), lignin content is not normally altered by treatment with alkalis agents. Balieiro Neto et al. (2007), when testing the addition of 0; 0.5; 1 and 2% of quicklime at fresh matter of silage, observed, at the moment of silo opening, that the lignin content was reduced in silages with 2% of quicklime. Amaral et al. (2009) verified reduction in lignin content in sugarcane silages treated with 1% of limestone when compared with the

control group (without additive). However, these researchers did not observe difference when quicklime was used as additive, similarly to the present study.

Linear increase in soluble carbohydrates concentration was observed (Figure 1H) when sodium hydroxide ($\hat{Y} = 9.61 + 8.8x$; $P = 0.0001$; $R^2 = 0.92$) or sodium bicarbonate ($\hat{Y} = 9.35 + 2.9x$; $P = 0.0156$; $R^2 = 0.47$) was added to the silage. However, the concentration of soluble carbohydrates linearly decreased when quicklime was added ($\hat{Y} = 9.18 - 1.95x$; $P = 0.0109$; $R^2 = 0.50$). There was linear deviation with hydrated lime addition ($\hat{Y} = 8.77 + 5.48 - 3.00x^2$; $P = 0.0170$; $R^2 = 0.51$). There was no effect of limestone or urea addition on soluble carbohydrates content. However, the effect of sodium bicarbonate and quicklime on soluble carbohydrates content was statistically significant and the coefficient of determination was relatively low, indicating that only 47 and 50%, respectively, of all variability observed for the concentrations of soluble carbohydrates was explained by the level of additive added to silage.

Probably, the increase in soluble carbohydrates concentration with the addition of sodium hydroxide or sodium bicarbonate occurred due to hemicellulose hydrolysis or to higher preservation of soluble carbohydrates of silages that received these treatments. Van Soest (1994) mentioned that the hydroxyl (OH) radical attacks fenolic groups and can break connections type ester between the uronic acid of hemicelluloses.

There was linear increase in IVDMD (Figure 1I) of sugarcane silages treated with sodium hydroxide ($\hat{Y} = 46.18 + 14.34x$; $P = 0.0001$; $R^2 = 0.90$), limestone ($\hat{Y} = 46.32 + 3.85x$; $P = 0.0138$; $R^2 = 0.45$), sodium bicarbonate ($\hat{Y} = 45.67 + 3.24x$; $P = 0.0317$; $R^2 = 0.41$) and hydrated lime ($\hat{Y} = 46.17 + 4.89x$; $P = 0.0101$; $R^2 = 0.5085$). Schmidt et al. (2007) observed, comparing with the control group, increase of IVDMD when 0.5% of urea was added to sugarcane silage. The authors justified this result by the lower content of structural carbohydrates of silage with urea addition. Pedroso et al. (2007), who evaluated the addition of crescent levels of sodium hydroxide to sugarcane silages (1, 2 or 3% of fresh matter), observed increase of 8.6 to 10.6% in IVDMD when compared with the silage without additive. In the present study, the increase in IVDMD with 1% addition of sodium hydroxide was of 38% and when 2% was added, this value went up to 63%, when compared with the control. These results of IVDMD are in agreement with the results obtained for NDF and ADF, once all additives were efficient in decreasing these fractions. The effect of some additives in increasing IVDMD probably occurred due to three factors, alkalis hydrolysis, which increased cellulose and hemicellulose digestion; dilution effect and effect of soluble carbohydrates preservation.

There was no effect ($P > 0.05$) of urea or quicklime addition on IVDMD, although these silages presented digestibility values numerically higher than control silage. Contrarily to the result obtained in this study, Santos et al. (2008) observed that the addition of 1 or 1.5% of quicklime or limestone significantly increased IVDMD of sugarcane silage. In the present study, it was also expected that the addition of quicklime would increase IVDMD, as there was a decrease in cell wall fractions of sugarcane silages that received these treatments. Probably, the decrease in fiber fractions was not sufficient to increase silage digestibility.

Buffer capacity linearly increased (Figure 1J) in silages treated with sodium hydroxide ($\hat{Y} = 20.01 + 23.35x$; $P = 0.0001$; $R^2 = 0.98$), limestone ($\hat{Y} = 18.61 + 18.60x$; $P = 0.0001$; $R^2 = 0.97$) and urea ($\hat{Y} = 19.21 + 5.61x$; $P = 0.0001$; $R^2 = 0.85$). Linearity deviation was verified in silages treated with sodium bicarbonate ($\hat{Y} = 19.24 + 6.88x + 3.08x^2$; $P = 0.0474$; $R^2 = 0.97$), quicklime ($\hat{Y} = 19.24 + 3.93x + 28.99x^2$; $P = 0.0001$; $R^2 = 0.9989$) and hydrated lime ($\hat{Y} = 19.24 + 22.45x + 6.65x^2$; $P = 0.0010$; $R^2 = 0.99$).

The reader must be aware that the fermentative data presented high coefficients of variation. Probably, it was due to the fact that these coefficients of variation account for data, and not for the model. So, it is common to observe high coefficients of variation when the effect of treatment is quite evident, resulting in large differences between treatments, as it could be observed for ethanol, propionic, butyric and lactic acids concentrations. All the tested additives were efficient in reducing ethanol production ($P < 0.05$). Urea addition linearly decreased the ethanol content of silages and linearity deviation was observed when treatments were sodium hydroxide, limestone, sodium bicarbonate, quicklime and hydrated lime (Table 2).

Unlike the result obtained in the present study, Pedroso et al. (2007) evaluated the addition of 0; 1; 2 or 3% of sodium hydroxide to sugarcane silage and observed that this additive was not able to reduce ethanol concentration in treated silages when compared with the control silage. When the authors evaluated the addition of 0; 0.5; 1.0 or 1.5% of urea in the sugarcane silage, they observed that urea was also not able to reduce ethanol content in silages. Urea, when in contact with ensiled silage, is hydrolyzed to ammonia, which has inhibitor effect on yeast and mould population, showing to be able to reduce ethanol production in sugarcane silages (Alli et al., 1983).

Amaral et al. (2009) verified reduction of 72% in ethanol concentration in sugarcane silages treated with 1% of quicklime or limestone when compared with control. Santos et al. (2008) observed reduction of 92% in ethanol concentration, when 1 or 1.5% of quicklime was added to
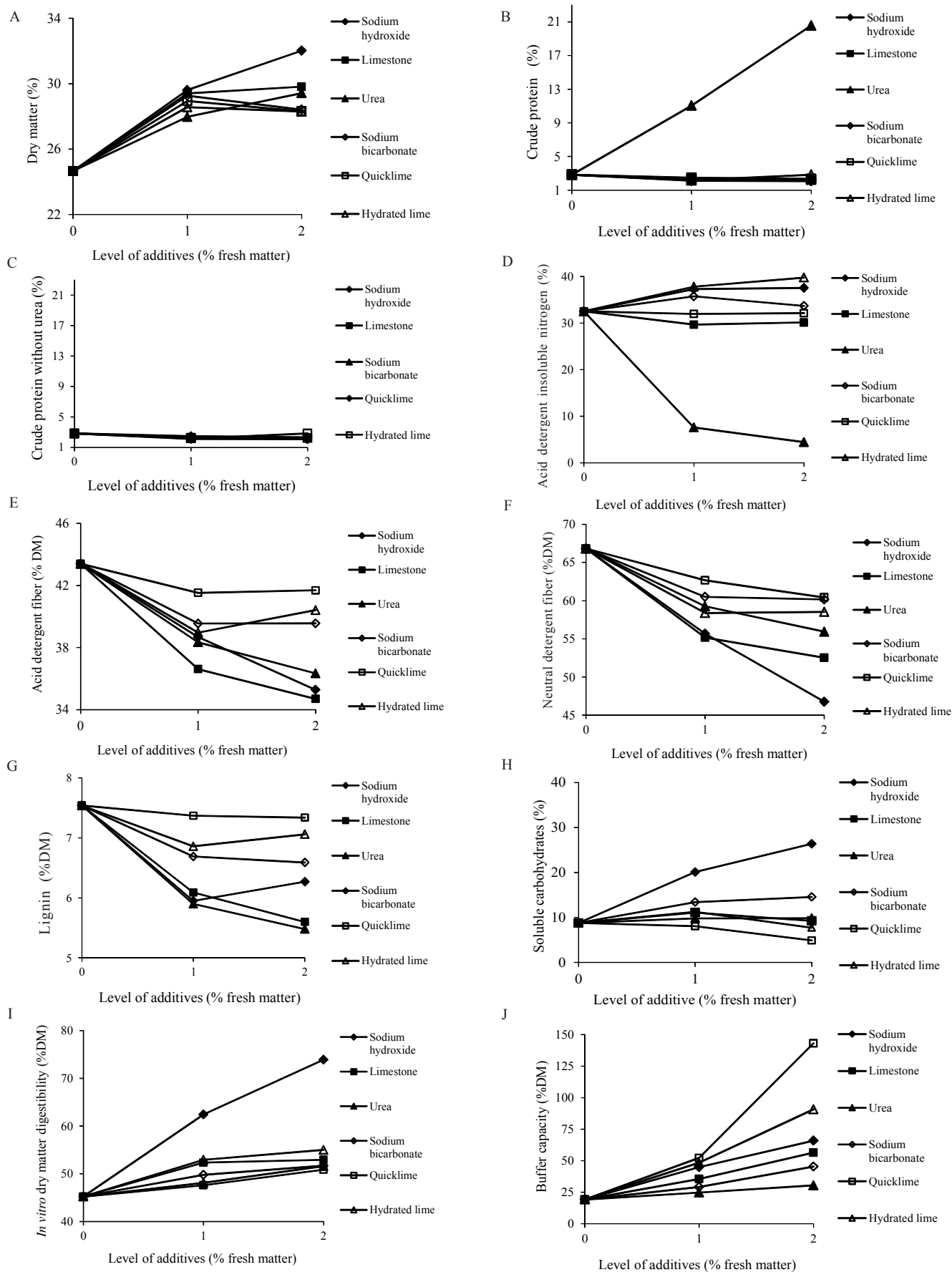
Figura 1 - Dry matter (A), crude protein (B), crude protein without urea (C), acid detergent insoluble nitrogen (D), acid detergent fiber (E), neutral detergent fiber (F), lignin (G), soluble carbohydrates (H), *in vitro* dry matter digestibility (I) and buffer capacity (J).

sugarcane silage. When the additive tested was limestone, in the same concentrations, a reduction of 71% in ethanol concentration was verified with addition of 1% and of 65% with the addition of 1.5% of limestone to silages. The authors mentioned that sugarcane treatment with alkalis agents suggests inhibitor effect on yeast growth, as silages treated with these products presented significant reduction in alcoholic fermentation, high concentration of soluble carbohydrates and lower total dry matter losses. However, the alkalization resulted from additive use can neutralize the acidifying action of lactic acid, allowing lactic bacteria to continue to unfold soluble carbohydrates into more lactic acid, resulting in low substrate for yeast to unfold to ethanol.

Silva et al. (2008) evaluated the effect of soluble carbohydrates on the ethanol content in sugarcane silages and verified that ethanol production was reduced with the decrease in soluble carbohydrates of silages. The authors correlated this result to the lower metabolism of soluble carbohydrates to ethanol by yeasts.

For acetic acid concentration, linearity deviation was observed ($P<0.05$) with the inclusion of limestone, quicklime or hydrated lime in the sugarcane silage (Table 2). Sodium hydroxide addition linearly increased this acid concentration and there was no influence of urea or bicarbonate addition. Acetic acid concentrations of silages are a little above the standard cited as normal (1 to 3% DM) by Kung Jr. & Stokes (2001) for forage silages. It is important to emphasize that although sugarcane is a forage, it differs from the other forages, even from those of temperate climate, for its higher concentration of soluble carbohydrates which are available for much more intense fermentations during ensiling. Amaral et al. (2009) added 0 and 1% of quicklime to sugarcane silage and observed values of 1.3 and 1.6% in DM of acetic acid concentration, respectively. These values were lower than what was observed in this study. When the tested additive was limestone, also at the doses of 0 or 1%, the authors obtained concentrations of 1.3 and 1.5% of this acid in DM, also lower than those obtained in the present study. Schmidt et al. (2007) did not find difference in acetic acid concentration when 0 or 0.5% of urea was added to the sugarcane silage (2.18 vs. 1.93% DM).

Propionic acid concentration linearly increased with sodium hydroxide or limestone inclusion in the silages ($P<0.05$) (Table 2). When the inclusion of quicklime or hydrated lime was evaluated, there was linearity deviation ($P<0.05$). No effect of urea or bicarbonate addition was observed on the concentration of this acid ($P>0.05$). Except for the treatment with 2% of quicklime, propionic acid concentrations are within the range of 0 to 1%, classifying

them as high quality silages. Few are the studies found in the literature, using additives for sugarcane silage, that presented the organic acids concentrations.

For butyric acid concentration, linearity deviation was observed ($P<0.05$) when sodium hydroxide, limestone or hydrated lime was added (Table 2). The concentration of this acid linearly increased with the addition of sodium bicarbonate or quicklime ($P<0.05$). No effect on this acid concentration was verified when urea was tested ($P>0.05$). In silages treated with quicklime or hydrated lime, the values of butyric acid found were higher and could be explained by the high buffering capacity of the additives, allowing the development of Clostridium, which, according to Pahlow et al. (2003), besides converting sugars into butyric acid, also use lactic and acetic acids for this conversion. In sugarcane silages treated with alkalis additives, increase in lactic acid production was usually observed, which could have contributed along with the highest pH, for the higher butyric acid concentration. Although in lower value, Amaral et al. (2009) observed that the addition of 1% of quicklime increased butyric acid concentration compared with the control silage (3.1 vs. 0.2% DM). In the same study, these authors did not observe effect of limestone addition (1% fresh matter) on the concentration of this acid when compared with the treatment without additive.

Lactic acid concentration linearly increased ($P<0.05$) with inclusion of sodium hydroxide, limestone or urea. There was linearity deviation ($P<0.05$) when the additives used were sodium bicarbonate, quicklime or hydrated lime (Table 3).

The reason why all additives promoted increase in lactic acid concentration could be explained by the fact that the inclusion of alkalis substances neutralizes the acidifying action of lactic acid, leading more soluble carbohydrates to be unfolded by yeasts and moulds. According to Santos et al. (2008), buffering of acids produced by fermentation is a stimulus for higher conversion of soluble carbohydrates into lactic acid, increasing the concentration of this final product and avoiding ethanol production. Castro Neto et al. (2008) evaluated lactic acid production in silages with 0 or 0.5% of urea or 0.5% of urea + 0.5% of zeolite and observed that the additions of urea or urea + zeolite increased lactic acid concentration in sugarcane silages.

In the present study, all additives were able to decrease the alcoholic fermentation and increase lactic acid concentration in the silage. However, this effect was more pronounced when sodium hydroxide or limestone was used.

The study of protein fractions in sugarcane silage presents small practical importance due to the low

Table 2 - Values of ethanol and organic acid (acetic, propionic and butyric) concentrations obtained with and without the addition of additives in sugarcane silage

Additive	Doses (%)			CV	Probability		Equation	R^2
	0	1	2		Linear	Deviation		
Ethanol (%DM)								
NaOH	15.30	1.57	0.23	131.02	0.0001	0.0083	$\hat{Y} = 15.3-19.92x+6.19x^2$	0.9076
$CaCO_3$	15.30	1.70	1.12	118.97	0.0001	0.0001	$\hat{Y} = 15.3-20.11x+6.51x^2$	0.9083
$CO(NH_2)_2$	15.30	5.56	1.78	86.18	0.0001	0.7529	$\hat{Y} = 14.31-6.76x$	0.7858
$NaHCO_3$	15.30	4.39	4.72	72.90	0.0012	0.0219	$\hat{Y} = 15.3-16.53x+5.62x^2$	0.7970
CaO	15.30	3.03	3.55	86.60	0.0001	0.0001	$\hat{Y} = 15.3-18.67x+6.40x^2$	0.8788
$Ca(OH)_2$	15.30	1.80	2.04	108.82	0.0001	0.0001	$\hat{Y} = 15.3-20.38x+6.88x^2$	0.9014
Acetic acid (%DM)								
NaOH	2.77	3.43	3.97	20.48	0.0096	0.8688	$\hat{Y} = 2.79+0.60x$	0.5458
$CaCO_3$	2.77	3.55	3.21	14.46	0.1076	0.0290	$\hat{Y} = 2.77+1.33x-0.55x^2$	0.5243
$CO(NH_2)_2$	2.77	2.98	2.88	9.50	0.5913	0.4099	-	-
$NaHCO_3$	2.77	2.62	2.69	8.69	0.6495	0.4829	-	-
CaO	2.77	0.69	0.71	77.19	0.0001	0.0010	$\hat{Y} = 2.77-3.13x+1.05x^2$	0.9089
$Ca(OH)_2$	2.77	0.82	0.83	67.95	0.0001	0.0008	$\hat{Y} = 2.77-2.94x+0.98x^2$	0.9152
Propionic acid (%DM)								
NaOH	0.040	0.078	0.088	35.21	0.0004	0.1000	$\hat{Y} = 0.044+0.024x$	0.7088
$CaCO_3$	0.040	0.035	0.075	49.73	0.0240	0.0750	$\hat{Y} = 0.033+0.018x$	0.3603
$CO(NH_2)_2$	0.040	0.043	0.053	33.50	0.2822	0.7012	-	-
$NaHCO_3$	0.040	0.045	0.060	31.60	0.0697	0.5671	-	-
CaO	0.040	0.145	1.303	122.91	0.0001	0.0001	$\hat{Y} = 0.04-0.421x+0.526x^2$	0.9610
$Ca(OH)_2$	0.040	0.105	0.418	100.01	0.0001	0.0364	$\hat{Y} = 0.04-0.059x+0.12x^2$	0.8424
Butyric acid (%DM)								
NaOH	0.020	0.858	0.090	124.65	0.2188	0.0001	$\hat{Y} = 0.02+1.640x-0.803x^2$	0.9716
$CaCO_3$	0.020	0.015	1.298	154.33	0.0002	0.0064	$\hat{Y} = 0.02-0.649x+0.644x^2$	0.8453
$CO(NH_2)_2$	0.020	0.083	0.155	121.63	0.0786	0.9343	-	-
$NaHCO_3$	0.020	0.038	0.508	152.57	0.0042	0.0722	$\hat{Y} = -0.055+0.244x$	0.5234
CaO	0.020	4.865	12.145	92.86	0.0001	0.0594	$\hat{Y} = -0.386+6.063x$	0.9620
$Ca(OH)_2$	0.020	6.278	7.370	77.27	0.0001	0.0036	$\hat{Y} = 0.02+8.840x-2.583x^2$	0.9229

Additive - NaOH - sodium hydroxide; $CaCO_3$ - limestone; $CO(NH_2)_2$ - Urea; $NaHCO_3$ - sodium bicarbonate; CaO - quicklime; $Ca(OH)_2$ - hydrated lime; Linear - probability of linear effect; Deviation - probability for linear deviation; CV - coefficient of variation (%); DM - dry matter.

contribution of this forage in protein to attend animals requirements. When NH_3-N concentration was evaluated (Table 3), linearity deviation was observed with sodium hydroxide or quicklime addition (P<0.05). There was linear increase in NH_3-N concentration (P<0.05) when the tested additive was limestone or sodium bicarbonate. When urea or hydrated lime was added, linear decrease in NH_3-N concentration was observed. The decrease caused by urea can be explained by the effect of increase in the denominator concentration. So, it is expected that the maintenance of NH_3-N concentration (numerator) with substantial increase of CP in silage (denominator) results in considerable decrease in the concentration of that form of nitrogen when expressed in relation to total nitrogen. However, unlike the results of the present study, Siqueira et al. (2007) observed that silages treated with urea (1.5% FM) presented higher

concentrations of NH_3-N, in relation to total nitrogen, when compared with the control silage.

There was linearity deviation (P<0.05) of pH for silages treated with sodium hydroxide, sodium bicarbonate and hydrated lime (Table 3). The pH linearly increased (P<0.05) in silages treated with limestone, urea or quicklime. According to Pedroso et al. (2007), sugarcane silages treated with alkalis agents generally present higher pH than the maximum level considered adequate for ensiled forages stabilization (3.7 to 4.2). Kung Jr. et al. (2003), in literature review about additives for ensilage, concluded that forages treated with urea and with efficient transformation of this urea in ammonia resulted in silages with higher pH than the non-treated silages, as ammonia is a substance with alkalis power, which hamper pH reduction. Santos et al. (2008) observed that the addition of 1% of quicklime or limestone

to sugarcane silage promoted higher pH values. According to the authors, higher pH values in silages treated with alkalis additives were caused by buffering capacity, once the dissociation of ions present in chemical additives generates anionic charges able to neutralize the hydrogen ions from organic acids produced during the fermentation. The pH increase due to the addition of alkalis agents is a predominant factor for the alkalinization of sugarcane fiber fraction to occur, besides promoting alterations in the other nutrients. This statement is verified in the present study because a decrease in NDF and ADF fractions was observed, as well as an increase in IVDMD.

Sodium hydroxide, chosen as a standard additive (100% Relative Biological Efficiency - RBE), considerably decreased the alcoholic fermentation and, consequently, resulted in a silage with higher content of lactic acid, without substantially altering the other organic acids.

All tested additives linearly increased the relation of desirable and undesirable products of fermentation with increased level of their inclusion in the silage (Table 4).

Table 3 - Values of lactic acid concentration, ammonia nitrogen concentration and pH obtained with or without the addition of additives in the sugarcane silage

Additive	Doses (%)			CV	Probability		Equation	R^2
	0	1	2		Linear	Deviation		
Lactic acid (%DM)								
NaOH	3.42	8.37	13.04	50.61	0.0001	0.8220	$\hat{Y} = 3.47+4.81x$	0.9591
$CaCO_3$	3.42	9.60	15.43	54.16	0.0001	0.5434	$\hat{Y} = 3.48+6.00x$	0.9932
$CO(NH_2)_2$	3.42	4.75	7.54	36.99	0.0001	0.1760	$\hat{Y} = 3.19+2.06x$	0.8206
$NaHCO_3$	3.42	6.35	11.76	50.60	0.0001	0.0019	$\hat{Y} = 3.42+1.68x+1.24x^2$	0.9864
CaO	3.42	10.90	7.34	47.64	0.0037	0.0001	$\hat{Y} = 3.42+5.08x-0.13x^2$	0.9593
$Ca(OH)_2$	3.42	6.22	12.17	53.83	0.0001	0.0287	$\hat{Y} = 3.42+1.21x+1.58x^2$	0.9475
NH_3-N (% of total N)								
NaOH	8.91	5.97	5.00	27.34	0.0001	0.0419	$\hat{Y} = 8.91-3.93x+0.99x^2$	0.8910
$CaCO_3$	8.91	13.35	14.00	27.38	0.0467	0.3179	$\hat{Y} = 9.71+2.45x$	0.3599
$CO(NH_2)_2$	8.91	5.23	1.12	71.87	0.0001	0.7630	$\hat{Y} = 9.00-3.91x$	0.9137
$NaHCO_3$	8.91	11.78	12.68	18.57	0.0117	0.3337	$\hat{Y} = 9.32+1.84x$	0.5262
CaO	8.91	6.53	1.40	62.50	0.0001	0.0168	$\hat{Y} = 8.91-0.99x-1.38x^2$	0.9614
$Ca(OH)_2$	8.91	5.47	2.04	57.40	0.0001	0.9988	$\hat{Y} = 8.91-3.44x$	0.9303
pH								
NaOH	3.48	4.40	4.71	13.11	0.0001	0.0003	$\hat{Y} = 3.48+1.221x-0.304x^2$	0.9789
$CaCO_3$	3.48	3.68	3.95	5.74	0.0001	0.4091	$\hat{Y} = 3.47+0.235x$	0.8884
$CO(NH_2)_2$	3.48	3.83	4.05	6.80	0.0001	0.2814	$\hat{Y} = 3.50+0.284x$	0.8833
$NaHCO_3$	3.48	4.05	4.29	9.12	0.0001	0.0032	$\hat{Y} = 3.48+0.730x-0.163x^2$	0.9719
CaO	3.48	4.48	5.57	19.99	0.0001	0.6643	$\hat{Y} = 3.47+1.041x$	0.9708
$Ca(OH)_2$	3.48	4.49	4.80	13.94	0.0001	0.0001	$\hat{Y} = 3.48+1.365x-0.353x^2$	0.9840

Additive - NaOH - sodium hydroxide; $CaCO_3$ - limestone; $CO(NH_2)_2$ - Urea; $NaHCO_3$ - sodium bicarbonate; CaO - quicklime; $Ca(OH)_2$ - Hydrated lime; Linear - probability of linear effect; Deviation - probability of linearity deviation; CV - coefficient of variation (%).

Table 4 - Values of relation of desirable and undesirable products (D/U ratio = [lactic/(ethanol + acetic + butyric)]) and relative biological efficiency (RBE) obtained with or without the addition of additives in sugarcane silage

Additive	Doses (%)			CV	Probability		Equation	R^2	RBE
	0	1	2		Linear	Deviation			
D/U relation									
NaOH	0.02	1.48	3.08	79.18	0.0001	0.3757	$\hat{Y} = 0.15+1.436x$	0.9525	100.0a
$CaCO_3$	0.02	1.83	2.77	70.15	0.0001	0.0250	$\hat{Y} = 0.32+1.284x$	0.9486	89.4a
$CO(NH_2)_2$	0.02	0.59	1.62	85.11	0.0001	0.1376	$\hat{Y} = 0.10+0.707x$	0.7738	49.2b
$NaHCO_3$	0.02	0.94	1.57	70.16	0.0001	0.7799	$\hat{Y} = 0.22+0.685x$	0.8469	47.7b
CaO	0.02	1.28	0.46	77.85	0.0614	0.0001	$\hat{Y} = 0.20+2.029x-0.951x^2$	0.9093	-
$Ca(OH)_2$	0.02	0.70	1.19	61.37	0.0001	0.8990	$\hat{Y} = 0.21+0.492x$	0.9586	34.3b

Additive - NaOH - sodium hydroxide; $CaCO_3$ - limestone; $CO(NH_2)_2$ - Urea; $NaHCO_3$ - sodium bicarbonate; CaO - quicklime; $Ca(OH)_2$ - hydrated lime; RBE - Relative Biological Efficiency; Linear - probability for linear effect; Deviation - probability for linearity deviation.
[a,b] - letters within each variable differ for the slope ratio method.

Considering the regression coefficient (Slope) of sodium hydroxide as standard (100% of Relative Biological Efficiency), the other additives presented RBE of 89.4; 49.2; 47.7 and 34.3% for limestone, urea, sodium bicarbonate and hydrated lime, respectively. The additive with the best Relative Biological Efficiency, without differing from sodium hydroxide, was limestone (89.4%). Even though this additive presented deviation effect, a linear component was adopted, once the R^2 between the linear component and the deviation was very close (0.95 vs. 0.97). The RBE of urea (49.2%) did not differ from the efficiency found for sodium bicarbonate (47.7%) or for hydrated lime (34.3%), and was lower than the RBE of sodium hydroxide and limestone. The addition of quicklime to silage resulted in decreased alcoholic fermentation and increased lactic acid concentration, with high increase in butyric acid concentration. This fact resulted in lack of equation linearity, without the possibility of generating RBE value for this additive.

Conclusions

In general, the addition of alkalis agents, at the moment of sugarcane ensiling, furthers the conservation, improving the fermentative pattern of silages compared with the silage without additive and inhibiting alcoholic fermentation, resulting in silages with better nutritive value. Sodium hydroxide and limestone presented the best relations of desirable and undesirable fermentation products, indicating that the evaluated doses of these additives were efficient to improve the fermentative pattern of sugarcane silage.

Acknowledgments

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