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Fermentation Characteristics of Silage of Sugar Cane Treated with Calcium Oxide, *Lactobacillus buchneri* and Their Associations

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Abstract

We evaluated the fermentation products, the gaseous and effluent losses of sugarcane silages without calcium oxide (CaO) or with CaO addition, at the levels of zero, 0.8%, 1.6% and 2.4%, in association with the microbial additive *Lactobacillus buchneri* inoculated at the levels of zero, 50.000, 100.000 e 150.000 ufc/g of sugarcane, wet bases. The variety RB855536, harvested after 12 months of first growth was used. The experiment design was the completely randomized design, in a 4 × 4 factorial arrangement. They were evaluated in the silages, the contents of volatile fatty acids, lactic acid, ethanol, and the pH, as well as the gaseous and effluent losses. In the analysis of the data, the SAS system was utilized. It was observed on interaction effect of the chemical and microbial additive over the contents of lactic acid, acetic acid, propionic acid, butyric acid, ethanol and over the gaseous and effluents losses. However, there was no interaction effect regarding to pH. When it was observed on interaction effect of additives, the effects of the levels of one additive were evaluated by regression analysis in each level of each other, and vice-versa. The level 1.6% of CaO associated to the level 50,000 ufc/g of natural matter of *Lactobacillus buchneri* provided adequate levels of lactic acid (superior to 4.5%), and of acetic acid (around 1%), mod-

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erate content of propionic acid (0.55%), low content of butyric acid (0.05%) and controlled the production of ethanol and the gaseous and effluent losses. The pH of the silages were influenced by CaO addition, but were not affected consistently by microbial inoculation.

Keywords

Sugarcane Silage; Calcium Oxide; *Lactobacillus buchneri*; Organic Acid

1. Introduction

The sugar cane (*Saccharum officinarum* L) is used long ago as a forage crop by farmers in Brazil. Stands out for being the sort of greater forage production potential of DM and energy per unit area in single cut, productions reaching between 15 and 20 tonnes (t) of total digestible nutrients (TDN) per hectare, compared with maize, sorghum and cassava produce about 8 t of TDN/ha [1].

The cane sugar is a food that has two components in larger numbers at the expense of others. They are sugar and fiber fraction (NDF) [2]. These components are used in greater proportions of well differentiated by rumen microorganisms, *i.e*., modes as soon as the sugar is fermented in the rumen and is easy to use by the animal. The contents of NDF are used slowly [3].

The daily supply of cane sugar *in natura* numerous flocks, supplemental feeding or total containment, demand daily hand labor for cutting, loading, straw removal, processing and delivery, in addition to causing great imbalance of system regrowth. The ensiling of sugar cane, potentially, would optimize the use of hand labor and obtain uniform regrowth of sugarcane plantation. However, the cane silage, promoted the alcoholic fermentation by yeasts, significantly reducing the nutritional value of the final product [4].

The use of additives can inhibit the proliferation of unwanted microorganisms to the process, such as yeasts, molds and fungi and have desirable actions on the fiber fraction of the sugar cane become essential [5].

The CaO has been introduced as an additive in ensiling of sugar cane as a way to improve the fermentation process, aiming to stimulate lactic acid fermentation and reduce the production of ethanol, and hydrolyzed fiber components, increasing digestibility [6]. Biological additives, among which *Lactobacillus buchneri*, have also been shown to control the fermentation, by promoting hetero lactic fermentation with lactic, acetic and propionic acid. Acetic acid controls the alcoholic fermentation in anaerobic condition by inhibiting the growth of yeasts and increases the aerobic stability of silage after opening the silo [7].

The objective of this research was to evaluate the effect of levels of CaO and *Lactobacillus buchneri* and their associations on the chemical composition, *in vitro* digestibility of dry matter and gas losses and silage effluent from cane sugar.

2. Methods

Silages containing chemical and microbial additives were evaluated. We studied the association of four levels of calcium oxide (CaO) with four levels *Lactobacillus buchneri* (buch), in an experiment conducted at the State University of North Fluminense Darcy Ribeiro-UENF, in Campos-RJ, Brazil.

We used a variety of cane sugar RB855536, harvested by cutting 5 cm of soil at twelve months old cane plant, with a total sugar concentration of 210 BX. Sixteen treatments were established by the association of four levels of the chemical additive CaO with four levels of microbial additive *Lactobacillus buchneri* (**Table 1**) in a completely randomized design in a factorial design with three replications, totaling 48 plots.

The chemical additives (CaO) was applied in commercial form, micro - pulverized, and biological additive *Lactobacillus buchneri* (buch) was applied using hand sprayer with a capacity of 0.5 L for cane sugar before filling of minced experimental silos in order to obtain uniform distribution of the silage mass, diluting the doses used in 100 mL of water. As experimental silos, 15 L plastic buckets were used. At the bottom of each experimental silo, 2 kg of silage dry sand separated by two layers of plastic screen with fine mesh and cotton fabrics were placed, to prevent contact with sand and silage permit absorption and subsequent quantitative determination of the effluents.

In the silage was compacted in sequence, layers of 5 to 10 cm of thickness of cane sugar sting. The quantity of

Table 1. Levels of chemical additives (CaO) and biological (Buch) added at ensiling of cane sugar, natural matter in different treatments.

Treatment - Levels of additives			
1 - 0 CaO* + 0 Buch**	5 - 0 CaO + 50.000 Buch	9 - 0 CaO + 100.000 Buch	13 - 0 CaO + 150.000 Buch
2% - 0.8% CaO + 0 Buch	6 - 0.8 CaO + 50.000 Buch	10 - 0.8 CaO + 100.000 Buch	14 - 0.8 CaO + 150.000 Buch
3% - 1.6% CaO + 0 Buch	7 - 1.6 CaO + 50.000 Buch	11 - 1.6 CaO + 100.000 Buch	15 - 1.6 CaO + 150.000 Buch
4% - 2.4% CaO + 0 Buch	8 - 2.4 CaO + 50.000 Buch	12 - 2.4 CaO + 100.000 Buch	16 - 2.4 CaO + 150.000 Buch

*Buch—*Lactobacillus buchneri* (UFC/g MN); **CaO—Óxido de cálcio.

sugar to be stored in each experimental silo previously weighed, based on the volume of the silo, in order to obtain the closest possible density of 650 kg/m^3 in the ensiled material. After filling, each experimental silo was closed with a plastic tarp, tied with circular strips of automotive tire air chamber, sealed with duct tape and heavy. The dry matter loss in gas form was calculated using the formula: PG = ((PSI - PSF)/MSI) × 100, wherein gas loss (% DM); weight of the ensilage silo (kg); PSF = weight of the silo after fermentation (kg) and dry matter silage (amount of forage in kg × % DM) After removal of silage, the set silo, sand, fabric and nylon fabric was heavy for quantification of effluent produced. The loss effluent was calculated using the formula: PE = ((PSAF - PSAI)/MNI) × 1000, where: effluent production (kg effluent/ton of fresh forage ensiled), weight of the whole silo, sand, nylon fabric and cotton fabric after opening (kg), weight of the whole silo, sand, nylon fabric and cotton fabric before ensiling (kg) and number of ensiled forage (kg);

The pH was measured with the aid of a digital potentiometer, according to the methodology described by [8]. To determine the ethanol content of the silage, fresh solutions of samples by taking 25 g of silage by adding 225 mL of distilled water if processing in blender for a minute were prepared. The solutions were filtered in a first step with the aid of a household sieve, and then using nylon fabric with a mesh aperture of 50 μm. After the solutions were centrifuged filtered a rotation of 12,000 rpm [9]. After the centrifugation procedure, the supernatant solution was transferred to Eppendorf tubes with volumetric capacity of 2 mL were stored in a freezer.

The ethanol content was determined by gas chromatograph with flame ionization detector capillary column coupled with a Model LM- 100 liquid phase, according to the procedure described by [10].

The organic acids were identified and quantified by HPLC (High Performance Liquid Chromatography) using a chromatograph Shimadzu (LC CLASS 10) with UV detector (SPD- M10A) at a wavelength of 230 nm using column C-18 reverse phase (250 × 4.6 mm) as described by Silva *et al*. 2002. The data on the levels of organic acids and ethanol reviews were expressed based on dry matter. Statistical analysis was carried out initially variance analysis including in the model the effects of the biological additive, chemical additive and interaction of biological and chemical additives, plus the error term. Because it is growing quantitative levels of chemical and biological additives, the effects of both were broken into linear, quadratic and cubic effect. When there was an interaction effect of biological and chemical additive regression equations of additive levels for each level of the other were adjusted. Cubic made were discarded due to lack of biological significance.

3. Results and Discussion

There was no interaction effect (P > 0.05) in the level of buch with the level of CaO for silage pH. The interaction effect was, however, to lactic acid, acetic, propionic, butyric acid, ethanol, gas loss and loss effluent. Thus, we proceeded to the unfolding of interactions, evaluating the effect of additive levels at each level of the other. The results for levels of lactic acid, acetic acid, propionic acid, butyric acid, ethanol, pH, and the loss of gases and effluents, depending on the level of addition of *Lactobacillus buchneri* (buch) for each level of calcium oxide, are shown in **Figures 1** and **2**. The regression equations of fermentation products depending on the microbial additive CaO level in each level are shown in **Table 2**.

There was no regression effect (P > 0.05) on the level of buch content ác. lactic silage to levels of CaO and 1.6% to 0.8% (**Figure 1(a)**). The average content of lactic acid to the level of 1.6% CaO was high, exceeding 4.5%, while for the level of 0.8% CaO was found to average content of ác. lactic 3.24%. At the level of 2.4% CaO, there was a quadratic effect of levels of *Lactobacillus buchneri*, with minimum point for the level of buch of 64,860 cfu / GMN, which correspond to the lactic acid content of 1.84% in the MS silage. In the absence of CaO (CaO level zero), there was a quadratic effect buch for lactic acid (**Figure 1(a)**), with the point of

Table 2. Regression equations of lactic acid (LAT), acetic acid (ACET), propionic (PROP), butyric acid (BUT) and ethanol (ETN), pH, loss gases (PG) loss effluent (PE), according *Lactobacillus buchneri* level (Buch), at each level of calcium oxide in silage.

Variable	%CaO	Equation	Pr > F	R^2	CV(%)
LAT	0	$\hat{y} = 0.9742 + 0.0118x - 0.000031x^2$	<0.0001	0.94	7.38
	0.8	$\hat{y} = 3.2427$ (nef)			
	1.6	$\hat{y} = 4.8553$ (nef)			
	2.4	$\hat{y} = 3.4360 - 0.0493x - 0.00038x^2$	<0.0001	0.96	8.27
ACET	0	$\hat{y} = 0.9188 + 0.0679x - 0.00047x^2$	0.0002	0.85	31.99
	0.8	$\hat{y} = 0.6828 - 0.0313x + 0.00038x^2$	<0.0001	0.90	39.05
	1.6	$\hat{y} = 1.1123 - 0.0064x + 0.000044x^2$	0.0062	0.68	8.66
	2.4	$\hat{y} = 0.3848 + 0.0622x - 0.00038x^2$	0.0527	0.48	69.27
PROP	0	$\hat{y} = 0.4658$ (nef)			
	0.8	$\hat{y} = 0.2190 + 0.0068x - 0.000019x^2$	<0.0001	0.97	8.1
	1.6	$\hat{y} = 0.2587 - 0.0039x$	<0.0001	0.94	8.36
	2.4	$\hat{y} = 0.2828 - 0.0004x$	<0.0001	0.93	2.67
BUT	0	$\hat{y} = 0.0494$ (nef)			
	0.8	$\hat{y} = 0.0691$ (nef)			
	1.6	$\hat{y} = 0.0770 - 0.00089x + 0.000005x^2$	0.0007	0.80	14.48
	2.4	$\hat{y} = 0.2080 - 0.0031x + 0.000013x^2$	<0.0001	0.97	15.87
ETN	0	$\hat{y} = 10.2802 - 0.1259x + 0.00048x^2$	<0.0001	0.96	13.86
	0.8	$\hat{y} = 1.1777 + 0.0131x - 0.00008x^2$	0.0163	0.60	13.50
	1.6	$\hat{y} = 0.5075 + 0.0069x - 0.000051x^2$	0.0167	0.60	22.09
	2.4	$\hat{y} = 0.9782 - 0.0113x + 0.000036x^2$	<0.0001	0.95	20.56
pH	-	$\hat{y} = 2.7175$ (nef)			
PG	0	$\hat{y} = 19.3648$ (nef)			
	0.8	$\hat{y} = 11.0795 - 0.0186x + 0.00017x^2$	0.0161	0.60	5.20
	1.6	$\hat{y} = 9.4511$ (nef)			
	2.4	$\hat{y} = 7.5898 - 0.0173x$	<0.0001	0.86	3.99
PE	0	$\hat{y} = 38.5659$ (nef)			
	0.8	$\hat{y} = 21.4747 + 0.3684x - 0.0020x^2$	<0.0001	0.90	7.67
	1,6	$\hat{y} = 11.5618$ (nef)			
	2,4	$\hat{y} = 9.4133 + 0.0504x - 0.00018x^2$	<0.0001	0,96	2,56

nef—no regression effect.

maximum at 190.323 ufc/gMN buch and corresponding content of 3.21% of the acid, which is an extrapolation to be higher than the highest level tested inoculant.

In the pH range normally found in silages, acetic acid is less dissociated than lactic acid. In the non- ionized form, acetic acid penetrates by passive diffusion in the yeast cell, which can affect both the absorption of phosphate, by chemical interference with the plasma membrane, such as the activity of glycolytic enzymes, or reduce the intracellular pH, after their cleavage, causing increased consumption of ATP by the organism to remove H + ions inside cells. This mechanism leads to energy exhaustion of the yeast cell, preventing it [11]. Thus, the acetic acid may inhibit fermentation promoted by yeast, responsible for high levels of ethanol to silage cane sugar. Furthermore, acetic acid formed from lactic acid, which has been used as an indicator of good quality silage, by lowering the pH to favorable levels of silage preservation reduce waste losses of dry matter and and having no limiting effect on consumption [12]. In the specific case of silage from cane sugar, acetic acid production is desirable, within certain limits, for a limited levels, has the favorable effect of reducing the fermentation, improve the aerobic stability of silage by inhibiting the growth of yeasts and molds [13]. At high concentrations, acetic acid limits the voluntary intake by reducing animal performance [12].

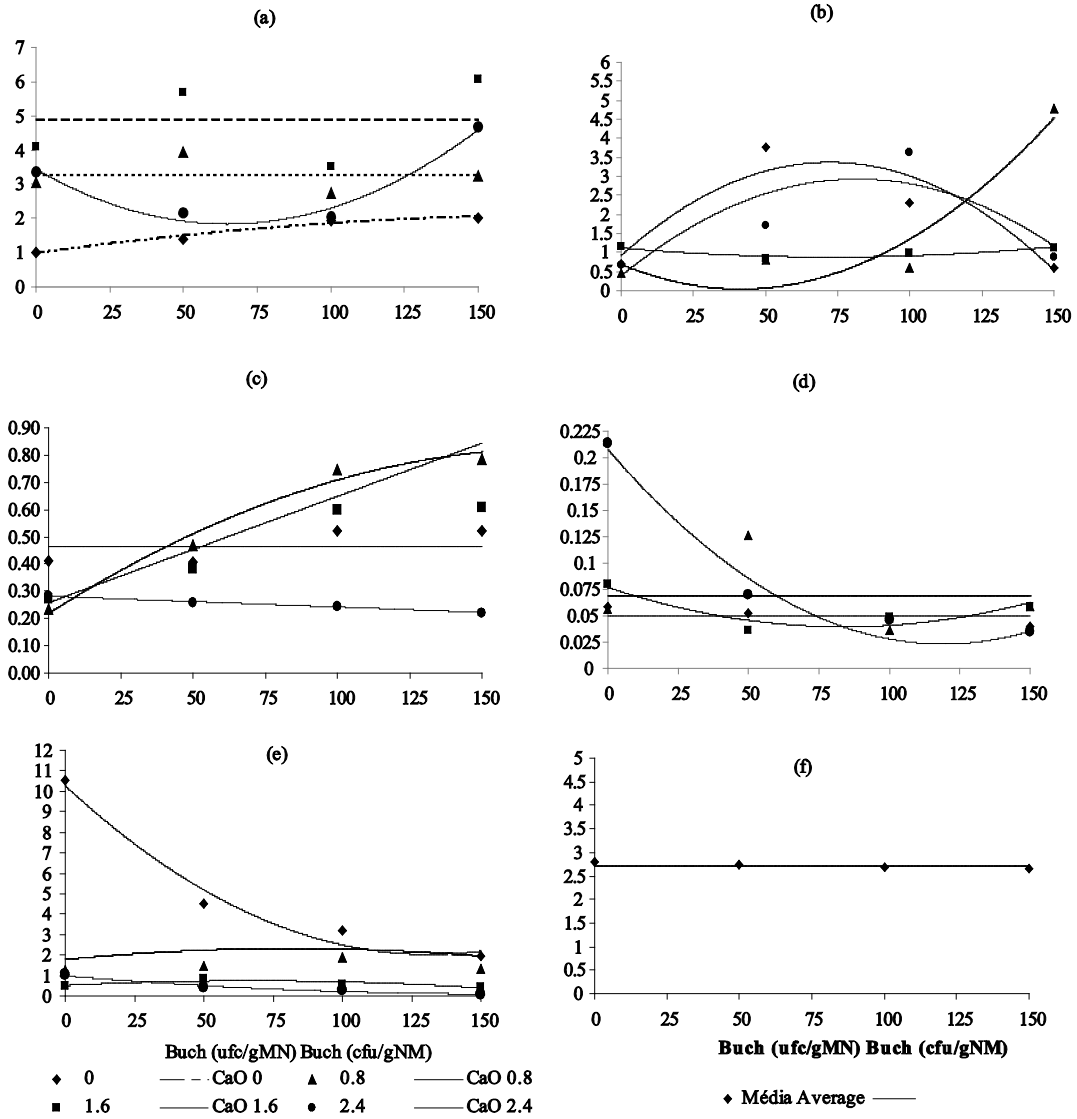

Figure 1. Levels of lactic acid (a), acetic acid (b), propionic (c), butyric acid (d), ethanol (e) and pH (f) of silages of cane sugar depending on the level of addition of *Lactobacillus buchneri* (buch) for each level of calcium oxide.

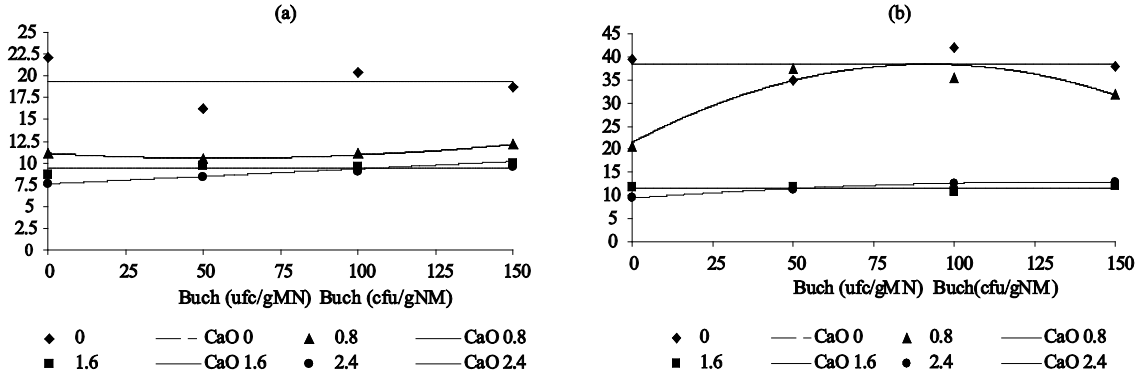

Figure 2. Levels of gaseous loss (a) and loss effluent (b) of silages of cane sugar depending on the level of *Lactobacillus buchneri*, at each level of calcium oxide.

In **Figures 1(a)** and **(b)**, one can see that there is a negative relationship between the levels of lactic and acetic acid. The inclusion of the CaO level of 1.6% gave the highest levels of lactic acid in the silage at the same time, low levels of acetic acid. The zero level of CaO (biological additive only) resulted in lower levels of lactic acid and higher levels of acetic acid. To the level of 2.4% CaO inclusion, there was a quadratic effect of buch with minimum point for lactic acid corresponding to 64,800 cfu / GMN while for acetic acid there was a quadratic effect of buch, with point maximum to 81 840 cfu/GMN. The inclusion of CaO in the level of 2.4% still showed the second lowest levels of lactic acid and the second highest levels of acetic acid, among the four levels of inclusion of CaO. In turn, the level of 0.8% CaO resulted in the second highest levels of lactic acid and the second lowest levels of acetic acid. The negative relationship described above between acetic and lactic acids are concerns, as already mentioned, the fact that the production of acetic acid occurs by metabolism of lactic acid by *Lactobacillus buchneri* [14].

All combinations of doses from biological additives described CaO and quadratic effects for acetic acid. In the absence of CaO, there was an initial increase of acetic acid concentration, increasing the level of buch through a point of maximum at 72.200 cfu/GMN. At the level of 2.4% of calcium oxide there was similar to that observed in the absence of CaO behavior. However, it was found lower acetic acid content at maximum (81.840 ufc/gMN). At the level of 0.8% CaO Quadratic effects of buch, from a point of minimum for 41.180 ufc/gMN, rising to follow and reaching the highest value of ác. Acetic among treatments at level buch of 150.000 ufc/gMN. The level of 1.6% CaO yielded average to near 1% acetic acid, with a tendency to decrease with increasing dose buch values. In the absence of CaO, there was no effect of reducing the level of buch on the level of propionic acid, estimating the average propionic acid levels of 0.46% for all levels of buch. The treatments consisted of the levels of calcium oxide and 1.6% to 0.8% combined with different levels of buch, respectively, there was linear and quadratic effects of level buch (**Table 2**). However, when analyzing the curves described by their equations on the graph (**Figure 1(c)**) are observed successive increases in propionic acid levels with increasing level of buch, with little difference between the two treatments. The level of 2.4% CaO in combination with the levels of buch resulted in slightly downward to the level of propionic acid linear effect ($P < 0.05$), corresponding to this level of CaO lower levels of propionic acid.

The butterfat is undesirable during the fermentation process of ensilage for demonstrating the occurrence of proteolytic fermentation product generating low acceptability to direct and negative effects on animal consumption. Values for butyric acid in silage of cane sugar between 0.04 and 0.1 are adequate and allow us to infer the efficiency of the fermentation process [5]. No regression effect ($P > 0.05$) on the level of buch butyric acid at levels of zero and 0.8% CaO (**Figure 1(d)**) was observed, with an estimated average grades of 0.05% and 0 07% of this acid, respectively. At levels of 1.6 and 2.4% CaO, there was a quadratic effect of buch ($P < 0.05$) in the content of ác. butyric, with minimum points for levels of 89,000 and 119.231 cfu/GMN buch and levels of 0.04 and 0.02% of this acid, respectively. When the CaO was added to the 2.4% level in the absence of buch occurred high production of butyric acid, the level fell dramatically with each increase in Buch, to achieve the lowest levels of all silages, the two higher levels of buch (**Figure 1(d)**). The addition of 1.6% CaO provided a satisfactory values for all butyric acid levels buch.

The ethanol content, direct determinant of losses during the fermentation process of silage cane sugar, is the main obstacle to the production of forage silage with this process, since its synthesis occurs at the expense of soluble carbohydrates (sugars) present the ensiled material. The principal to be achieved in the ensiling of cane sugar, the goal is to minimize the content of this component. Accordingly, it can be seen that the additives tested were effective in controlling the fermentation. When analyzing **Figure 1(e)** it turns out that for the treatment consists of the exclusive silage cane sugar (no added buch and CaO) values of ethanol were above 10% of dry matter and that the dose of inoculant recommended by the manufacturer (50.000 ufc/gMN) when in the absence of CaO, provided the amount of 5% ethanol MS. However, higher levels of buch and association with CaO at different levels, maintained the ethanol content below 2% DM. Thus, it is necessary to highlight the positive synergistic effect of the association between higher doses of additives tested were able to almost cancel the ethanol produced in silage (**Figure 1(e)**).

For variable pH no effect of interaction between additives and effect of reducing the level of buch noted, observing the average pH value of 2.72 in the silages, for four levels of CaO in association with the four levels buch. This average pH enables the preservation of the material inhibiting deleterious microorganisms that compromise the conservation and nutritive value of silage by anaerobic condition. However, the pH in isolation should not be considered as a parameter for evaluating the quality of silage studied, since the yeast microorgan-

ism harmful to the main cane silage, are not inhibited by low pH, surviving on limited pH range 3.5 to 6.5, and some species are able to survive even at pH below 2.0 [11] and can promote fermentation of the silage after aeration, with the opening of the silo. There was an interaction effect of the biological additive (buch) and CaO on gas losses and effluents. Therefore, we studied the effect of levels of a factor at each level of the other.

Losses commonly occurring during silage fermentation contribute to the elevation of the final product cost, silage, representing economic losses for the activity under the quantitative and qualitative points of view. Noteworthy is the loss by gases from the metabolism of mainly soluble carbohydrates in the ensiled mass either aerobically due to the presence of air in the same or anaerobic, due to favorable conditions for the activity of undesirable microorganisms in the process, such as yeasts, fungi, mildews and molds. These losses may reach high levels in the silage of cane sugar. The use of substances that inhibit or minimize gas loss becomes essential to the success of activity, since the cost justifiable.

Gas losses were substantially minimized by the association between CaO additives and buch, checking for linear and quadratic effects of buch for CaO levels of 0.8 and 2.4%, respectively. There was no regression effect ($P > 0.05$) on the level of buch gas losses in the absence of CaO (CaO zero level). In this case, there was an average loss corresponding to 19.34% DM for the various levels of buch gases. This indicates that the addition of buch not reduced losses in silage verified by the absence of CaO gases. There was no regression effect ($P > 0.05$) in the level of buch to CaO level of 1.6%. At this level of CaO, were smaller losses in silage gases, with estimated 8.77% of DM for the four levels of buch average.

The other route is the loss gravimetrically determined by effluent quantified the content of silage leachate slurry mass for the absorbent material commonly used in experimental silos. In the present study, there was a quadratic effect on the loss per buch effluent levels of 0.8 and 2.4% of CaO. For the level of CaO 0.8%, there was a point corresponding to the maximum level 92.100ufc/gMN buch with loss estimated by wastewater 38.44 kg / ton of fresh forage ensiled. The loss at maximum to the level of 0.8% of CaO was similar average loss recorded in silage without adding CaO containing only buch, for which there was no effect of reducing the level of buch ($P < 0.05$). To the level of 2.4% of CaO, there was a quadratic effect with minimum point to losses in effluent level buch of 140.000 ufc/gMN, corresponding to loss of 12.94 kg/ton of fresh forage ensiled. There was no regression effect ($P > 0.05$) in the level of buch on effluent losses to the level of 1.6% CaO, with estimated mean value of 11.56 kg/ton of fresh forage ensiled. There was also the regression effect ($P > 0.05$) on the level of buch losses effluent in the absence of CaO (zero), observing the greatest losses, with an estimated 38.60 kg/ton average of ensiled green matter.

Jointly analyzing the influence of different levels in association buch levels of CaO, it can be inferred that the level of buch 50.000 cfu/GMN recommended by the manufacturer, in combination with 1.6% CaO, was adequate, keeping the alcoholic fermentation, acetic and butyric, and gas losses and effluents at low levels. Furthermore, the level of 1.6% of CaO in association with buch, provided the highest levels of lactic acid, acetic acid, moderate levels of slightly above 1%, moderate levels of propionic acid and maintaining low values of pH. Furthermore, it was as efficient as the level of 2.4% CaO in reducing losses and waste gases.

As noted above, there was no interaction effect of level of CaO with the level of buch for silage pH. The interaction effect occurred, however for lactic, acetic, propionic, butyric acid, ethanol, gas loss and loss effluents. Thus was also evaluated for these variables, the effects of CaO levels within each level buch

The results for concentrations of lactic acid (4a) acetic acid (4b) propionic acid (4c) butyric acid (4d), pH (4e), ethanol (4f), loss of gases (5a) and effluent (5b) depending on the level of CaO, at each level of buch are shown respectively in **Figures 3** and **4**. The regression equations adjusted for different levels of CaO variables within buch level are presented in **Table 3**.

There was a quadratic regression effect ($P < 0.05$) the level of CaO on ác content. lactic at all levels of buch (**Table 3** and **Figure 4**). In the absence of buch (buch zero), there was a point of maximum at 1.66% CaO, corresponding to a yield of 3.99% lactic acid. At the level of 50.000 ufc/gMN buch, we found the second highest level of this acid all silages, from 5.22% at the point corresponding to the maximum level of 1.31% CaO. At the level of 100.000 ufc/gMN buch, there was much point to the level of 1.28% CaO and corresponding production of lactic acid of 3.29%, which was approximately 2 percentage units below the maximum level observed to the level of 50.000 ufc/gMN. At the level of 150.000 ufc/gMN buch higher levels of CaO (from 1.6% to 2.4%) provided the highest values recorded for lactic acid, estimating the level of 5.28% in the raw dried at maximum corresponding to 1.87% of CaO. This value was slightly higher than the 5.22% of that observed for the acid level 50.000 ufc/gMN buch in combination with 1.31% CaO. Thus, the use of 50.000 ufc/g buch MN in

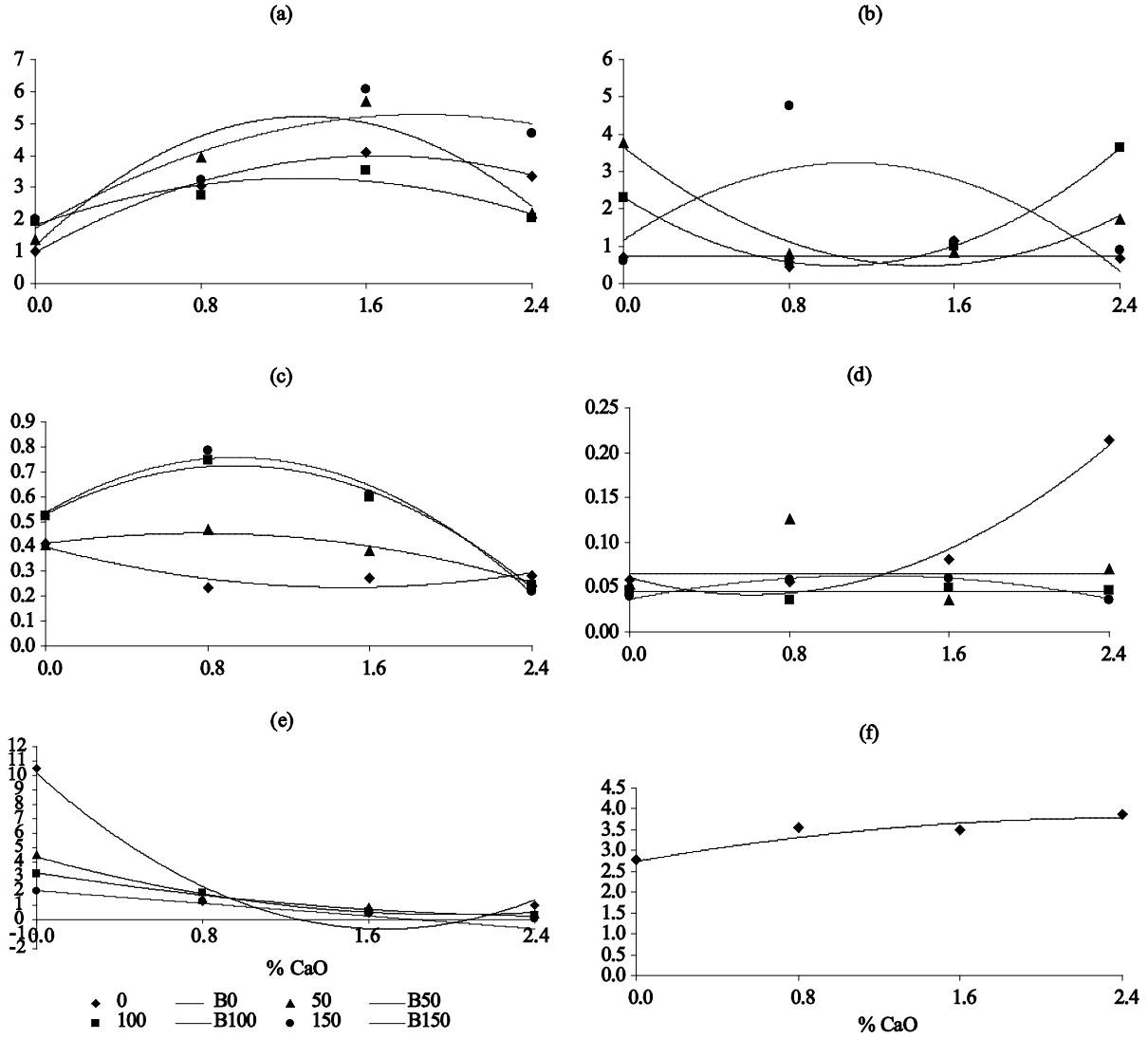

Figure 3. Lactic acid values (a), acetic acid (b) propionate (c) butyric acid (d), ethanol (e) pH and (f) of the silage cane sugar depending on the level of calcium oxide in each level of *Lactobacillus buchneri.*

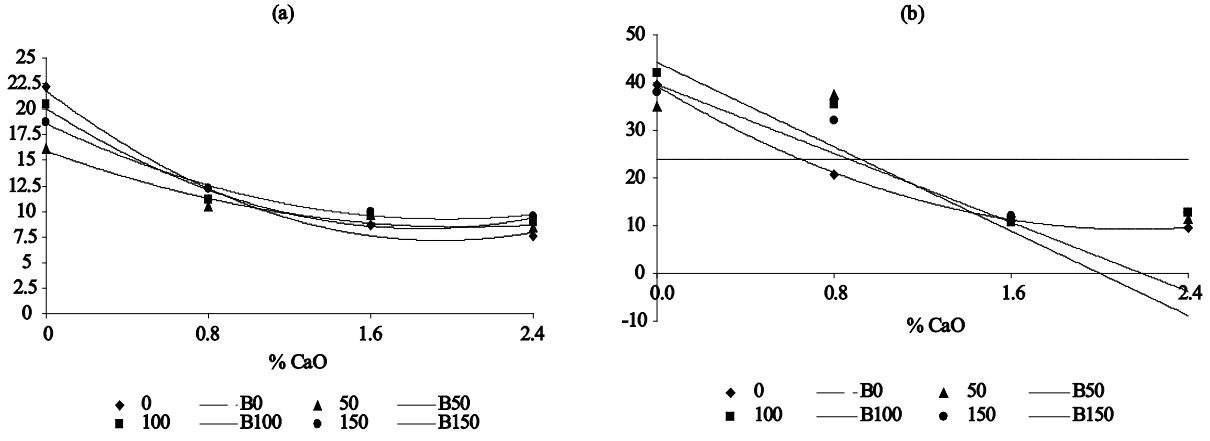

Figure 4. Levels of gaseous loss (a) and effluent (b) of silages of cane sugar depending on the level of calcium oxide, at each level of *Lactobacillus buchneri*.

Table 3. Regression equations for the concentrations of lactic acid (LAT), acetic acid (ACET), propionic (PROP), butyric acid (BUT), ethanol (ETN), loss gases (PG) and effluent (PE) based on the level of adding calcium oxide to each level of *Lactobacillus buchneri*.

Variable	Buch	Equation	Pr > F	R^2	CV%
LAT	0	$\hat{y} = 0.9637 + 3.6483x - 1.0990x^2$	<0.0001	0.99	4.16
	50	$\hat{y} = 1.1713 + 6.1830x - 2.3620x^2$	<0.0001	0.90	17.94
	100	$\hat{y} = 1.8152 + 2.3002x - 0.8971x^2$	0.0006	0.81	12.82
	150	$\hat{y} = 1.7170 + 3.8150x - 1.0208x^2$	0.0006	0.81	19.33
ACET	0	$\hat{y} = 0.7363$ (nef)			
	50	$\hat{y} = 3.6540 - 4.3679x + 1.5026x^2$	0.0097	0.64	56.98
	100	$\hat{y} = 2.3185 - 3.5352x + 1.6992x^2$	<0.0001	0.99	6.63
	150	$\hat{y} = 1.1603 + 3.7712x - 1.7161x^2$	0.0670	0.45	78.86
PROP	0	$\hat{y} = 0.3977 - 0.2175x + 0.0729x^2$	0.0005	0.81	11.07
	50	$\hat{y} = 0.4113 + 0.1100x - 0.0729x^2$	<0.0001	0.94	5.48
	100	$\hat{y} = 0.5287 + 0.4212x - 0.2265x^2$	<0.0001	0.98	4.86
	150	$\hat{y} = 0.5348 + 0.4748x - 0.2539x^2$	<0.0001	0.97	7.45
BUT	0	$\hat{y} = 0.0610 - 0.0633x + 0.0520x^2$	<0.0001	0.97	11.94
	50	$\hat{y} = 0.0713$ (nef)			
	100	$\hat{y} = 0.0447$ (nef)			
	150	$\hat{y} = 0.0367 + 0.0437x - 0.0182x^2$	<0.0001	0.92	7.96
ETN	0	$\hat{y} = 10.1575 - 12.8760x + 3.8398x^2$	<0.0001	0.96	28.16
	50	$\hat{y} = 4.3632 - 4.0023x + 0.9987x^2$	<0.0001	0.96	19.33
	100	$\hat{y} = 3.2603 - 2.2704x + 0.4245x^2$	<0.0001	0.96	18.23
	150	$\hat{y} = 2.0108 - 1.0969x$	<0.0001	0.94	22.84
pH	-	$\hat{y} = 2.7373 + 0.8622x - 0.1780x^2$	<0.0001	0.83	5.66
PG	0	$\hat{y} = 21.7957 - 15.0762x + 3.8776x^2$	<0.0001	0.97	9.19
	50	$\hat{y} = 15.9108 - 7.2052x + 1.7461x^2$	<0.0001	0.94	7.28
	100	$\hat{y} = 20.0832 - 12.7148x + 3.4362x^2$	<0.0001	0.96	8.03
	150	$\hat{y} = 18.5903 - 9.4787x + 2.4036x^2$	<0.0001	0.97	5.5
PE	0	$\hat{y} = 39.2655 - 27.8702x + 6.4648x^2$	<0.0001	0.99	2.88
	50	$\hat{y} = 23.9128$ (nef)			
	100	$\hat{y} = 44.2378 - 22.1173x$	0.0001	0.86	22.97
	150	$\hat{y} = 39.5970 - 18.0767x$	<0.0001	0.88	19.24

nef = there was no effect of nef regression.

association to 1.6% CaO, enable the production of similar levels 150.000 ufc/gMN lactic acid and 1.8% of CaO economy 0.56% CaO and 100.000 ufc/gMN buch.

There was no regression effect ($P > 0.05$) in the level of CaO in the absence of buch (level zero buch) about the content of ác. acetic, whose average value was approximately 0.74%. However, for other levels of buch there was a quadratic regression effect ($P < 0.05$) the level of CaO on the content of this acid. At the level of 50.000 ufc/gMN buch there was a quadratic effect ($P < 0.05$) the level of CaO with minimum point to the level of 1.45% CaO and acetic acid content estimated 0.48%. Also to the level of 100.000 ufc/gMN buch was found at the point of minimum content of 0.48% ác. acetic level of 1.04% of CaO. The inclusion of 150.000 ufc/gMN buch associated with CaO resulted in higher levels of ác. acetic point with maximum at 1.1% CaO and 3.23% acetic acid content, which demonstrates the ability of biological inoculant to promote the synthesis of this acid. From a nutritional point of view, however, high levels of acetic acid are undesirable for depressing the consumption of silage.

There was a quadratic regression effect (P < 0.05) the level of CaO association at all levels buch about the content ác. propionic silages. In the absence of CaO (buch zero level) was found at the point of minimum 0.23% of said acid corresponding to the addition of 1.49% of CaO. By associating the levels of CaO with the level of 50.000 ufc/gMN buch, gave maximum point to 0.75% CaO and 0.45% corresponding content of ác. propionic. At the level of 100.000 ufc/gMN buch, hit up the point with the maximum value of 0.72% of the acid level of 0.93% CaO. The highest content of ác. propionic acid was observed in the level of 150.000 ufc/gMN buch, at maximum, corresponding to the level of 0.93% CaO and estimated production of acid of 0.76%. This value, however it was very close to the value of 0.72% estimated for the same level of alkali -level 100.000 ufc/gMN buch.

For the content ác. butyric there was a quadratic regression effect (P < 0.05) the level of CaO in the absence of buch (level zero buch) with minimum point to 0.61% CaO and ships acid content of 0.04%. Moreover, there was no effect regression (P > 0.05) in the level of CaO associated with levels of 50,000 and 100.000 ufc/gMN buch on the content of acid. However the mean levels of butyric acid levels in these buch remained within acceptable limits, with averages of 0.07 and 0.04%, respectively. At the level of 150.000ufc/gMN buch observed quadratic regression effect (P < 0.05) the level of CaO on ác content. butyric acid with maximum point of 1.2% CaO and 0.06% of said acid.

There was a quadratic regression effect (P < 0.05) the level of CaO at zero, and 50.000 100.000ufc/gMN buch on the ethanol content of the silage levels. In the absence of buch (buch zero level) was estimated minimum point level of 1.68% CaO, corresponding to the null ethanol. It was found, however, that the dog was able to control the alcoholic fermentation in the absence of buch when in the next level of 1.6%, and the ethanol content decreased about 10% in the absence of CaO to zero. In 50.000 ufc/gMN buch level was found at the point of minimum content of 0.35% ethanol, corresponding to the level of 2.0% CaO. For the level of 100,000 cfu/gmn buch was estimated minimum point level of 2.67% of CaO and alcohol content of 0.22%. This would be a point of minimum extrapolation since the higher CaO level tested in this study was 2.4%. To the level of 150.000 ufc/gMN buch there was a decreasing linear effect (P < 0.05) the level of CaO on ethanol content.

When analyzing **Figure 3(e)**, although all tested combinations have reduced the ethanol content of the silage, it appears the buch pronounced effect in controlling the fermentation taking as basis the intercept buch of each level (level zero CaO), which was gradually reduced due to the increased level of biological inoculant, which is related to the values of acetic acid produced, estimated at 0.60, 1.30, 1.34 and 2.39%, respectively, to zero, 50.000, 100.000 and 150.000 ufc/gMN buch levels, in association with CaO.

There was no interaction between the levels of CaO and buch and quadratic regression effect (P < 0.05) in the level of CaO on the pH of the silage, reaching the maximum point at the estimated level of 2.42% of CaO, corresponding pH of 3.78. However, this level is an extrapolation of CaO, since it is higher than the highest level tested in this study alkali (2.4%), which provides the same pH (3.78). Therefore, it can be inferred that the pH observed for the various levels of CaO remained within suitable for silage conservation under anaerobic conditions limits.

To loss of gases, there was a quadratic regression effect (P < 0.05) in the level of CaO, at all levels of buch (**Table 3** and **Figure 4**). In the absence of buch (zero buch level) there was gas at the point of minimum loss of 7.14% DM, corresponding to the level of 1.94% CaO. For inclusion level of 50.000ufc/gMN buch observed minimum point to the level of 2.06% CaO corresponding to the loss of 8.48% DM. The estimated loss of gases at the point of minimum when the inclusion level 100.000ufc/gMN, was 8.32% value, the level of 1.85% of CaO. Furthermore, in the gas loss in the level of 150.000ufc/gMN buch were checked for point of minimum 1.97% of CaO, corresponding to loss of 9.24% of DM. One can therefore conclude that the level of buch had little influence on gas losses and minimum points for all levels of buch, minor gas losses occurred to varying levels of CaO in a narrow range between 1 85 and 2.06%.

In the absence of buch (zero buch level) there was a quadratic regression effect (P < 0.05) the level of CaO on the loss effluent (**Table 3**), with estimated loss at the point of minimum of 9.22 kg/ton of ensiled green matter, corresponding to the level of 2.15% CaO. For the level of buch 50.000 ufc/gMN there was no effect of reducing the level of CaO (P > 0.05) on the loss effluent is estimated the average level of loss of 23.91 kg/ton of fresh forage ensiled. However, this result is unexpected when confronted with the response of the other levels of CaO buch and does not allow a logical explanation. For levels of 100,000 and 150.000 ufc/gMN buch there was a decreasing linear regression effect (P < 0.05) the level of CaO on effluent losses. Except for the inclusion level of buch of 50.000 ufc/gMN, raising the level of CaO always brought reduce effluent losses, which in part can be

attached to hygroscopic effect of alkali.

The joint analysis of the effect of increasing levels of buch in association levels of CaO can be inferred that the level of 50.000 ufc/gMN associated with the level of 1.6% CaO brought favorable results, since ensured high acid content lactic, moderate levels of acetic and propionic acid and butyric inhibited and fermentation. In addition, the pH level of 1.6% CaO was 3.66, which is consistent with the high content of lactic acid checked, that was the second highest among treatments. Acrescente up that provided the content lowest average of butyric acid, ethanol levels, less than 1%, and was as efficient as the level of 2.4% CaO in reducing losses and waste gases.

4. Conclusion

The association of the level of 1.6% of calcium oxide level with 50.000 cfu/gmn Lactobacillus buchneri, such as silage additives for sugar may be recommended to provide high levels of lactic acid, moderate levels of acetic acid and propionic, control butterfat alcoholic fermentations, minimize gaseous effluents and losses, and generate favorable pH value of the conservation of silage.

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