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Association of Biological and Chemical Additives on Nutrient Composition, Total Losses, Microbiological and Fermentative Profile of Sugarcane Silage

Research Article

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ABSTRACT

The aim of this study was to determine the effects of a bacterial inoculant and chemical additives on nutrient composition, *in vitro* degradation, total loss, aerobic stability, microbiological quality, and fermentative profile of sugarcane silage. Treatments were distributed to forty-eight mini-silos in a 2 × 4 factorial arrangement: two levels of microbial inoculant (INO, 0 or 4 g/t of fresh sugarcane and three chemical additives (CHE, CaO, NaCl and urea at 10 g/kg on as-is basis). The association of INO and urea had the highest values of dry matter (DM) and neutral detergent fiber *in vitro* degradation. Treatments with inoculant and chemical additives reduced the gas loss (g/kg as-is basis). The association of INO and CHE increased the amounts of lactic acid bacteria. The association of INO and CHE resulted in a synergetic effect to decrease ethanol production and to increase lactic acid production. Inoculant and CHE showed a positive synergetic effect on total losses, dry matter recovery, microbiological profile, and production of ethanol and lactic acid. Treatments containing urea had greater DM *in vitro* degradation and aerobic stability compared with the other chemical additives. The association of INO and CHE positively affected the chemical composition, *in vitro* degradation, total losses, aerobic stability, microbiological and fermentative profile of sugarcane silage.

KEY WORDS aerobic stability, alcoholic fermentation, ethanol, *in vitro* degradation, lactic bacteria, sugarcane (*Saccharum officinarum*).

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is an alternative feed source for cattle in tropical area, since the harvesting phase matches with the drought season which is a period of shortage of feed, and sugarcane crop may produce up to 40 t of DM per hectare (Ávila *et al*. 2009). However, feeding fresh sugarcane to cattle requires daily manpower and it chemical composition may alter from the beginning to the end of harvesting. Despite sugarcane has a high water-soluble sugar concentration and a low buffering capacity, its fermentation produces high amounts of ethanol which increases the DM losses and decreases the DM digestibility (Kung Jr and Stanley, 1982; Santos *et al*. 2009).

In order to alter the sugarcane fermentation and inhibit the proliferation of non-desired microorganisms, such yeasts, chemical additives and bacterial inoculants have been used.

Cai *et al.* (1997) evaluated the effects of salt and salt-tolerant bacteria (*Lactobacillus plantarum*) on sorghum silage and reported an increase of the lactic acid bacteria amounts and a decrease of pH with both additives. Santos *et al.* (2009) reported that CaO reduced the ethanol content and the DM losses of sugarcane silage possibly due to the reduction of water activity and inhibition of yeast growth. In addition, alkalis (such as CaO) may cause hydrolysis of bonds between lignin and plant cell wall components, increasing fiber degradation (Van Soest, 1994). The urea during the fermentation process is hydrolyzed into ammonia which inhibits the yeast and mold proliferation, reducing the ethanol production and DM losses in sugarcane silage (Alli *et al.* 1983). The inclusion of chemical additives in sugarcane silage has the function of providing anaerobic environment more favorable to the conservation of sugarcane, mainly by the reduction of counts of fungi and yeasts, *clostridium* and *enterobacteria* (Gandra *et al.* 2017). On the other hand, homofermentative bacteria (i.e. *Lactobacillus plantarum*) are added to rapidly increase lactic acid, decreasing the pH of sugarcane silage (Filya *et al.* 2004).

However, lactic acid can be oxidized by yeasts during the aerobic exposure of silage (Pahlow *et al.* 2003). In order to diminish the problem of aerobic deterioration of silage, other types of inoculants have been used. Propionic acid bacteria (i.e. *Propionibacterium acidipropionici*) can ferment sugars and lactate to acetate and propionate, which have antimycotic properties (Moon, 1983).

The objective of the current study was to evaluate the effects of both microbial inoculant and chemical additives on nutrient composition, *in vitro* degradation of DM, microbiology and fermentative profile of sugarcane silage. Our hypothesis was that either inoculant or chemical additives would improve the aerobic stability and fermentative profile of sugarcane silage, and they would show a positive synergetic effect.

MATERIALS AND METHODS

This study was carried out between January and September at the Department of Animal Science, Federal University of Grande Dourados, Dourados, Brazil (22 ˚14´S latitude, 54 ˚49´W longitude and 450 m altitude).

Ensiling and treatments

The sugarcane variety, harvesting, cut length, sampling and ensiling procedures are described in Gandra *et al.* (2017).

Forty-eight mini-silos were used in a 2 × 4 factorial arrangement, composed by two levels of microbial inoculant (INO, 0 or 4 g/t of fresh sugarcane; INO was composed by *Lactobacillus plantarum* at 3.0×10^{10} cfu/g and *Propionibacterium acidipropionici* at 3.0×10^{10} cfu/g), three chemical additives (CHE, CaO, NaCl and urea at 10 g/kg on as-is basis), and a control treatment without additives. The doses of CHE additives used were established, based mainly on the dose of urea widely used in the literature that can improve the nutritional value of sugar cane without harm to animal health. The commercial inoculant used was Kera SIL cana (Kera Nutrição Animal, Bento Gonçalves, Brazil) diluted in water (2 g/L) and sprayed onto the forage. Chemical additives were top dressed and mixed into the fresh forage. The same amount of water was added to all mini-silos. Treatments were provided separately to each mini-silo to provide true replicates.

Chemical and *in vitro* degradation

Chopped fresh sugarcane was collected before the ensiling and stored at -20 ˚C. Dry matter (method #950.15), organic matter (DM-ash), and ash (method #942.05) were determined according to AOAC (2002). Crude protein (CP) was calculated as Kjeldal N × 6.25. Neutral detergent fiber (aNDF), acid detergent fiber (aADF) and lignin (sulphuric acid method) were determined according to Van Soest *et al.* (1991). The non-fiber carbohydrate (NFC) content was estimated according to Hall (2000): NFC= 100 – ash – ether extract – NDF – (CP–CP from urea+urea content), in which all terms are expressed in % DM. The chemical composition of fresh sugarcane before the ensiling is reported in Table 1. Mini-silos were opened after 60 d and samples (n=5, 0.2 kg) from different sites of each mini-silo were collected to form a composite sample and DM, CP, aNDF, aADF, lignin and ash were determined, as previously described. The *in vitro* degradation of DM and NDF was performed according to Tilley and Terry (1963).

Total losses and aerobic stability

Mini-silos were weighed on days 15, 30, 45 and 60 of ensiling. On day 60, mini-silos were opened to determine gas losses. The silage, silo assembly, sand layer and nylon screen were weighed to quantify the effluent production. Gas losses were calculated according to the equation:

$$GL= (SWE–WSO) / (DME\times100)$$

Where:
GL: gas losses (% DM).
SWE: silo weight prior to the ensiling (kg).
WSO: silo weight after the silos opening (kg).

DME: dry matter ensiled (kg of forage×% DM).

Effluent production was calculated as follows:

EP= (WSAO–WSAE) / (DME×100)

Where:
EP: effluent production (kg of effluent/t of as-is basis ensiled).
WSAO: weight of the silo assembly after the silos opening (kg).
WSAE: silo weight before the ensiling.
DME: DM ensiled (kg of forage×% DM).

Dry matter recovery (DMR) was calculated as:

DMR= (FDM/IDM) × 100

Where:
FDM: dry matter after the mini-silos opening (kg) and IDM: dry matter before the ensiling.

Changes of DM content were calculated as the difference in module of DM percentage at the ensiling moment and the DM percentage at the mini-silos opening. Silo temperatures were determined by an infrared digital thermometer every 8 h during 7 d after the silos opening. The aerobic stability was defined as the period (h) in which silage remained stable before rising more than 1 ˚C above the room temperature (Driehuis *et al*. 2001).

Microbiological profile

Five samples (0.2 kg) were collected on day 60 from different sites of all silos and homogenized to form a composite sample. Subsamples of 10 g of each treatment were diluted in 90 mL of sterilized sodium chloride solution (0.9%) and a serial dilution was performed from 10^{-1} until 10^{-6} in test tubes. The microorganism counting was performed in triplicate from each dilution using culture medium of agar De Man, Rogosa and Sharpe (MRS) to lactic-acid bacteria, nutrient agar to aerobic and anaerobic bacteria (48 h of incubation at 37 ˚C) and agar PDA (potato dextrose agar, 120 h of incubation at 26 ˚C) for mold and yeast.

Fermentation profile

After the opening of mini-silos (on day 60), one sample (500 g) of each bucket was collected to extraction of juice by a hydraulic press. Silage juice aliquots (50 mL) were used to determine the pH with a digital potentiometer. Aliquots of 2 mL of silage juice were transferred to test tubes containing 1 mL of sulfuric acid (1 N) and stored at -20 ˚C. Ammonia nitrogen analysis was performed by colorimetric method described by Kulasek (1972) and adapted by Foldager (1977). The analyses of short-chain fatty acids, ethanol and acid lactic concentration were carried out at the Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science – University of São Paulo, Pirassununga, Brazil, according to the methods described by Rodrigues *et al.* (2012). Briefly, aliquots (1 mL) of silage juice were mixed with formic acid (0.2 mL) in amber glass bottles and stored at -20 ˚C until analysis. Short-chain fatty acids and ethanol concentrations were determined by a gas chromatograph (Focus GC, Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with an automatic sample injector (model AS-3000, Thermo Electron Corporation®, MA, USA), a glass packed column (2.0 m×1/5", 80/120 Carbopack® B-DA/4% Carbowax® 20M phase) and a flame ionization detector set at 270 ˚C. The chromatograph oven and injector temperatures were set to 190 ˚C and 220 ˚C, respectively.

Hydrogen was used as the carrier gas flowing 30 mL/min. The acid lactic concentration was measured by high performance liquid chromatography (LC-10ADVP Shimadzu HPLC system, Shimadzu Inc., Kyoto, Japan) according to Ding *et al*. (1995).

Statistical analysis

Data were submitted to analysis of variance using the PROC MIXED (SAS, 2004) verifying the normality of residuals and homogeneity of variances using PROC UNIVARIATE, according to the following model:

$$Y_{ij} = \mu + I_i + C_j + I_i \times C_j + e_{ij}$$

Where:
Y_{ij}= dependent variable.
μ: overall mean.
I_i: fixed effect of inoculant.
C_j: fixed effect of chemical additive.
$I_i \times C_j$: inoculant by chemical additive interaction effect.
e_{ij}: residual error.

The degrees of freedom were calculated by DDFM= kr. Significance level was set at ≤ 0.05. For the evaluation of possible interactions between microbial inoculant and chemical additives, multiple comparisons of the means were performed through the PDIFF command of the SAS, in order to show the effects of the interactions

RESULTS AND DISCUSSION

An interaction effect (P≤0.029) of INO by CHE was observed for DM. organic matter, NFC and ash content of sugarcane silage (Table 2).

Table 1 Chemical composition of sugarcane before ensiling (g/kg DM, otherwise stated)

Dry matter (g/kg)	282
Organic matter	948
Neutral detergent fiber	532
Acid detergent fiber	288
Lignin (sa)[1]	62.7
Ash	36.2
Crude protein	25.2
Brix[2]	17.8

[1] Lignin (sulphuric acid method)= lignin determined by the sulfuric acid method, according to Van Soest *et al*. (1991).
[2] Brix= is a numerical refractive index scale (how much light deviates from the deviation caused by distilled water) from a solution commonly used to indirectly determine the amount of soluble carbohydrates in a sucrose solute.

Table 2 Effects of both inoculant and chemical additives on chemical composition and *in vitro* neutral detergent fiber degradation of sugarcane silage (g/kg DM, otherwise stated)

Item	CON[1]				INO[2]				SEM	P-value		
	-	NaCl	CaO	Urea	-	NaCl	CaO	Urea		INO	CHE	INO × CHEM
Dry matter (g/kg)	218^{b}	248^{b}	254^{b}	267^{a}	275^{a}	271^{a}	271^{a}	283^{a}	15.6	0.348	0.456	0.012
Organic matter	946^{a}	927^{c}	910^{c}	936^{b}	955^{a}	934^{b}	927^{b}	936^{b}	26.7	0.457	0.045	0.029
Neutral detergent fiber	636	642	656	620	623	637	646	662	30.9	0.987	0.545	0.654
Acid detergent fiber	342	345	340	338	352	375	346	342	17.8	0.312	0.129	0.665
Non-fiber carbohydrate[3]	273^{a}	247^{b}	223^{b}	204^{c}	291^{a}	258^{b}	239^{b}	202^{c}	25.6	0.234	0.458	0.023
Lignin	70.2	67.8	64.5	65.5	63.4	64.5	63.7	62.4	7.8	0.431	0.432	0.786
Ash	54.4^{c}	73.3^{a}	88.6^{a}	64.4^{b}	45.3^{c}	65.5^{a}	73.3^{a}	64.4^{a}	2.1	0.002	0.017	0.022
Crude protein	34.5^{b}	35.6^{b}	30.9^{b}	110^{a}	38.9^{b}	37.8^{b}	39.8^{b}	120^{a}	1.67	0.035	0.005	0.002
***In vitro* degradation**												
Dry matter	606^{c}	589^{c}	592^{c}	622^{b}	637^{b}	649^{a}	632^{a}	662^{a}	47.8	0.043	0.033	0.006
Neutral detergent fiber	623^{c}	635^{c}	629^{c}	669^{b}	653^{b}	665^{a}	659^{ab}	689^{a}	46.7	0.050	0.041	0.038

[1] CON: sugarcane silage treated or not (-) with chemical additives (addition of 10 g/kg fresh sugarcane).
[2] INO: sugarcane silage with microbial inoculant treated or not (-) with chemical additives (addition of 10 g/kg fresh sugarcane). Inoculant contained *Lactabacillus plantarum* 3.0 × 10^{10} cfu/g and *Propionibacterium acidipropionici* 3.0 × 10^{10} cfu/g and was added at 4g/t fresh forage.
[3] According to Hall (2000).
INO: probabilities for effects of inoculant; CHE: chemical additive and INO × CHEM: inoculant by chemical additive interaction.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The lowest value of DM content in sugarcane silage was observed in mini-silos with no inoculant or CHE. The highest values of organic matter were observed in those silos without CHE. Interesting, the highest values of NFC were found in treatments without chemical additives, and intermediate values were observed for treatments containing NaCl, and lowest values of NFC detected to treatments containing urea.

As expected, treatments containing urea showed the highest CP content. In addition, the association of INO and CHE positively affected (P≤0.050) the *in vitro* degradation of DM and NDF in which the highest values of DM and NDF degradation were found for those silos treated with INO and urea.

Either INO or CHE decreased (P≤0.043) the gas losses (% DM) and effluent losses (kg/t as-is basis; Table 3). The association of INO and CHE decreased (P≤0.021) the gas losses (% as-is basis) and total losses (% DM), and increased (P≤0.050) the DMR and aerobic stability of sugarcane silage. Inoculants provided with CHE increased (P≤0.011) the amounts of lactic acid bacteria, anaerobic bacteria and total bacteria in sugarcane silage (Table 4). In addition, the INO association with CHE decreased (P=0.004) the fungi proliferation in the silage.

Treatments did not affect pH of silage juice but increased (P=0.036) it butyrate concentration (Table 5). An interaction effect was observed on ammonia nitrogen (P=0.003), acetate (P=0.012), ethanol (P=0.022) and lactic acid (P=0.021) concentration in silage juice. The highest ammonia concentration values were observed in those silages treated with urea, and intermediate values for other treatments, except for CON without CHE which had the lowest value of ammonia nitrogen. The highest values of acetate concentration were observed when silages were treated both by INO and CHE. The lowest values of ethanol concentration in silage were also observed when they were treated by both INO and CHE. Finally, we observed the highest values of lactic acid bacteria in silages treated by both INO and CHE, intermediate values for those silage treated only with CHE and lowest values for silages without additives.

The association of INO and CHE increased the DM content, but CHE decreased the organic matter content of sugarcane silage. The decreased organic matter content is related to the high ash content of some CHE (NaCl and CaO). Chemical additives seem to decrease NFC content of silages, but this result is related to the high ash (NaCl and CaO) and CP (urea) values, since NFC is a estimation and depend on these values.

Table 3 Effects of both inoculant and chemical additives on total losses and aerobic stability of sugarcane silage

Item	CON[1]				INO[2]				SEM	P-value		
	-	NaCl	CaO	Urea	-	NaCl	CaO	Urea		INO	CHE	INO × CHE
Gas losses (g/kg as-is basis)	29.9	27.6	27.2	22.4	16.9	16.6	16.4	17.8	0.30	0.006	0.004	0.105
Gas losses (g/kg DM)	264	207	201	219	224	193	196	182	1.2	0.003	0.012	0.405
Effluent losses (kg/t as-is basis)	38.9	29.9	30.5	30.9	25.6	23.5	22.8	27.9	0.16	0.023	0.043	0.567
Effluent losses (g/kg DM)	31.8	24.3	23.0	27.8	27.8	21.3	21.2	21.9	0.40	0.653	0.133	0.876
Total losses (g/kg DM)	287	225	239	247	251	214	217	204	1.80	0.009	0.016	0.145
Dry matter recovery (g/kg DM)	736	799	793	761	773	795	783	781	25.6	0.003	0.005	0.766
Stability (˚C)	31.9	31.2	31.1	30.5	34.7	33.6	32.2	31.7	3.78	0.003	0.456	0.587
Stability (h)	32.0	43.2	41.6	49.6	52.0	48.7	49.6	51.7	4.09	0.044	0.045	0.765

[1] CON: sugarcane silage treated or not (-) with chemical additives (addition of 10 g/kg fresh sugarcane).
[2] INO: sugarcane silage with microbial inoculant treated or not (-) with chemical additives (addition of 10 g/kg fresh sugarcane). Inoculant contained *Lactabacillus plantarum* 3.0 × 10^{10} cfu/g and *Propionibacterium acidipropionici* 3.0 × 10^{10} cfu/g and was added at 4g/t fresh forage.
INO: probabilities for effects of inoculant; CHE: chemical additive and INO × CHEM: inoculant by chemical additive interaction.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of both inoculant and chemical additives on microbiological profile of sugarcane silage (log_{10} cfu/g fresh silage, otherwise stated)

Item	CON[1]				INO[2]				SEM	P-value		
	-	NaCl	CaO	Urea	-	NaCl	CaO	Urea		INO	CHE	INO × CHE
Lactic bactéria	4.29^{c}	4.40^{c}	4.38^{c}	4.19^{c}	5.99^{ab}	5.40^{b}	5.38^{b}	6.19^{a}	1.43	0.001	0.656	0.002
Aerobic bactéria	5.38	5.56	5.30	5.53	5.38	4.56	4.30	4.53	1.78	0.439	0.654	0.431
Anaerobic bactéria	4.25^{b}	4.92^{b}	4.97^{b}	5.12^{ab}	5.25^{a}	5.92^{a}	5.87^{a}	5.98^{a}	0.79	0.002	0.688	0.011
Total bactéria	5.74^{bc}	5.08^{c}	5.50^{c}	5.97^{b}	6.43^{ab}	6.27^{b}	6.37^{b}	6.69^{a}	1.56	0.002	0.032	0.003
Fungi (log_{10}/g fresh silage)	6.75^{a}	5.09^{c}	5.70^{b}	5.02^{c}	4.75^{cd}	4.39^{d}	4.72^{cd}	4.02^{e}	0.67	0.001	0.534	0.004

[1] CON: sugarcane silage treated or not (-) with chemical additives (addition of 10 g/kg fresh sugarcane).
[2] INO: sugarcane silage with microbial inoculant treated or not (-) with chemical additives (addition of 10 g/kg fresh sugarcane). Inoculant contained *Lactabacillus plantarum* 3.0 × 10^{10} cfu/g and *Propionibacterium acidipropionici* 3.0 × 10^{10} cfu/g and was added at 4g/t fresh forage.
INO: probabilities for effects of inoculant; CHE: chemical additive and INO × CHEM: inoculant by chemical additive interaction.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of both inoculant and chemical additives on fermentative profile of sugarcane silage

Item	CON[1]				INO[2]				SEM	P-value		
	-	NaCl	CaO	Urea	-	NaCl	CaO	Urea		INO	CHE	INO × CHE
pH	4.22	3.33	4.34	3.32	3.22	3.37	4.04	3.28	0.01	0.298	0.453	0.653
Amoniacal nitrogen (mg/dL)	3.47	4.68	3.15	18.13	5.47^{c}	5.68	7.15	19.78	0.03	0.012	0.010	0.453
Acetate (g/kg DM)	5.68^{c}	5.12^{c}	6.87^{c}	8.15^{b}	9.87^{ab}	11.12^{a}	12.87^{a}	12.13^{a}	0.08	0.002	0.453	0.012
Propionate (g/kg DM)	0.12	0.09	0.31	0.12	0.52	0.59	0.61	0.52	0.05	0.321	0.653	0.976
Butyrate (g/kg DM)	0.42	0.75	0.31	2.52	1.76	2.75	2.31	1.79	0.04	0.036	0.328	0.776
Ethanol (g/kg DM)	54.1^{ab}	43.9^{b}	51.1^{ab}	61.4^{a}	34.1^{c}	23.9^{d}	21.1^{d}	11.4^{e}	0.23	0.001	0.539	0.022
Lactic acid (g/kg DM)	13.4^{d}	20.3^{c}	26.5^{c}	37.8^{b}	53.4^{a}	50.3^{a}	56.5^{a}	57.8^{a}	0.67	0.018	0.050	0.021

[1] CON: sugarcane silage treated or not (-) with chemical additives (addition of 10 g/kg fresh sugarcane).
[2] INO: sugarcane silage with microbial inoculant treated or not (-) with chemical additives (addition of 10 g/kg fresh sugarcane). Inoculant contained *Lactabacillus plantarum* 3.0 × 10^{10} cfu/g and *Propionibacterium acidipropionici* 3.0 × 10^{10} cfu/g and was added at 4g/t fresh forage.
INO: probabilities for effects of inoculant; CHE: chemical additive and INO × CHEM: inoculant by chemical additive interaction.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The CP content of sugarcane was increased by treatments containing urea, since it has approximately 282% of CP (NRC, 2001). In addition, forages treated with ammonia (raised from urea hydrolysis) have shown increased insoluble N and true protein due to ammonia reduces proteolysis (Buchanan-Smith, 1982). The inclusion of urea increased the *in vitro* digestibility of DM and NDF due to the higher availability of non-protein nitrogen available to ruminal microorganisms, which improved the symbiotic conditions between ruminal environment and the substrate in question. The *in vitro* degradation of DM and NDF were improved by the association of urea and microbial inoculants, but the reasons are unclear.

A possibly reason is related to the lower gas losses (% as-is basis) and effluent losses (kg/as-is basis) of the association treatment compared to the others, since the gas and effluent losses from the alcoholic fermentation may lead to an accumulation of cell wall components and a reduction of *in vitro* degradation (Santos *et al.* 2013). Despite we did not observe effects on fibrous components, the treatment association (chemical additives and inoculant) increased the CP content of sugarcane silage, increasing it *in vitro* degradation. Agreeing with our results, Pedroso *et al.* (2008) found a decrease in yeasts and higher digestibility of silage treated with urea. In addition, urea and CaO are alkaline substances with hydrolytic action on the cell wall components, which

reduces the consumption of non-fiber carbohydrates during the fermentation, increasing the digestibility of sugarcane silage (Balieiro Neto *et al*. 2007). Furthermore, the addition of *L. plantarum* and *P. acidipropionici* in corn silage has increased the DM recovery in silage, but did not increase the DM and NDF *in vivo* digestibility in sheep.

The association of CHE and INO decreased the gas losses (% as-is basis) and total losses because they diminished the development of molds and yeast, which produce CO_2 and jeopardize the wall cell integrity resulting in release of cellular content and increase of gas and effluent losses, respectively (Filya *et al*. 2004). Chemical additives, such as urea, NaCl and CaO, have some properties that keep the holding water capacity on vegetal cell, and consequently decrease losses by effluent, avoiding the nutrient losses (Balieiro Neto *et al*. 2009). Martins *et al.* (2015) found that the addition of 1% of CaO (on fresh matter) decreased the gas losses and increased the DM recovery of sugarcane silage and they suggested that the results were related to altered osmotic pressure of the forage mass which inhibited the development of yeasts during the fermentation of sugarcane silage.

However, Yitbarek and Tamir (2014) claimed that mineral such calcium and salt usually either have no effect on fermentation or act in buffer capacity, and the only reason for adding minerals to the silage is make it more nutritionally complete.

The majority of silage lactic acid bacteria grow better at temperatures between 20 and 40 ˚C, in which the optimum temperature is 30 ˚C (Driehuis *et al*. 2011), achieved in the current experiment by all treatments. In fact, treatments with INO increased the temperature of aerobic stability and may related to the numerically increase of DM content of silage. The rise in temperature is greater in silages with higher DM, because more heat is required to raise the temperature of wetter material than is necessary for dried material (McDonald *et al.* 1991).

Ohyama *et al.* (1980) reported that the significant factors which influence the aerobic stability are DM, acetic acid, butyric acid and concentration of fungi, whereas there is a positive correlation for acetic and butyric acids with aerobic stability. In the current experiment the highest period in which silage remained stable was found in INO without CHE treatment and in the association of INO with urea. The latter treatments also showed the lowest values of fungi amounts and highest concentration of acetate in sugarcane silage. Some additives, including NaCl and CaO can be used as antiseptic for undesirable microorganism (Rezende, *et al*. 2011). In addition, CHE additives, mainly CaO and NaCl contributed to better stability of the cane silages due to the possible antimicrobial action and also the ability of these additives to maintain the osmotic conditions of the medium, which resulted in greater DM content, greater DM recovery and reduction of losses, besides the substantial improvement of the nutritional value of the sugar cane in the silage process.

As expected, INO increased the lactic acid bacteria in the current experiment. Muck (2013) claimed that lactic acid bacteria inoculant strains have been selected for growth rapidly in a homofermentative manner under several temperatures and DM contents, and thus is expected that inoculants will be highly competitive and produce largely lactic acid compared to untreated silages. The association of INO and CHE or just the INO treatment had lower amounts of fungi, compared to treatments without CHE and INO or those treatments with CHE. Some strains of lactic acid bacteria may produce antifungal compounds. Broberg *et al.* (2007) reported that *L. plantarum* isolated from grass silage produced antifungal compounds (3-phenyllactic acid and 3-hydroxydecanoic acid) in MRS broth, and these compounds were higher in inoculated silage compared to those untreated. Ammonia concentration in silages is related to proteolysis in silo caused by clostridial microorganisms or a slow drop of pH (McDonald *et al.* 1991). Martins *et al.* (2015) also reported increase of ammonia nitrogen of sugarcane silage treated with chemical additives, including urea and CaO, compared to control. In addition, lactic acid bacteria (i.e. *L. plantarum*) are able to decarboxylase amino acids, forming ammonia and CO_2 (McDonald *et al*. 1991).

A slow drop in the pH prolongs the fermentation and increase the production of acetic acid (McDonald *et al.* 1991). Furthermore, a relatively high buffering capacity favors the production of acetate, butyrate and in ammonia, increasing the aerobic stability of silages (Wilkinson and Davies, 2012). Yeasts produce ethanol from the fermentation of sugars causing up to 49% loss of substratum (McDonald *et al.* 1991). Since the association of INO and CHE decreased the amounts of fungi in silages, consequently it should reduce the ethanol production. The increase of lactic acid in sugarcane silage treated INO is expected due to the inoculant used in the current study is based on lactic acid bacteria.

CONCLUSION

The association of INO and CHE positively affected the chemical composition, *in vitro* degradation, total losses, aerobic stability, microbiological and fermentative profile of sugarcane silage.

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