











# Fermentative characteristics and nutritional value of sugarcane silage added with two types of urea

*Características fermentativas y valor nutricional del ensilaje de caña de azúcar con adición de dos tipos de urea*

*Características fermentativas e valor nutricional da silagem de cana-de-açúcar aditivada com dois tipos de ureia*

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## Abstract

**Background:** Dry matter (DM) loss is high in sugarcane silage due to its high content of soluble carbohydrates which favors yeast growth and ethanol production, thus reducing the nutritional value of the preserved forage. **Objective:** To determine the effect of adding two types of urea at two concentration levels to sugarcane silage on the nutritional value and fermentative characteristics of the silage. **Methods:** A completely randomized 2×2+1 factorial design was used, based on two types of urea (conventional and protected), two urea concentration (0.5 and 1.0% of the natural matter), and a control (sugarcane silage with no additives). Six replicates were used. The mixture was placed in PVC silos and opened after 60 days. **Results:** There was no difference in DM content or effluent losses by the addition of urea. In regards to neutral detergent fiber, acid detergent fiber, and lignin, decreased levels were observed compared to the control, regardless of urea type. Urea type and concentration had an effect on ammoniacal nitrogen content, with higher values for conventional urea, but not affecting the pH. The addition of urea to sugarcane silage reduced gas losses and raised the levels of crude protein, ether extract, and effective degradability. **Conclusion:** Adding protected or conventional urea at 1% of natural matter at the time of sugarcane ensiling reduces gas losses and improves nutritional composition of the silage.

**Keywords:** conservation; ensilage; fermentation; forage; fiber; losses; protein; rumen degradability; ruminant; sugarcane; silage; urea.

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## Resumen

**Antecedentes:** La caña de azúcar ensilada presenta una elevada pérdida de materia seca (MS) debido a su alto contenido de carbohidratos solubles que favorece el crecimiento de levaduras y la producción de etanol, reduciendo así el valor nutricional del ensilado. **Objetivo:** Determinar el efecto de dos tipos de urea en dos concentraciones sobre las características fermentativas y el valor nutricional del ensilaje de caña de azúcar. **Métodos:** Se utilizó un diseño completamente al azar, en esquema factorial 2x2+1, basado en dos tipos de urea (común o protegida), dos concentraciones de urea (0,5 y 1,0% de la materia natural) y un control (ensilaje de caña de azúcar sin aditivos). Se utilizaron seis réplicas de cada tratamiento. El material fue ensilado en silos de PVC, los que fueron abiertos después de 60 días. **Resultados:** No hubo diferencia en el contenido de MS o en las pérdidas por efluentes con la adición de urea. En relación a la fibra detergente neutra, fibra detergente ácida y lignina, se observó disminución de éstos con respecto al control, independientemente del tipo de urea. Se observó un efecto de la concentración y del tipo de urea para el contenido de nitrógeno amoniacal, con mayores valores para la urea común, pero sin efecto sobre el pH. La adición de urea en el ensilado de caña redujo las pérdidas por gases y elevó los niveles de proteína bruta, extracto etéreo y degradabilidad efectiva. **Conclusión:** La adición de urea común o protegida, al 1% de materia natural, al inicio del ensilaje de caña de azúcar reduce las pérdidas por gases y mejora la composición nutricional del ensilaje.

**Palabras clave:** caña de azúcar; conservación; degradabilidad ruminal; ensilado; ensilaje; fermentación; fibra; forraje; pérdidas; proteína; rumiante; urea.

## Resumo

**Antecedentes:** A ensilagem de cana-de-açúcar sem aditivos apresenta elevada perda de matéria seca (MS) devido ao alto teor de carboidratos solúveis, favorecendo o crescimento de leveduras e produção de etanol, reduzindo assim o valor nutricional da forragem conservada. **Objetivo:** Determinar o efeito do tipo de ureia (comum ou protegida) e diferentes concentrações nas características fermentativas e valor nutritivo das silagens de cana-de-açúcar. **Métodos:** Utilizou-se um delineamento inteiramente casualizado em esquema fatorial  $2 \times 2 + 1$ , sendo dois tipos de ureia (comum ou protegida), e duas concentrações (0,5 e 1,0% com base na matéria natural) mais o controle (silagem exclusiva de cana-de-açúcar); com seis repetições. O material foi ensilado em silos de PVC e abertos após 60 dias. **Resultados:** Não houve diferença no teor de MS e nas perdas por efluentes com adição da ureia. Em relação à fibra em detergente neutro, fibra em detergente ácido e lignina observou-se diminuição destes em relação ao controle, independente do tipo de ureia. Observou-se efeito das concentrações e do tipo de ureia para o teor de nitrogênio amoniacal, com maiores valores para a ureia comum, porém sem efeito no pH. A adição de ureia na ensilagem de cana reduziram as perdas por gases e elevaram os teores de proteína bruta, extrato etéreo e degradabilidade efetiva. **Conclusão:** O uso de 1% de ureia, independente do tipo, promove um incremento no valor nutricional da silagem de cana-de-açúcar e reduz as perdas por gases.

**Palavras-chave:** cana-de-açúcar; conservação; degradabilidade ruminal; ensilagem; fermentação; fibra; forragem; perdas; proteína; ruminante; uréia.

## Introduction

The use of sugarcane (*Saccharum* spp L.) in the *in natura* form to feed cattle is an established practice among Brazilian cattle ranchers. However, the daily cutting of sugarcane is burdensome and causes logistical and operational problems owing to the daily demands of labor. Thus, sugarcane is generally used as silage to feed cattle (Rigueira *et al.*, 2018). However, when sugarcane is ensiled, it presents a fermentative pattern characterized by high ethanol production and loss of soluble carbohydrates, which is mediated by yeasts; reducing its nutritional value and losing dry matter (DM) (Moreira *et al.*, 2014; Roth *et al.*, 2016).

Urea is often used as an additive to silage. This is based on the transformation of urea into ammonia, which reacts with water to form ammonium hydroxide, raising the pH, and inhibiting the metabolism of undesirable microorganisms such as yeasts (Kung Junior *et al.*, 2003). The buffering effect of ammonium hydroxide on silage pH can be advantageous, although this depends on the extent to which it is able to delay pH decrease. If the buffering effect is moderate, then there is greater competition between lactic acid bacteria and yeasts, since the latter are predominant in fermentation at pH values below 4. However, if the buffering effect is large, then development of undesirable microorganisms that act at higher pH is favored, causing forage deterioration.

Recently, a product termed 'protected urea' has been developed. It generates a controlled release of ammonia and reduces the buffering effect caused by conventional urea in addition to allowing higher concentration to be used at the time of ensilage.

Therefore, the objective of this work was to compare the fermentative characteristics of adding protected or conventional urea to sugarcane silage and to assess the nutritive value of the silage.

## Materials and methods

### *Ethical considerations*

The experiment was approved by the Ethics Committee for Animal Experimentation of Universidade Estadual de Montes Claros (number 155/2018). The study was performed at the Departamento de ciências agrárias da Universidade Estadual de Montes Claros (UNIMONTES), Campus Janaúba-MG, Brazil (GPS coordinates: latitude 15°52'38" S, longitude 43°20'05" W).

### *Experimental design*

A completely randomized 2×2+1 factorial design was used, based on two types of urea (conventional and protected), two urea concentrations (0.5 and 1% of the natural matter), and a control (sugarcane silage with no additives). Six replicates were used.

### *Procedures*

The sugarcane variety used was IAC 86-2480, obtained from the experimental farm of UNIMONTES. The forage was cut manually. One year after the last cut, the stalks were chopped using a stationary forage harvester (Nogueira, São João da Boa Vista, SP, Brazil) with the knives adjusted to obtain 1 to 2 cm particle size. Four 20 kg piles of fresh sugarcane were harvested, protected or conventional urea was added at the desired proportions, and then the mixture was homogenized.

The silages were prepared in PVC silos, 50 cm in height and 10 cm in diameter, with a *Bunsen* valve. At the bottom of the silos, 0.4 kg of dry sand was added to drain the effluents, and a layer of foam to prevent contact between the silage and the sand. The experimental set, consisting of silo plus a lid, sand, and foam, was weighed to obtain the empty weight of the silo. Subsequently, the mixtures to be ensiled were placed into the silos and compacted to a density of 550 kg m<sup>-3</sup> (Ruppel *et al.*, 1995). The silos were sealed, stored at room temperature (25 °C), and opened only after 60 days. Before opening, the silos were reweighed to quantify gas losses.

After removal of the silage, the experimental set containing silo plus lid, sand, and foam were reweighed to quantify the produced effluent (Jobim *et al.*, 2007).

After the silage was removed from the silos, it was homogenized; one part was pressed with a hydraulic press (Bovenau®, Rio do Sul, SC, Brazil) to extract silage juice. The pH of this liquid was determined using a potentiometer (Tecnal®, Piracicaba, SP, Brazil), and the level of ammoniacal nitrogen (N-NH<sub>3</sub>) was estimated by distillation with magnesium oxide and calcium chloride using a boric acid receptor solution and titration with 0.1 N hydrochloric acid (AOAC, 1980). The remainder of the silage was dried in a forced ventilation oven at 55 °C for 72 h and ground in a mill (Thomas Model 4 Wiley; Thomas Scientific, Swedesboro, NJ, USA) with 1 mm sieve screens for chemical analysis, and 5 mm for *in situ* incubation.

Analyses were carried out according to the analytical procedures of the National Institute of Science and Technology in Animal Science (INCT-CA; Detmann *et al.*, 2012). Dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were assessed.

To evaluate ruminal degradation kinetics, four 450 ± 50 kg crossbred steers were used. All the animals had a gastric cannula. The animals received 3.0 kg concentrate in two equal portions in the morning and afternoon, as well as diet based on cane silage. The *in situ* degradability technique described by Casali *et al.* (2009) was performed using 7.5 × 15 cm non-woven fabric bags with an approximate porosity of 60 µm. The number of samples was determined from the ratio of 20 mg DM cm<sup>-2</sup> of bag surface area (Nocek, 1988).

The bags were placed in 20 × 30 cm fillet bags along with 100 g lead weights. The fillet bags were tied with a nylon thread, leaving a length of 1 m so that the bags could move freely in the solid and liquid phases of the rumen. The fillet bags were then deposited in the ventral sac region of the rumen with the end of the nylon

thread remaining attached to the cannula for 0, 6, 12, 24, 48, 72, 96, 120 and 144 h in reverse order, i.e., starting with the duration of 144 h. The 0 h time samples were inserted into the rumen for 5 min. Subsequently, all samples were collected and washed in ice water to stop ruminal fermentation. Afterward, the samples were placed in greenhouses at 55 °C for 72 hours and then cooled in a desiccator and weighed.

The residues remaining in the non-woven fabric bags following collection from the rumen were analyzed for DM and NDF contents. The percent degradation was calculated by the proportion of food remaining in the bags after ruminal incubation, and NDF was analyzed according to the methods proposed by Van Soest *et al.* (1991) without the use of α-amylase. The obtained data were adjusted to a non-linear regression by the Gauss-Newton method (Neter *et al.*, 1985) using the SAS software (SAS, 2008), according to the equation proposed by Ørskov & McDonald (1979):  $Y = a + b(1 - e^{-ct})$ , where Y = is the cumulative degradation of the nutritional component analyzed after time t; a = the degradation curve intercept when t = 0, which corresponds to the water-soluble fraction of the analyzed nutrient component; b = the degradation potential of the water-insoluble fraction of the analyzed nutrient component; a+b = the degradation of the analyzed nutritional component when time is not a limiting factor; c = the rate of degradation per fermentative action of b; and t = incubation time.

Once calculated, the a, b, and c coefficients were applied to the equation proposed by Ørskov & McDonald (1979):  $ED = a + (bxc/c+k)$ , where ED = is the effective degradability of the nutritional component, and k = the feed passage rate. The particle passage rate through the rumen was estimated to be 5% h<sup>-1</sup>, as suggested by AFRC (1993).

#### Statistical analysis

A completely randomized design in a 2 x 2 +1factorial arrangement with two urea types (conventional and protected) in two

concentrations (0.5 and 1% of the natural matter) plus one control treatment (sugarcane silage with no additives) and six replicates was used for fermentation traits and chemical composition variables. The data were submitted to analysis of variance using PROC GLM of SAS, version 9.2 (SAS Institute, Inc., Cary, NC, USA). When the "F" test was significant for the treatments, urea concentrations and types were analyzed by t test. For all tests,  $\alpha=0.05$  was used. The mathematical model used was  $Y_{ijk} = \mu + \tau_i + \text{doses } j + \tau_i \times \text{doses } j + \epsilon_{ijk}$ , where:  $Y_{ijk}$  is the observation  $ijk$ ;  $\mu$ , the overall mean;  $\tau_i$ , the effect of the treatment urea types, with  $i = 1$  and  $2$ ; concentrations, the effect of urea concentrations; the random effect  $k$ ;  $\tau_i \times \text{doses } j$ , the interaction effect of urea types  $i$  and concentrations  $j$ ;  $\epsilon_{ijk}$  is the random error with mean 0 and variance  $\sigma^2$ .

For the *in situ* degradability variables, a split-plot randomized complete block was used, with five treatments (plots), nine incubation times

(subplots), and four blocks. The different animal weights were the blocking factor. Data were tested via analysis of variance and regression using the MIXED procedure of SAS, at  $\alpha=0.05$ , according to the model:  $Y_{ijk} = \mu + \tau_i + \text{Time } j + \tau_i \times \text{Time } j + \epsilon_{ijk}$ , where:  $Y_{ijk}$  is the observation  $ijk$ ;  $\mu$ , the overall mean;  $\tau_i$ , the fixed effect of the treatment applied to the plot, with  $i = 1, 2, 3, 4$  and  $5$ ; Time, fixed effect of incubation time  $j$  to subtract; the random effect of animal  $k$ ;  $\tau_i \times \text{Time } j$ , the interaction effect of treatment  $i$  and time  $j$ ;  $\epsilon_{ijk}$ , the random error with mean 0 and variance  $\sigma^2$ . The treatment means were compared to the control by Dunnett's test ( $\alpha=0.05$ ).

## Results

The DM contents of the silages did not differ with the addition of urea. The pH values were within a suitable range for forage conservation, with a difference only for the silage with 1% conventional urea compared with that of the control silage ( $p<0.05$ ; Table 1).

**Table 1.** Fermentation profile of sugarcane silage supplemented with two types of urea and two concentrations.

Concentrations	Urea		Mean	Control	SEM	p-value <sup>1</sup>			
	protected	conventional				Type	Dose	TxC	AxCTL
	pH				0.217	NS	NS	NS	*
0.5%	3.41	3.48							
1.0%	3.47	3.50*		3.37					
	N-NH <sub>3</sub> /% total nitrogen				0.602	**	**	NS	*
0.5%	14.06	16.58	15.32 <sup>B</sup>						
1.0%	15.02*	22.21*	18.61 <sup>A</sup>	11.87					
Mean	14.54 <sup>b</sup>	19.39 <sup>a</sup>							
	Gas losses (kg t <sup>-1</sup> )				0.807	NS	NS	NS	**
0.5%	7.13*	7.78*							
1.0%	7.03*	7.13*		13.55					
	Effluent loss (kg t <sup>-1</sup> )				0.674	NS	NS	NS	NS
0.5%	29.22	29.14							
1.0%	25.46	28.13		25.81					

<sup>1</sup>Type, effect of urea type; Concentration, effect of urea concentration; TxC: interaction effect between type and concentration of urea; AxCTL: effect of the additive compared to the control.

\* and \*\* significant at the level of 5 or 1% significance, respectively.

\* Means followed by an asterisk differ from the control by Dunnett's test ( $p<0.05$ ).

Means followed by different superscript upper-case letters (<sup>A, B</sup>) within the same column, and different superscript lower-case letters (<sup>a, b</sup>) within the same row, are significantly different by the t test ( $p<0.05$ ).

SEM – standard error of the mean.



The treatments with 1% urea had a higher fraction of N-NH<sub>3</sub> than both the control and the 0.5% treatment (p<0.05). In relation to urea type, conventional urea showed higher N-NH<sub>3</sub> fraction than that of protected urea (p<0.05). Effluent losses were not influenced by the addition of urea (p>0.05). Lower gas losses were observed in all urea treatments when compared with the control silage (p<0.05; Table 1).

There was a significant interaction between dose and type of urea for crude protein (p<0.05), and all treatments differed from the control (p<0.05). The EE content was higher

for silages with 1% urea (p<0.05). Regarding the different types of urea, protected urea showed the highest levels (p<0.05; Table 2). Reductions in NDF, ADF, and lignin were observed in the fibrous fraction compared with that in the control treatment (p<0.05; Table 2). All treatments differed from the control for NDF (p<0.05). For ADF, only silage with 1% conventional urea differed from the control; for lignin, all treatments with 1% urea, regardless of type, differed from the control. There was no interaction effect between type and dose of urea (p>0.05).

**Table 2.** Chemical composition of sugarcane silage supplemented with two types of urea and two concentrations.

Concentrations	Urea		Mean	Control	SEM	p-value <sup>1</sup>			
	protected	conventional				Type	Dose	TxC	AxCTL
	Dry matter				0.309	NS	NS	NS	NS
0.5%	23.22	23.17		22.43					
1.0%	23.87	23.46							
	Mineral matter (% DM)				0.536	NS	NS	NS	NS
0.5%	7.46	7.70		6.86					
1.0%	8.03	7.48							
	Crude protein (% DM)				0.642	NS	**	*	**
0.5%	8.83 <sup>*aB</sup>	9.67 <sup>*aB</sup>		3.88					
1.0%	14.64 <sup>*bA</sup>	15.46 <sup>*bA</sup>							
	Ether extract (% DM)				0.602	*	*	NS	NS
0.5%	0.75	0.46	0.61 <sup>A</sup>	0.59					
1.0%	1.27	0.77	1.02 <sup>B</sup>						
Mean	1.01b	0.62a							
	Neutral detergent fiber (% DM)				0.360	NS	NS	NS	**
0.5%	46.91*	44.68*		52.02					
1.0%	44.78*	44.35*							
	Acid detergent fiber (% DM)				0.428	NS	NS	NS	**
0.5%	25.46	24.74		28.16					
1.0%	24.29	24.04*							
	Lignin (% DM)				0.445	NS	NS	NS	**
0.5%	17.52	17.29		19.60					
1.0%	16.07*	16.16*							

<sup>1</sup>Type, effect of urea type; Dose, effect of urea dosage; TxC: interaction effect between type and dose of urea; AxCTL: effect of the additive compared to the control.

\* and \*\* significant at the level of 5 or 1% significance respectively.

\* Means followed by an asterisk differ from the control by Dunnett's test (p<0.05). Means followed by different superscript upper-case letters (<sup>A,B</sup>) within the same column, and different superscript lower-case letters (<sup>a,b</sup>) within the same row, are significantly different by the t test (p<0.05). SEM – standard error of the mean.

Treatment of sugarcane silage with urea (conventional or protected) reduced the "a" fraction by 11.8% in relation to the control (mean of 44.07%; Table 3). There was a significant interaction between dose and type of urea, with the highest average observed in sugarcane silage supplemented with 1% conventional urea. The inclusion of 0.5% urea decreased potential degradability of the DM compared with that of the control regardless of urea type. There was no interaction effect for fraction "b" ( $p>0.05$ ). However, there were effects for both doses, with a higher average at the 0.5% dose and urea type, with a higher mean for protected urea. There was also a difference between the treatment with 0.5%

protected urea and the control for fraction "b" ( $p<0.05$ ). For fraction "c", no difference was observed between treatments, with means of 2.2% per hour ( $p>0.05$ ).

There was a significant interaction between concentrations and urea types for the potential degradability of dry matter (DM), with the highest average found after 1% urea, regardless of type. The inclusion of urea in the sugarcane silage increased the undegradable fraction of DM by 11.73% in relation to the control silage. There was a significant interaction between dose and urea type, with the highest average observed for 0.5% protected urea.

**Table 3.** Ruminal degradation parameters of dry matter of sugarcane silages supplemented with two types of urea and two concentrations.

Concentrations	Urea		Mean	Control	SEM	p-value <sup>1</sup>			
	protected	conventional				Type	Dose	TxC	AxCTL
	Fraction "a", %				0.190	**	*	**	*
0.5%	32.95 <sup>*Bb</sup>	39.02 <sup>*Ba</sup>		44.07					
1.0%	41.38 <sup>*Ab</sup>	42.06 <sup>*Aa</sup>							
	Insoluble fraction "b", %				0.448	*	*	NS	*
0.5%	24.00 <sup>*</sup>	20.65	22.33 <sup>A</sup>	19.13					
1.0%	21.40	18.97	20.18 <sup>B</sup>						
Mean	22.70 <sup>a</sup>	19.81 <sup>b</sup>							
	Potential degradability of "c", % hour <sup>-1</sup>				0.741	NS	NS	NS	NS
0.5%	2.2	2.2		2.5					
1.0%	2.2	2.2							
	Potential degradability %				0.241	*	**	*	*
0.5%	56.96 <sup>Bb*</sup>	59.67 <sup>Ba*</sup>		63.2					
1.0%	62.78 <sup>Aa</sup>	61.93 <sup>Aa</sup>							
	Effective degradability, %				0.141	*	*	*	*
0.5%	40.34 <sup>Bb*</sup>	45.72 <sup>Ba*</sup>		50.20					
1.0%	48.04 <sup>*Ab</sup>	49.20 <sup>*Aa</sup>							
	Undegradable fraction, %				0.298	*	**	*	*
0,5%	43.04 <sup>A*</sup>	40.32 <sup>Ab*</sup>		36.79					
1,0%	37.22 <sup>Ba</sup>	38.06 <sup>Ba</sup>							

<sup>1</sup>Type, effect of urea type; Dose, effect of urea dosage; TxC: interaction effect between type and dose of urea; AxCTL: effect of the additive compared to the control.

\* and \*\* significant at the level of 5 or 1% significance respectively.

Means followed by an asterisk differ from the control by Dunnett's test ( $p<0.05$ ). Means followed by different superscript upper-case letters (<sup>A, B</sup>) within the same column, and different superscript lower-case letters (<sup>a, b</sup>) within the same row are significantly different by the t test ( $p<0.05$ ). SEM – standard error of the mean.

## Discussion

Sugarcane has intrinsic characteristics, such as low buffering power and high soluble-carbohydrate concentration, which allow adequate conservation, even with a DM content below 28%.

Monteiro *et al.* (2011) suggested that 28% DM is the minimum requirement for proper fermentation since the rapid drop in pH inhibits the development of undesirable microorganisms. The additives did not alter the DM content of the silages, which were all below 28%. The absence of any effect of the additives can be explained by the low proportions of urea added to the silage, and the fact that they are deterioration-inhibiting and not absorbent additives (Castro Neto *et al.*, 2008) (Table 1).

Even with DM values below those recommended for adequate fermentation, the pH values were all less than 4.1, which is sufficient to restrict the development of undesirable microorganisms (Arriola *et al.*, 2011; Ogunade *et al.*, 2017). The low pH values of silage are due to the low buffer capacity of sugarcane, even when a relatively small amount of acid is produced during silage (Pedroso *et al.*, 2014). However, the pH of the silage with 1% conventional urea was higher than the control, although within the limit mentioned above. The high pH observed on the 1% conventional urea treatment was associated with increased ammonia release compared to treatment with the same dose of protected urea. Additionally, the high pH raised the buffering power of the ensiled material, as ammonia absorbs H<sup>+</sup> ions present in the medium, neutralizes them, and retards the decrease of the pH (Ferreira *et al.*, 2007) (Table 1).

Treatments with 1% urea showed higher values of N-NH<sub>3</sub> compared to 0.5% urea treatments. This increase in N-NH<sub>3</sub> content is related to the transformation of urea to ammonia; the higher concentration of urea released higher amounts of N-NH<sub>3</sub> in the

medium. This result is consistent with the report of Freitas *et al.* (2017), who observed a linear increase in the percentage of N-NH<sub>3</sub> with increasing urea concentrations in millet silage (Table 1).

With regard to urea type, protected urea presented the lowest N-NH<sub>3</sub> values. This is related to the polymers that cover urea molecules, reducing urease action on urea and controlling ammonia release into the medium. Lopes and Evangelista (2010) reported that ammonia originating from the urease action on urea has an inhibitory effect on yeast growth, thereby reducing DM losses in the silage. Thus, a better evaluation of the N-NH<sub>3</sub> level must be made for sugarcane silage, since it can improve silage conservation when it does not exceed a maximum of 10%, according to Costa *et al.* (2016).

The addition of urea, independently of concentration, reduced gas losses compared to the control treatment. This reduction was associated with the inhibition of breakdown of soluble carbohydrates to release carbon dioxide (Yuan *et al.*, 2017), the main metabolic product related to gas losses. This inhibitory effect is attributed to ammonia production inside the silo, which inhibits fungal and yeast growth (Kung Jr. *et al.*, 2000; Cao *et al.*, 2014). These microorganisms ferment soluble carbohydrates, producing ethanol, CO<sub>2</sub>, and volatile compounds that cause DM losses (McDonald *et al.*, 1991). The addition of urea did not influence effluent losses; this is another source of nutrient loss as leaching of soluble compounds can occur, leading to a reduction in the nutritional value of silage (Gebrehanna *et al.*, 2014).

The CP was higher in the silage with 1% urea (15.05%) compared with the silage with 0.5% urea (9.25%), which resulted from the greater addition of non-protein nitrogen in this treatment. Urea is a source of non-protein nitrogen, with 45% N for conventional urea and 39% for protected urea. This inclusion



increases the total nitrogen content in silage, which is computed as CP.

Urea type and concentration had variable influences on the fibrous fraction of sugarcane silage compared to the control silage. All the silage treated with urea differed from the control in NDF content, suggesting that the released ammonia resulted in alkaline hydrolysis with partial solubilization of hemicellulose (Lopes and Evangelista, 2010; Carvalho *et al.*, 2012). Another possible mechanism is that the greater preservation of non-fibrous carbohydrates promoted by the additive caused a dilution effect on NDF content (Jacovaci *et al.*, 2017) (Table 2). Only the silage treated with 1% conventional urea showed difference with the control silage; this result may be associated with a higher release of ammonia, causing a more intense alkaline hydrolysis of this fraction.

Lignin content only differed from the control for the silage treated with 1% urea. Van Soest (1994) mentioned that during alkaline treatment, some lignin and silica could be dissolved in the process; so, a higher dose of urea might induce a higher rate of hydrolysis of these constituents.

There was an increase of the EE contents in the silages treated with either 1% conventional or protected urea. A similar result was reported by Silva *et al.* (2014a), who found an increase in EE content for sugarcane silage after 1% urea treatment. With regard to urea type, an increase in EE values was observed in the silage treated with protected urea. This increase in EE content (Table 2) may be related to the coatings used in protected urea; these are soluble in organic solvents and are counted as lipids. Among the urea encapsulation techniques used by the industry, we can highlight the use of elemental sulfur, polymers, and waxes (Valderrama *et al.*, 2014).

The highest degradability was observed in silage treated with either 1% protected or conventional urea. These treatments were

associated with higher ammonia release, providing a higher rate of hemicellulose solubilization. According to Van Soest (1994), alkaline agents, such as urea, act on covalent ester-type bonds between lignin and hemicellulose to increase solubility of the fibrous fraction.

The use of urea in sugarcane silage decreases alcoholic fermentation and soluble carbohydrate losses and promotes quantitative gains due to lower gas losses (Vieira *et al.*, 2017) and qualitative gains due to increased digestibility (Araki *et al.*, 2017). However, in this study, urea reduced the effective degradability by 8.71% compared to silage without the additive. Other studies, such as that by Fortaleza *et al.* (2012), reported a similar result for sugarcane silage following the addition of 1% of urea. The latter report pointed out that the cause of this effect is unclear. Nevertheless, forage treatment with alkaline substances generally provides an increase in the effective degradability of DM (Rossi Junior and Schogor, 2006). Schmidt *et al.* (2007) compared sugarcane silage without additives or with 5% urea, showing that the DM in silage with urea had high effective degradability and NDF content was reduced to produce this high effective degradability.

In the present study, the NDF content also decreased in treated silages compared to the control, but there was no improvement in effective DM degradability. Schmidt *et al.* (2007) reported that qualitative variations of NDF fraction might influence degradability.

Based on this premise, together with technology advances and genetic improvements, new varieties of sugarcane for animal feed have been developed and made available in the market. Thus, variety IAC 86-2480 used in this study presents higher values of ruminal parameters when compared to variety RB72-454 used by Fortaleza *et al.* (2012). In these two varieties (IAC 86-2480 and RB72-454) no effect of urea inclusion on ensiling was observed with regard to effective degradability. However, a study on another

variety at Instituto Agronômico do Paraná found that the addition of 1% urea in sugarcane silage increases effective degradability of DM to 15.36% (Rossi Junior and Schogor, 2006). A study with four sugarcane varieties (RB 855536, RB 765418, SP 80-1842, and SP 80-1816) observed variation in the effective degradability of DM and the readily soluble fraction, because the quality and structural arrangement of the fibers were different (Spínola *et al.*, 2017). This variation in ruminal kinetic behavior using different varieties and additives has also been reported by Silva *et al.* (2014b).

With respect to ruminants, DM effective degradability of sugarcane silage tends to increase as the amount of ammonia in the silage rises. Ammonia is a source of nitrogen for fibrolytic and cellulolytic bacteria, and increased bacterial activity improves degradation of the fibrous fraction (Van Soest, 1994). This contribution of urea to the increase of nitrogen compounds is supported by the ammoniacal nitrogen and crude protein results. However, in the present study nitrogen increase was not used by ruminal bacteria since degradation rate of the insoluble fraction "b" to fraction "c" was constant, at 2.2% per hour. More studies are needed to elucidate the basis of this effect.

The use of protected or conventional urea at 1% of natural matter at the time of ensiling of sugarcane reduces losses by gases and improves the nutritional composition, with increase of crude protein (CP) content and reduction of fibrous fraction.

## Declarations

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### *Conflict of interest*

The authors declare they have no conflicts of interest with regard to the work presented in this report.

### *Author contribution*

João Paulo Sampaio Rigueira, Ignácio Aspiazú and Vicente Ribeiro Rocha Júnior designed the project. Wagner Sousa Alves, Diego Lucas Soares de Jesus, Marielly Maria de Almeida Moura carried out the experiment. Flávio Pinto Monção determined the statistical model of the experiment and the data analysis. Wagner Alves Sousa carried out the writing of the manuscript. All authors provide critical feedback of the writing and editing.

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