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Fermentation parameters, quality and losses in sugarcane silages treated with chemical additives and a bacterial inoculant

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ABSTRACT - The objective of this trial was to evaluate chemical additives and a bacterial inoculant on the inhibition of alcoholic fermentation and reduction of losses in sugarcane silages. Treatments were (doses on a fresh forage basis): without additive (control); urea (10 g/kg); urea (5 g/kg) + sodium benzoate (0.5 g/kg); sodium benzoate (1 g/kg); urea + ammonium sulfate in a 9:1 relation (10 g/kg); *Lactobacillus buchneri* (5×10^4 cfu/g). Silages were produced in 10.16- × 30-cm PVC tubes, provided with tight lids adapted with Bunsen valves for gas losses quantification. Minisilos were opened 139 days after ensiling. Ethanol content (227 g/kg dry matter - DM) and total DM loss (30%) were high in the control silage. All additives, except benzoate, decreased ethanol concentration in silages. Inoculation with *L. buchneri* increased acetic acid content in the silage, resulting in a 41% reduction in ethanol content and the lowest gas loss among treatments (15.2%). There was synergistic effect between additives for the combined use of urea and benzoate. Silage treated with urea + ammonium sulfate has higher content of total digestible nutrients than the silage treated with urea exclusively.

Key Words: ammonium sulfate, ethanol, *L. buchneri*, sodium benzoate, urea, volatile fatty acids

Introduction

Sugarcane is usually fed fresh to cattle during winter in Brazil. Recently, the pursuit for better feed and field management has led to an increase in its use as silage, although some hurdles must still be overcome. While the forage's high sugar content and low buffering capacity favor lactic acid production and fast pH drop, its normally high yeast population leads to intense alcoholic fermentation and excessive dry matter loss (DML) during ensilage (Pedroso et al., 2005).

Several anti fungi products have been tested to control yeasts in sugarcane silages. Some have shown poor efficiency and even deleterious effects, like inoculants containing homolactic bacteria (Freitas et al., 2006; Pedroso et al., 2008) while others, although efficient in controlling alcoholic fermentation, may be hazardous to the environment and farm personnel, like NaOH. Sodium benzoate, urea and inoculants containing *Lactobacillus buchneri* are some of the most studied additives, but the normal variability of results among experiments indicates that further investigations are necessary to broaden database, allowing more accurate predictions (Schmidt, 2008).

Adding urea to sugarcane has long been known as an effective way to correct protein content in the forage (Alvarez & Preston, 1976). Urea is frequently mixed with ammonium sulfate (9:1) to achieve adequate nitrogen/sulfur balance in sugarcane-based diets (Ferreiro et al., 1977). That way, if urea were proved efficient in controlling alcoholic fermentation during the ensilage of sugarcane, a secondary benefit would be the improvement of crude protein (CP) content in the silage, facilitating feed management. Feed management could be further facilitated if application of urea + ammonium sulfate had the same or better effects than urea applied solely.

Few experiments have been carried out to evaluate the combination of additives on sugarcane ensilage (Pedroso et al., 2007; Siqueira et al., 2010). The eventual occurrence of a synergistic effect creates the possibility of using additives in lower doses, possibly reducing the cost of application.

The objective of this experiment was to test additives on the control of alcoholic fermentation and losses in sugarcane silages, evaluating the possibility of occurrence of synergistic effect in the combined use of urea and sodium benzoate, reassessing the effects of urea and benzoate applied exclusively; the feasibility of applying a pre-mixture of ammonium sulfate and urea and the effects of inoculation with *L. buchneri*.

Material and Methods

Silages were produced with sugarcane (IAC86-2480), approximately 12 months old, mechanically harvested with

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Mentamit® adjusted for cut length between 5 and 10 mm. Approximately 1,800 g of the chopped forage were packed into 10.16 x 30-cm PVC tubes (minisilos), provided with tight lids adapted with Bunsen valves for gaseous losses quantification. Forage density in the minisilos averaged 724 kg/m^3.

Treatments differed according to the type of additive applied to the chopped sugarcane before ensiling (doses in a fresh forage basis - FF): without additive (control); urea (10 g/kg) - UR; urea (5 g/kg) + sodium benzoate (0.5 g/kg) - UR + SB; sodium benzoate (1 g/kg) - SB; urea + ammonium sulfate in a 9:1 ratio (10 g/kg) - UR + AS; *Lactobacillus buchneri* (5 x 10^4 cfu/g) - BUCH. Urea and urea + AS were added to the forage without dilution while sodium benzoate and *L. buchneri* were applied in aqueous solutions, using manual sprayers. The solution of sodium benzoate was applied at the rate of 4.5 L/t FF. The inoculant containing *L. buchneri* (strain NCIMB 40788, Lalsil Cana®, Lallemand S.A., Blagnac, Fr.) was applied according to label (2 g/t) using 1.5 L of solution/t FF.

Minisilos were weighed and sampled on day 0 and 139 days after ensiling. Dry matter loss was calculated by DM weight loss in the silage. Samples were dried in a forced ventilation oven (65 ºC, 48 h) and ground in a Wiley mill through a 1-mm screen and analyzed for acid detergent fiber (ADF); neutral detergent fiber (NDF) and lignin, according to Van Soest & Robertson (1985); DM, ash, crude protein (CP), ether extract and N-ADF, according to AOAC (1990). Content of total digestible nutrients (TDN) in silages was calculated according to Weiss et al. (1992).

Samples for ethanol, pH, volatile fatty acids (VFA) and lactic acid determinations were frozen (–10 ºC) until processing for analysis. On the day of processing, samples were thawed and extracts were produced by means of a hydraulic press (2 kgf/cm^3). Approximately 300 g of silage from each sample were used to produce 50 mL of juice in which pH was determined with a digital potentiometer. Extracts were centrifuged at 3,000 rpm for 15 min and 5 mL of supernatants transferred to 10 mL test tubes containing 1 mL of formic acid P.A. From these extracts, 1 mL was filtered through a Millex filter (0.45 µm) and stored (–10 ºC) until analysis. Ethanol and VFA were analyzed by gas chromatography according to Sigma-Aldrich, Co. (1998) and lactic acid using high performance liquid chromatography (HPLC) according to Wilson (1971).

Data were analyzed as a completely randomized design, with six treatments and four replicates, and subjected to ANOVA by the GLM procedure of SAS (SAS, 2003). Differences between means were tested using *t* test. Significant differences were declared if P<0.05.

Results and Discussion

The control silage presented concentrations of lactic, acetic, propionic and butyric acids and pH (Table 1) indicative of adequate fermentation and conservation in traditional silages (Kung & Shaver, 2001). Nonetheless, ethanol content was extremely high (227 g/kg DM) in the untreated silage (Figure 1), indicating undesirable and intense yeast development.

Silages with intense alcoholic fermentation tend to show inadequate final pH (Driehuis & Wikselaar, 2000) but the low buffering capacity of sugarcane allows rapid drop in pH even with relatively small amounts of acids in the silage (Alli et al., 1983) and, despite high levels of ethanol, these silages normally present final pH around 3.5 (Pedroso et al., 2005).

Yeasts are not inhibited by pH levels normally found in silages (McDonald et al., 1991) and lactic acid has weak direct fungicidal action (Moon, 1983). Consequently, the low pH and the lactic acid content in the control silage were unable to restrict yeast development, resulting in high gaseous and total DM losses in the silage (19.3% and 29.8%, respectively; Figure 1). High DM loss was expected for the control silage considering that fermentation of sugars by yeasts results in proximately 49% loss of substratum as CO_2 and H_2O (McDonald et al., 1991). Accordingly, fermentation in the control silage resulted in a 26% reduction in DM content, relative to the fresh forage (Table 1).

Uncontrolled yeast fermentation during the ensilage of sugarcane may consume up to 70% of sugars originally present in the forage, causing other components to become more concentrated in DM and a substantial reduction in silage nutritional value (Pedroso et al., 2005). Accordingly, NDF, ADF, CP, ash, Ca and P concentrations were higher and TDN was 22% lower in the control silage compared with the fresh sugarcane (Table 1). All these aspects are typical of sugarcane silages produced without additives and have been well documented (Pedroso et al., 2005; Siqueira et al., 2010).

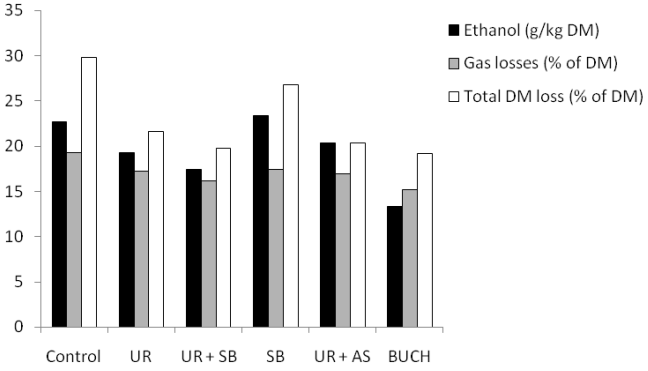
All silages treated with additives, except for the silage treated with sodium benzoate, had lower ethanol content than the control silage (P<0.05) but, since alcoholic fermentation was not eliminated, gas losses and total DML were still high (above 15% and 19% of DM, respectively) during ensilage (Figure 1). The characteristic loss of substratum (sugars) due to alcoholic fermentation caused fiber components, ash, Ca and P to become more concentrated in these silages, relative to the fresh sugarcane. Partial control of alcoholic fermentation was sufficient to

make silages treated with additives higher in TDN compared with control (Table 1).

Silages treated with urea exclusively (UR) and urea + benzoate (UR + SB) had similar contents of lactic, propionic and butyric acids but, acetic acid concentration and pH were higher in UR silage (Table 1). During ensilage, plant cell urease converts urea into ammonia. The alkaline property of ammonia causes delay in pH drop, favoring acetic acid production and higher DM losses (Kung & Shaver, 2001). Accordingly, the higher dose of urea when the additive was applied alone resulted in higher acetic acid content, pH above recommended (4.5) and higher ($P<0.05$) gaseous losses (17.2% vs. 16.2% of DM) compared with UR + SB silage (Figure 1). Ethanol content was similar ($P>0.05$) in both silages (average of 184 g/kg DM), representing approximately an 18% reduction in alcohol concentration relative to control (Figure 1). Total DML did not differ ($P>0.05$) between UR and UR + SB silages (average of 20.7% of DM), representing a 31% reduction in DML compared with control. The higher amount of nitrogen added to the forage in UR resulted in higher CP content compared with UR + SB silage (Table 1). Lignin and ash contents were lower and TDN was approximately 7% higher for the UR + SB silage compared with the silage treated with urea alone.

The reduction in alcohol production observed in UR and UR + SB silages may be credited to the toxic effect of ammonia on yeasts (Alli et al., 1983). Despite indication that conversion of urea into ammonia may be low in sugarcane silages (Nussio et al., 2006), Castro Neto et al. (2008) reported 33% N-NH_3 (relative to total N) in DM and reduced ethanol yield in sugarcane silage treated with urea (5g/kg FF). Some results in this experiment agree with Pedroso et al. (2007), who also observed reduced total DML and higher nutritional value for silages treated with urea exclusively, in doses ranging from 5 to 15 g/kg FF, compared with untreated silage. Pedroso et al. (2008) reported reduction in yeasts, lower ethanol content and higher digestibility for silage treated with urea (5 g/kg FF), despite an elevation in pH, but these authors considered that intensification of effluent production enhanced total DML in the silage, compared with the silage produced without additive. Siqueira et al. (2010) obtained elevation in N-NH_3, from 2.9% to 14.7% of total N, pH in the upper limit (4.2) and high gaseous losses in silage treated with urea (15 g/kg FF), without alteration in silage buffering capacity.

Results for sodium benzoate applied exclusively (SB) were inferior compared with results for the additive combined with urea. Neutral detergent fiber and ADF contents were similar in both silages but lignin and ash were more



Control = silage without additive; UR = silage with urea (10 g/kg of fresh forage - FF); UR + SB = silage with urea (5 g/kg FF) + sodium benzoate (0.5 g/kg FF); SB = sodium benzoate (1 g/kg FF); UR + AS = urea + ammonium sulfate in a 9:1 relation (10 g/kg FF); BUCH = *Lactobacillus buchneri* (5 x 10^4 cfu/g FF).

Figure 1 - Ethanol concentration and dry matter losses in sugarcane silages treated with chemical additives or a bacterial inoculant.

Table 1 - Chemical composition of fresh sugarcane and experimental silages[1] (g/kg DM, unless otherwise stated)

Element	Sugarcane	Control	UR	UR + SB	SB	UR + AS	BUCH	SE
DM (g/kg FF)	272a	202c	224b	228b	209c	227b	229b	7
Crude protein	25.6d	42.0c	151a	72.3b	40.8c	144a	36.0c	5
NDF	387d	637ab	603c	645a	656a	582c	612bc	20
ADF	240d	442a	411b	382bc	398bc	390bc	376c	20
Lignin	37.6d	86.9a	86.9a	49.6c	62.9b	45.0cd	43.4cd	7
Ash	31.2c	47.1a	45.0a	42.4b	45.2a	44.9a	40.7b	1.6
Ca	16.2d	26.6a	25.5ab	25.1b	24.9b	24.4b	21.8c	0.8
P	4.30d	6.70a	5.82bc	5.67c	6.50ab	5.77c	6.10b	0.5
TDN	693a	538e	568d	605c	571d	637b	628b	13
pH	nd	4,2a	4,5a	3,7b	3,5b	4,2a	3,4b	0,26
Lactic acid	nd	52.0c	79.4a	76.2ab	70.1b	75.8ab	50.8c	4.8
Acetic acid	nd	10.1c	21.4b	12.1c	11.0c	12.7c	32.9[a]	3.0
Propionic acid	nd	0.09b	0.13a	0.11ab	0.12ab	0.12ab	0.05c	0.03
Butyric acid	nd	0.99bc	1.08ab	1.03bc	1.23a	1.02bc	0.92c	0.11

[1] Control = silage without additive; UR = silage with urea (10 g/kg FF); UR + SB = urea (5 g/kg FF) + sodium benzoate (0.5 g/kg FF); SB = sodium benzoate (1 g/kg FF); UR + AS = urea + ammonium sulfate in a 9:1 relation (10 g/kg FF); BUCH = *Lactobacillus buchneri* (5 × 10^4 cfu/g FF).

a, b, c, d Means within a row with different superscripts differ ($P<0.05$) by *t* test.

FF = fresh forage; DM = dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; nd = not determined.

concentrated in SB resulting in lower TDN, compared with UR + SB (Table 1). Appling benzoate in a dose 100% greater than the dose used in the mixture of additives did not reduce ethanol content in the silage (P>0.05) and, despite the reduction in gaseous losses (P<0.05), total DML (26.8% of DM) was not different (P>0.05) from control (Figure 1). Alcoholic fermentation was not inhibited despite the higher lactic acid content and lower pH in the silage treated with benzoate compared with control (Table 1), confirming the inefficacy of low pH and lactic acid in controlling yeasts (McDonald et al., 1991; Moon, 1983).

Sodium benzoate is a common food preservative, which has long been known as an effective inhibitor of yeast and molds (Woolford, 1975). At low pH, sodium benzoate converts into undissociated benzoic acid and, in this form, is able to cross the yeast cell membrane. The exact mechanism by which cell growth is inhibited is yet to be defined. It appears to involve cytosol acidification by acid dissociation on the higher pH inside the cell, disruption of membrane homeostasis and mitochondrial physiology, among others (Krebs et al., 1983; Hazan et al., 2004). In this trial, applying benzoate did not reduce alcoholic fermentation but somehow improved the nutritional value of the silage, which presented lower FDA and lignin contents and higher TDN compared with the untreated silage (Table 1).

Pedroso et al. (2007) reported unsatisfactory results when benzoate was applied solely (1 g/kg FF) at the ensiling of sugarcane, but in a subsequent evaluation, application of the additive reduced alcohol yield and losses, improving silage digestibility and aerobic stability (Pedroso et al., 2008). Siqueira et al. (2007) and Siqueira et al. (2010) observed improved DM recovery and aerobic stability in silages treated with this additive, but ethanol was not analyzed. It is possible to speculate that higher doses of benzoate could be more effective, but the consequent increase in production costs would probably make it impractical.

Results discussed until this point indicate the occurrence of a synergistic effect between additives when urea and sodium benzoate were applied simultaneously. Most of the quality parameters were improved in UR + SB silage compared with silages treated with urea or sodium benzoate alone. If this combination of additives proves to be efficient in future evaluations, its use may bring some other benefits to farmers besides the control of alcoholic fermentation in the silages: the low dose of urea allows partial correction of protein content in the silage without the negative aspects that may occur when urea is applied in higher doses, such as inadequate pH and higher DM losses in the silage; applying urea at ensiling poses less risk of intoxication to animals than mixing the product with the forage in the feed bunk, the traditional way to correct CP content in sugarcane (Alvarez & Preston, 1976).

Applying urea + ammonium sulfate (UR + AS) had the same effect (P>0.05) on ethanol content, gas losses and total DML in the silage as urea applied exclusively (Figure 1). Crude protein, NDF, ADF and mineral contents were similar in both silages but UR + AS silage had less lignin and acetic acid contents and higher TDN than the UR silage (Table 1). Although there was no difference in final pH among these silages, the slightly higher dose of urea in UR compared with UR + AS (10 vs. 9 g/kg FF) seems to have been sufficient to delay pH drop, allowing enterobacteria growth for a longer period, which could explain the higher acetic acid content in the silage. On the other hand, the more adequate fermentation pattern in Ur + AS silage, resulted in less acetic acid in the silage, somehow reducing lignin content and, consequently, elevating silage TDN compared with UR. Results indicate that applying urea + ammonium sulfate to sugarcane at ensiling improves fermentation and might be a practical way to obtain a more appropriate S:N balance in diets containing these silages. The indication that applying the mixture results in silage with higher TDN than urea applied alone must be confirmed in further trials.

Inoculation with *L. buchneri* caused significant increase in acetic acid concentration in the silage, but contents of lactic and butyric acids and pH did not differ from control (Table 1). The inoculated silage had the lowest ethanol content among all silages (130 g/kg in DM), corresponding to a 41% reduction (P<0.05) in alcohol concentration relative to the untreated silage (Figure 1). The higher efficiency of inoculation in controlling alcoholic fermentation resulted in the lowest (P<0.05) gaseous losses among silages (15.2% of DM), representing a 22% reduction compared with the silage without additive. Total DML was approximately 36% lower (P<0.05) in the inoculated silage compared with control (19.2 × 29.8% of DM). The inoculated silage presented NDF, ADF, lignin and ash concentrations in the lower and TDN in the highest level observed among silages (Table 1).

The heterolactic bacteria *Lactobacillus buchneri* ferment lactic acid to acetic acid and small amounts of 1.2 propanediol, propionic acid and carbon dioxide (Oude Eelferink et al., 2001). Acetic acid has high fungicidal effect (Woolford, 1975) and inoculation with *L. buchneri* has consistently increased its concentration in grass and corn silages, reducing yeast counts (Driehuis et al., 1999; Kleinschmit et al., 2005; Pedroso et al., 2010). Considering sugarcane ensilage, inoculation with these bacteria also enhanced acetic acid production in evaluations by Mendes et al. (2008) and Pedroso et al. (2007) and reduced

ethanol content and losses in trials by Pedroso et al. (2007), Pedroso et al. (2008) and Siqueira et al. (2010). Inoculation with *L. buchneri* had no effect on ethanol production in research by Freitas et al. (2006).

Conclusions

Inoculation with *L. buchneri* is effective in reducing ethanol content and losses in sugarcane silages. Urea and sodium benzoate are more efficient when applied together, indicating the occurrence of synergistic effect between these additives. Applying urea mixed with ammonium sulfate has the same overall effect as urea alone and may facilitate feed management in the farm. The natural variability in results from experiments involving silage fermentation indicates that further evaluations are necessary to broaden the database of additives for the ensilage of sugarcane.

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