An Acad Bras Cienc (2022) 94(2): e20190996 DOI 10.1590/0001-3765202220190996
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CROP SCIENCE

Chemical composition and *in situ* degradability of sugarcane tip hay subjected to alkaline treatment

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Abstract: The present study aimed to evaluate the effect of alkaline treatments with urea, NaOH and $Ca(OH)_2$ on chemical composition and *in situ* ruminal degradability of dry matter, crud protein and neutral detergent fiber of sugarcane tip hay. Samples were incubated in the rumen of three cannulated cattle for up to 72 hours in a *split plot* randomized block design. Ammoniation with 6% urea increased ($p<0.05$) the crude protein content by 13% and reduced the neutral detergent fiber and insoluble nitrogen content of the hay. When treated with the highest doses of the compounds, there was a high potential degradability of dry matter, crude protein and neutral detergent fiber, and a shorter neutral detergent fiber *lag time*. Ammoniation with urea promotes a reduction in the content of NDF, hemicellulose and insoluble nitrogen, with an increase in the content of CP in the hay, with emphasis for the level of 6% urea. The ruminal degradation of sugarcane tip hay increases with alkaline treatments using 6% urea or 3% NaOH, however, ammoniation with urea is indicated for the treatment of hay, as this is low cost and can be easily adopted by farmers in the semiarid region.

Key words: Hydrolysis, potential degradability, *Saccharum officinarum*, urea.

INTRODUCTION

Agribusiness waste has been commonly used in ruminant feeding aiming to store feed for critical times of the year, intensify production in small farms and especially the reduction in production costs, which are highly correlated with the price of feed supplied to animals (Nussio et al. 2009).

Tip of sugarcane (*Sacharum officinarum* L.) is the main waste of the stalk harvest for industrialization, consisting of blades, leaf sheathes and a portion of the immature stalks (Schogor et al. 2009), mostly discarded in the field. This residue is estimated to be 18% of total stem production (Gargantini et al. 2013), which represents approximately 21 t ha^{-1} green forage, which may be energy source for ruminants after chemical treatments aiming to improve its nutritional value.

Among the chemical treatments used, stand out the alkali treatments using urea, sodium hydroxide (NaOH) and calcium hydroxide ($Ca(OH)_2$), which act on the lignocellulosic bonds of the plant cell wall (Pires et al. 2010) increasing the degradation of the NDF fraction, in addition to the increase in crude protein in the forage when treated with urea, meeting part of the nitrogen requirements of the animals (Moreira Filho et al. 2013).

Ammoniation, in addition to the effects on the chemical composition and improvement of the nutritional value of hay and straw, helps in the conservation of these roughages through the control of microorganisms such as fungi, bacteria, and molds that cause a reduction in forage quality by consuming soluble carbohydrates available in the plant to maintain their metabolism, which results in increased structural carbohydrates (Pádua et al. 2011).

Sodium hydroxide (NaOH) and calcium hydroxide ($Ca(OH)_2$) have high alkalizing power, dissolving hemicellulose and part of lignin. However, there are uncertainties as to the efficient amounts for treatment recommending 3 to 4% dry matter, mainly NaOH, due to the risk of environmental contamination by excess sodium excretion in the environment (Ribeiro et al. 2009). Thus, the objective of this research was to evaluate the effect of alkaline treatments based on urea, NaOH and $Ca(OH)_2$ on the chemical composition and *in situ* ruminal degradability of DM, CP and NDF of sugarcane tip hay.

MATERIALS AND METHODS

The experiment was carried out at the Animal Production Department of the Federal University of Piauí (UFPI), and the sugarcane tip was harvested in a sugarcane plantation of a Rural Association in the municipality of Teresina, State of Piauí. Prior to execution, the research was submitted to the Animal Experimentation Ethics Committee of the UFPI and approved under Protocol 038/13. After harvesting, the sugarcane tip was dried for 36 hours with manual overturning. After reaching the hay point (approximately 15% DM), the forage was subjected to treatments with urea at doses of 2, 4 and 6% (37, 74 and 111 g), sodium hydroxide and calcium hydroxide at doses of 1, 2 and 3% (20, 40 and 60 g) based on DM and amount of treated hay (2.1 kg hay DM per repetition in each treatment).

Upon ammoniation, urea was dissolved in approximately 890 mL water to raise the moisture content of hay to 30% (Moreira Filho et al. 2013), and NaOH and $Ca(OH)_{2,}$ were dissolved in 1.26 L of water, to raise the moisture to 70% (Ribeiro et al. 2009), with uniform distribution with the aid of a watering can. The urea treated-material remained sealed for 35 days and subsequently aerated for 48 h to eliminate excess ammonia. The forage treated with NaOH and $Ca(OH)_2$ was placed in bags that remained open in a roofed and ventilated environment for 48 h.

Samples of treated forage were pre-dried in a forced air oven at 55°C for 72 hours and then ground to 1mm particles in a Wiley knife mill. Analyses were carried out according to methodologies of AOAC (2012) for the contents of dry matter (DM), mineral matter (MM) and crude protein (CP), and according to Licitra et al. (1996) for the contents of neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN), expressed as a percentage of CP. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (LIG) contents were obtained according to Mertens (1997) adapted for autoclave (105°C for 60 min) (Barbosa et al. 2015) using non-woven bags with 4 x 5 cm size and 100 µm mesh size (Valente et al. 2011). Hemicellulose (HEM) and cellulose (CEL) contents were obtained as follows: $\%HEM = \%NDF - \%ADF \wedge \%CEL = \%ADF - \%LIG$, respectively.

To evaluate ruminal degradability, 4 g hay sample were weighed in 12 x 8 cm nylon bags with 50 µm mesh size, according to the 42 mg cm^{-2} ratio, adopted by Campos et al. (2011). The bags were incubated in the rumen of three fistulated adult cattle for 6, 24 and 72 h (NRC 2001) using 4 bags per sample at each incubation time. After withdrawal from the rumen, the bags were immersed in ice water to stop fermentation, washed in a washing machine and pre-dried in a forced air oven at 55ºC for 72 h. The soluble fraction was obtained by washing non-incubated bags in a revolving water bath at 39°C for one hour, and then washed together with the non-incubated bags.

In situ degradability parameters (*a*, *b* and *c*) and potential degradability (PD) of DM and CP were estimated by the exponential model of Ørskov & McDonald (1979), expressed as: $DP = a + b\left(1 - e^{-ct}\right)$, where, *DP* = percentage of degraded nutrient after *t* hours of rumen incubation; *a* = soluble fraction, rapidly degraded; *b* = potentially degradable fraction of material remaining in nylon bag after time zero; *c* = degradation rate of fraction remaining in nylon bag after time zero; *t* = incubation time.

The effective degradability (ED) of DM and CP in the rumen was estimated considering the rate of passage (*k*) 2, 5 and 8% h^{-1} for each treatment by the equation proposed by Ørskov & McDonald (1979): $ED = a + \left[(b\,c)/(c + k)\right]$, where, *ED* = effective degradability (%); *a* = soluble fraction, rapidly degraded (%); *b* = insoluble fraction slowly degraded (%); *c* = fractional rate of degradation of *b* (% h^{-1}); *k* = rate of passage (% h^{-1}).

To estimate the NDF degradation parameters, the Mertens & Loften (1980) model was adopted: $Rt = B\,e^{-ct} + I$, where: *Rt* = fraction degraded in time *t*; *I* = undegradable fraction (%). After adjusting the NDF degradation equation, the fractions were standardized by the equations: $Bp = B/(B + I) \times 100$ and $IP = I/(B + I) \times 100$, where, *Bp* = standardized potentially degradable fraction (%); *IP* = standardized undegradable fraction (%).

The experiment was conducted in a completely randomized design, in a 3 x 3 + 1 factorial arrangement (hay subjected to the three alkaline compounds at three doses and untreated hay), with five replications. To determine the *in situ* degradability of DM, CP and NDF, a *split plot* randomized block design was adopted, with the cattle representing the blocks, treatments assigned to the *plots* and incubation times to the *subplots*. For means and standard deviation, PROC MEANS was adopted, and to obtain degradability parameters, PROC NLIN of SAS (Statistical Analysis System, version 8). Tukey's test was applied at 5% probability for comparison of means.

RESULTS AND DISCUSSION

The DM content of the sugarcane tip hay reduced ($p<0.05$) on average 15.5 and 19.3% when treated with NaOH and $Ca(OH)_2$ (Table I), respectively, which is related to the addition of water to the roughage when treating it with aqueous solutions of alkaline agents. There was an increase in crude protein (CP) content ($p<0.05$) with increasing doses of urea, due to the incorporation of non-protein nitrogen (NPN) in the roughage (Pires et al. 2010), with an increase of 9.7% in the treatment with the highest dose of urea. Similar effects were obtained by Garcez et al. (2014) showing an increase of 13.7% in CP when treating babassu pindoba hay with 4 or 6% urea, and Oliveira et al. (2011) and Carvalho et al. (2006), when treating sugarcane bagasse with up to 6% and 7.5% urea, respectively, roughages with chemical composition similar to the sugarcane tip hay used in this research.

The increase in N content with the addition of NPN sources in forages is expected and increases the availability of this element as ammonia (NH_3-N) for rumen microbial protein synthesis, especially for fibrolytic bacteria due to their low deamination ability, requiring NH_3-N for synthesis of amino acids and increase of their population and colonization capacity. Thus, the increase in N contents with the addition of doses above 2% urea in sugarcane tip hay keeps CP concentration above the recommended (7%) to maintain the minimum NH_3-N content (8 mg dL^{-1} rumen fluid) required for efficient fibrolytic

Table I. **Chemical composition of sugarcane tip hay treated or not with alkaline agents.**

Constituent	Non-treated	Alkaline agent¹									CV (%)
			Urea			NaOH		$Ca(OH)_2$			
		Dose									
		2%	4%	6%	1%	2%	3%	1%	2%	3%	
DM (%)²	92.8^{a}	88.2^{ab}	86.7^{ab}	87.2^{ab}	77.0^{cd}	83.4^{bc}	73.6^{d}	73.5^{d}	76.7^{cd}	73.1^{d}	3.9
% DM											
CP	4.6^{d}	10.1^{c}	14.3^{b}	17.6^{a}	4.0^{d}	4.2^{d}	4.6^{d}	4.0^{d}	4.4^{d}	5.0^{d}	5.4
NDFap	68.2^{bc}	68.8^{ab}	66.2^{bcd}	65.3^{d}	67.7^{bcd}	66.5^{bcd}	66.2^{cd}	68.6^{abc}	67.5^{bcd}	70.9^{a}	1.2
ADFap	40.0^{d}	43.3^{abc}	43.8^{ab}	44.6^{a}	41.2^{bcd}	41.2^{bcd}	41.5^{bcd}	41.7^{bcd}	41.6^{bcd}	40.7^{cd}	2.2
Cellulose	32.7ª	35.2ª	34.5ª	34.2ª	33.6ª	33.5ª	33.7ª	33.3ª	34.2ª	33.5ª	2.8
Hemicellulose	28.2^{ab}	25.5^{bc}	22.9^{cd}	20.7^{d}	26.5^{abc}	25.4^{bc}	24.7^{bc}	26.9^{ab}	25.9^{bc}	27.2^{a}	4.9
Lignin	7.3^{b}	8.2^{ab}	9.3^{ab}	10.4^{a}	7.6^{b}	7.7^{b}	7.8^{b}	8.4^{ab}	7.5^{b}	7.2^{b}	15.0
% total N											
NDIN	34.5^{a}	23.8^{b}	19.4^{b}	14.7^{cb}	38.7^{a}	38.9^{a}	37.3^{a}	36.4^{a}	37.2^{a}	39.3^{a}	11.0
ADIN	28.0^{abc}	15.2^{d}	11.2^{de}	9.5^{e}	32.4^{a}	28.5^{ab}	28.1^{ab}	23.5^{b}	27.2^{bc}	28.6^{ab}	5.0

¹Means followed by different letters in the same row are significantly different by Tukey's test ($p < 0.05$).
²DM = dry matter; NDFpa = Neutral detergent fiber corrected for protein and ash; ADFpa= Acid detergent fiber corrected for protein and ash; CP = crude protein; NDIN = Neutral detergent insoluble nitrogen; ADIN = Acid detergent insoluble nitrogen.

microbial fermentation (Naumann et al. 2013). In this sense, Carvalho et al. (2006) recommend the dose of 2.62% urea to sugarcane bagasse to provide the minimum CP level for good functioning of the rumen.

Lower NDF contents were observed in treatments with doses 4 and 6% urea (66.2 and 65.3%, respectively) and 3% NaOH (66.2%) in relation to untreated roughage, lower values than the reductions obtained by Barros et al. (2010) when treating sugarcane tip associated with bagasse (20:80) with 5% urea. This effect is related to the quality of the sugarcane tip hay fiber and reveals that the hydrolysis of the NDF fraction occurs more efficiently at higher doses of alkaline agents, solubilizing mainly hemicellulose, which presented lower values for treatments with 4 and 6% urea (22.9 and 20.7%, respectively) and 3% NaOH (24.7%).

The acid detergent insoluble nitrogen (ADIN) content of sugarcane tip hay exceeds the proportion of 20% total N, limiting roughage degradability by reducing N for microbial action (Clipes et al. 2010). However, ammoniation provided lower ($p<0.05$) contents of these insoluble nitrogen components (NDIN and ADIN), which contributes to a quantitative reduction in the proportion of N bound to the fiber fraction.

The increase in soluble fraction (*a* fraction) of DM, on average 15.1%, with higher doses of alkaline agents, is justified both by the increased solubility of fiber fractions with hydrolytic action, and by the addition of hydrophilic compounds in chemical treatments, as urea and hydroxides

(Table II). The greatest impact on the potential degradability of DM was evidenced with 4% urea and 3% NaOH, reflecting the effective action of these compounds on the fiber fraction (Table I).

Sugarcane tip hay presented DM degradation rate (*c*) higher than 2% h^{-1} (Table II), remaining in this range when ammoniated with up to 4% urea and by hydrolysis with 1% NaOH, considered ideal (2 to 6% h^{-1}) for good quality roughages and compatible with grass hay (Oliveira et al. 2013). However, the values of degradation rate (*c*) are lower than those obtained by Ribeiro et al. (2009) for hydrolyzed sugarcane with 2.25% NaOH or CaO, with mean values of 3.8 and 4.2% h^{-1}, respectively, which is associated with the higher fiber content of the sugarcane tip (68.2%) in relation to the whole plant (58.1%), with higher proportion of leaves and lignified components.

The effects on DM degradability in this study at doses above 4% urea or 2% NaOH or $Ca(OH)_2$ were greater than those obtained with 2% $Ca(OH)_2$ alkaline treatment in whole sugarcane by Macedo et al. (2011), with values of 35.2 and 70.8% for *b* fraction and potential degradation (PD), respectively, and Campos et al. (2011), with values 55.7% for PD, showing better effect of these alkali compounds on sugarcane tip hydrolysis only at higher doses due to the quality of the fiber fraction, with a higher proportion of cellulose (32.7%) and lignin (7.3%) in its composition. However, the *in vitro* DM digestibility of sugarcane bagasse, a lower quality roughage, increases linearly with up to 7.5% urea dose (Carvalho et al. 2006).

The soluble (*a*) and potentially degradable (*b*) fractions of CP were higher in all treatments when compared to untreated hay (52,7%), mainly in hay subjected to ammoniation (73, 78.3 and 82.8%, respectively for 2, 4 and 6% urea). (Table III).

Table II. Soluble (*a*) and potentially degradable (*b*) fractions, degradation rate (*c*), potential (PD) and effective (ED) degradability, and coefficient of determination (R^2) for dry matter of sugarcane tip hay treated or not with alkaline agents.

Alkaline agent	Dose	a (%)	b (%)	c (% h^{-1})	PD (%)	ED (%)			R^2
						k*=2	k=5	k=8	
Urea	2%	13.2	45.4	4.1	58.6	43.8	33.7	28.7	98.9
	4%	15.4	56.6	3.2	72.0	50.2	37.4	31.5	98.7
	6%	17.8	75.9	1.2	87.9	51.0	34.8	29.3	98.8
NaOH	1%	13.6	50.7	3.5	64.3	45.9	34.5	29.1	95.2
	2%	16.3	58.7	1.8	75.0	44.4	32.1	27.3	91.8
	3%	15.9	84.1	1.2	86.0	47.2	32.0	26.7	97.7
$Ca(OH)_2$	1%	16.0	48.5	2.2	64.5	41.7	31.1	26.7	96.1
	2%	16.4	62.9	1.4	79.3	41.8	29.8	25.5	98.1
	3%	10.9	72.2	1.2	83.1	38.5	25.3	20.6	98.7
Non-treated	-	13.1	50.6	2.4	63.7	40.5	29.3	24.6	98.6

***k* = rate of passage 2, 5 and 8% h^{-1}.**

Table III. **Soluble (*a*) and potentially degradable (*b*) fractions, degradation rate (*c*), potential (PD) and effective (ED) degradability, and coefficient of determination (R^2) for crude protein of sugarcane tip hay treated or not with alkaline agents.**

Alkaline agent	Dose	a (%)	b (%)	c (% h^{-1})	PD (%)	ED (%)			R^2
						k*=2	k=5	k=8	
	2%	73.0	40.6	1.1	83.7	81.3	79.2	78.0	97.1
Urea	4%	78.3	42.3	3.6	90.6	86.2	83.4	82.1	93.3
	6%	82.8	48.3	7.2	99.0	88.9	85.9	84.9	92.6
	1%	47.6	49.5	1.0	90.1	64.5	56.1	53.3	93.2
NaOH	2%	46.3	46.6	1.4	92.9	65.2	56.3	53.1	97.5
	3%	44.3	51.9	1.6	96.2	67.7	57.1	53.1	99.0
	1%	39.1	44.4	1.3	93.5	60.7	50.5	46.8	98.4
$Ca(OH)_2$	2%	52.8	32.6	1.4	85.5	66.1	59.8	57.6	94.8
	3%	50.9	37.5	1.5	88.5	67.2	59.7	56.9	98.3
Non-treated	-	52.7	37.7	1.3	90.4	67.5	60.5	57.9	94.1

***k* = rate of passage 2, 5 and 8% h^{-1}.**

The increase in potential degradability (PD) of crude protein occurred from treatments with doses of 4% urea and 2% NaOH, with values above 90%. The highest degradation rates (*c*) obtained for ammoniated hay from 4% and 6% urea (3.6 and 7.2% h^{-1}, respectively) resulted from the greater availability of ruminal NH_3-N from urea, with an increase in microbial protein synthesis and utilization of the degradable fraction of the components of hay DM.

Potential degradability (PD) of crude protein represents the proportion of potentially degraded nitrogen compounds in the rumen and should be correlated with effective degradability (ED), which represents the actual degradation in this environment, with direct influence on DM degradability (Suzuki et al. 2010). Higher ED values were obtained in treatments with 4 and 6% urea (86.2 and 88.9%, respectively), which justifies the increase in dry matter DP and ED when ammoniated with these doses (Table II).

The colonization time (*lag time*) obtained for NDF (Table IV) remained below the average values (5-6 hours) obtained for tropical roughages by Muniz et al. (2012). The potentially degradable fraction (*Bp*) increased from ammoniation with 4% urea, for $Ca(OH)_2$ and NaOH, it was effective only at doses of 2 and 3%, respectively. This effect can be attributed to the action of breaking the bonds between hemicellulose and lignin, and increasing the solubility of the fiber fraction, as verified by Ribeiro et al. (2009) and Freitas et al. (2011), with values of 86.5% and 87.8% for *Bp* when treating sugarcane with 2.25% and 5% of CaO and NaOH, respectively.

Associated with the increase in potential degradability (*Bp*), a shorter *lag time* was found due to the action of alkaline agents, especially treatments with 6% urea and 3% NaOH, which were effective in reducing 47.7 and 54.8% bacterial colonization time. This effect results in a shorter time for the beginning of hay NDF degradation by the action of alkalis

Table IV. **Colonization time (*lag time*), standardized potentially degradable fraction (*Bp*), standardized undegradable fraction (*Ip*), rate of passage (*k*), and coefficient of determination for NDF of sugarcane tip hay treated or not with alkaline agents.**

Alkaline agent	Dose	*Lag time* (h)	*Bp* (%)	*Ip* (%)	*k* (% h^{-1})	R^2
	2%	4.4	53.1	41.9	3.6	96.6
Urea	4%	3.5	65.2	35.3	3.2	94.4
	6%	2.3	84.9	15.1	1.7	99.8
	1%	4.2	55.1	45.0	3.6	95.9
NaOH	2%	3.8	58.1	41.9	3.2	95.3
	3%	1.9	85.6	14.4	1.4	99.4
	1%	3.9	52.9	47.1	2.9	98.5
$Ca(OH)_2$	2%	3.4	75.9	24.1	1.3	96.6
	3%	3.1	66.5	33.5	2.3	98.3
Non-treated	-	3.6	53.6	46.4	2.4	98.7

in solubilizing the fiber and removing the barriers to bacterial colonization, in addition to stimulating microbiota by increasing NH_3-N with ammoniation, which increases DM degradation, resulting in PD 87.9 and 86.0% for doses of 6% urea and 3% NaOH, respectively (Table II).

The greater alkalinizing effect of NaOH compared to $Ca(OH)_2$ is related to its molecular structure, which is formed by alkali metal, with higher solubility and dissociation capacity, which justifies its higher efficiency in hydrolyzing fiber constituents. According to Pires et al. (2010), the changes caused by alkaline agents in the cell wall constituents vary with the doses to be applied, forage quality, treatment period and ambient temperature.

CONCLUSIONS

Ammoniation with urea promotes a reduction in the content of NDF, hemicellulose and insoluble nitrogen, with an increase in the content of CP in the hay, with emphasis for the level of 6% urea. The ruminal degradation of sugarcane tip hay increases with alkaline treatments using 6% urea or 3% NaOH, however, ammoniation with urea is indicated for the treatment of hay, as this is low cost and can be easily adopted by farmers in the semiarid region.

Acknowledgments

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for financial support for this research. This article is part of the Magister Scientiae Dissertation of the first author.

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How to cite
FRANCO LJD, GARCEZ BS, ALVES AA, AZEVÊDO DMMR, PARENTE HN, FILHO MAM, MOREIRA AL & LIMA FWR. 2022. Chemical composition and *in situ* degradability of sugarcane tip hay subjected to alkaline treatment. An Acad Bras Cienc 94: e20190996. DOI 10.1590/0001-3765202220190996.

Manuscript received on September 19, 2019;
accepted for publication on June 07, 2020

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