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Digestion of feed fractions and intake of heifers fed hydrolyzed sugarcane stored for different periods[1]

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ABSTRACT - The objective of this study was to evaluate, in Nellore heifers, intake and digestibility of hydrolyzed sugarcane stored for different periods. The experimental design used was a 4 × 4 Latin square, four diets, four Nellore heifers with ruminal cannulas (initial body weight 285.4±23.08 kg and average initial age 14 months) and four periods of 21 days. The diets were composed by fresh sugarcane (time zero) or hydrolyzed sugarcane with addition of 0.5% of hydrated lime, stored for 24, 48 or 72 hours, as the unique forage. Intake and digestibility of feed fractions, nitrogen balance, microbial synthesis efficiency, total number of ruminal protozoans and ammoniacal nitrogen did not significantly change by storing sugarcane with addition of 0.5% of hydrated lime. Sugarcane pH varied quadratically for storage time, with maximum pH of 7.02 after 24 hours from lime addition. Ruminal liquid pH values were higher for heifers fed fresh sugarcane, in comparison with those fed hydrolyzed sugarcane. Sugarcane treated with 0.5% of hydrated lime stored for up to 72 hours does not change ruminal digestion to alter the amount of feed consumed by pubescent Nellore heifers. Thus, lime is a viable technology, once it allows long-duration storage and bee control on treated forage, which contributes to animal feeding logistics.

Key Words: digestibility, dry matter intake, hydrated lime, microbial protein, Nellore, nitrogen balance

Introduction

The use of quicklime or hydrated lime to improve the nutritional value of sugarcane has been researched by several authors (Oliveira et al., 2007; Balieiro Neto et al., 2007; Moraes et al., 2008ab), based on the premise that such products cause beneficial changes to the fibrous fraction of the forage by solubilizing components such as the hemicellulose on the cellular wall, improving fiber digestibility. However, the use of these products aims not only to improve the nutritional value of sugarcane, but also to maintain its nutritional characteristics, reducing the frequency of cuts to be provided to the animals (Domingues, 2009; Sforcini, 2009).

The reduced number of cuts is associated with reduced production costs, as operational and labor costs are also reduced. Moreover, reducing the amount of cuts results in better quality of life in breeding systems that depends on sugarcane as the unique forage during pasture-restricted periods (dry season of the year), because of higher flexibility in logistics on farms. Although the number of studies about hydrolyzed sugarcane has increased in the last years (Anualpec, 2009), only a few estimate the effects of the storage of hydrolyzed sugarcane with lime on bovine digestion.

In theory, the use of alkaline agents, such as micro-processed lime, to treat sugarcane can increase ruminal pH of bovines, promoting the growth of cellulolithic bacteria and improving the ruminal digestion of this feedstuff. Dias (2009) found increased ruminal pH in crossbred cows fed sugarcane treated with increasing doses of lime, as well as increased digestibility of the dry matter of sugarcane, though he did not measure variables related to the microbial ruminal growth. In order to improve the knowledge of the use of lime for sugarcane treatment, this experiment aimed to evaluate the intake of feed fractions and digestive aspects in heifers fed diets containing hydrolyzed sugarcane stored for different periods.

Material and Methods

The experiment was conducted after approval by the Ethics and Safety Commission of Faculdade de Ciências Agrárias e Veterinárias, in Jaboticabal, São Paulo, Brazil. It was conducted from October 2nd, 2009 to December 15th, 2009, at the Digestibility Sector of the institution, which is

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located at 21º14'05" South latitude and 48º17'09" West longitude, at 613.98 meters above the sea level. According to the Köppen classification, the region climate is Awa with warm summers and dry winters (Table 1).

Four Nellore heifers with rumen cannulas were used. Their initial body weight and average age were 285.4±23.1 kg and 14 months, respectively. Heifers were originally under feeding management based on sugarcane. At the beginning of the experiment, they were restricted to a Latin square with four animals, four diets and four 21-day periods.

The initial 15 days of each experimental period were used for the adaptation of animals to the facilities (covered, concrete-paved) and diets; and the remaining six days were used for data collection, totaling 84 experimental days. The animals remained in individual stalls provided with troughs and drinkers and were fed diets containing fresh sugarcane (time zero or after different storage time; Table 2).

Sugarcane cv. IAC 86-2480 (early cycle) was cultivated in the Dairy Cattle Sector in UNESP/Jaboticabal. On December 20, 2008, it was subjected to cover fertilization (400 kg of nitrogen per hectare) and invasion control by a tractor-pulled cultivator. The sugarcane was cut manually (10 cm above the ground) with a machete. It was 10 to 12 months old (4th cut) and chopped up by a stationary chopper. The resulting particles measured 8 to 10 millimeters, approximately.

After chopped up, part of the sugarcane was spread in layers of about 20 cm on concrete floor under roofed stall. A suspension of hydrated lime was added with a watering can (partial composition: MgO - 1.5%, total CaO - 72.5%, $Ca(OH)_2$ - 95.5%), at a rate of 0.5 kg of lime in 2 liters of water for each 100 kg of chopped sugarcane, according to Domingues (2009). After homogenizing the lime suspension with the sugarcane, the forage presented yellow color, pleasant smell and absence of bees. The material concentration was spread in 80 cm high heaps of 500 kg approximately, obtaining hydrolyzed sugarcane stored for 24, 48 or 72 hours. For the fresh sugarcane diet (time zero), the forage was provided immediately after chopped.

Table 1 - Means of maximum temperature (MaxT), minimum temperature (MinT), average temperature (AT), air humidity (AH) and precipitation

MaxT, ºC	MinT, ºC	AT, ºC	AH, %	Precipitation, mm
30.60	19.35	24.02	75.85	195.32

Source: Weather station at FCAV-Unesp in Jaboticabal, São Paulo, 2009.

Table 2 - Diet composition

Feedstuff (% of dry matter)	Treatment (diets)			
	FS	HS24	HS48	HS72
Sugarcane	56.1	55.7	56.3	56.3
Finely ground corn grain	18.7	18.9	18.7	18.7
Soybean bran	22.5	22.7	22.4	22.4
Mineral nucleus[1]	1.89	1.91	1.88	1.88
Calcitic limestone	0.42	0.42	0.41	0.41
Urea	0.34	0.34	0.34	0.34

FS - diet with fresh sugarcane (time zero); HS24, HS48 and HS72 - diets with hydrolyzed sugarcane stored for 24, 48 and 72 hours, respectively.
[1] Guaranteed levels: P - 40 g; Ca - 146 g; Na - 56 g; S - 40 g; M g - 20 g; Cu - 350 mg; Zn - 1300 mg; Mn - 900 mg; Iron - 1050 mg; Co - 10m g; I - 24 mg; Se - 10 mg; F (max.) 400 mg; excipient q.s. - 1000 mg.

To formulate the diets, an intake of 2.4 kg of dry matter per 100 kg of body weight was considered, according to the NRC (1996). Feed intake was recorded between days 15 and 20 of each period, by weighing the feed offered and the leftovers; the supply was kept 10% above voluntary intake and provided in two daily meals at 8 a.m. and 2 p.m. by mixing the forage and the concentrate in the troughs.

During the trial, samples of feed and leftovers were collected between the 15th and 20th days of each period. These samples were previously dried in a forced-ventilation oven at 55 ºC for 72 hours and ground in a Willey mill with 1 mm sieves. After, the diet was analyzed according to its chemical composition (Table 3). The composition of experimental diets was also determined (Table 4).

The contents of dry matter, crude protein (CP), ether extract (EE) and mineral matter (MM) were determined according to the AOAC (1990). Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were determined according to Licitra et al. (1996). The contents of neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin were determined by using the procedures of Van Soest (1973) and Van Soest et al. (1991), with neutral detergent fiber corrected for ash and proteins. Total carbohydrates (TC) and non-fibrous carbohydrates (NFC) were obtained through the equations: TC = 100 - CP - EE - MM and NFC = 100 - NDFap - CP - EE - MM, respectively (Van Soest et al., 1991), in which NDFap is the content of neutral detergent fiber corrected for ash and protein. The contents of total digestible nutrients (TDN) was determined according to Weiss (1993), in which TDN = (tDCP × CP) + (EE × 2.25) + [0.98 × (100 - NDFap - CP - ash - EE - 1)] + 0.75 × {(NDFn - lignin) × [1 - (lignin/NDFn)0.667]} - 7, in which tDCP stands for truly digestible crude protein; the forage was expressed by $tDCP = e^{-0.012} \times ADIP$ and for a concentrate equal to DCPap = 1 - 0.004 × ADIP; NDFn corresponds to NDF adjusted for nitrogen, determined from NDIP = -8.77 + (0.33 × CP) + (0.143 × NDF), in which NDIP and ADIP = neutral and acid detergent insoluble protein, determined from the NDF and ADF residues, respectively.

To determine the microbial protein synthesis, total urine collection was conducted between days 15 and 20 of each period, using a two-way Folley catheter no. 20, with

Table 3 - Chemical composition of forages and ingredients of the concentrate

% of dry matter	Sugarcane	HS24	HS48	HS72	Corn	Soybean	Urea
Dry matter[1]	24.03	25.06	25.97	26.80	90.89	91.99	99
Mineral matter	2.95	5.25	5.70	5.87	1.11	6.53	-
Crude protein	3.42	3.53	3.55	3.49	10.36	50.04	281
Ether extract	0.66	0.66	0.72	0.75	3.54	2.23	-
Neutral detergent fiber	55.25	55.08	59.79	61.95	17.10	15.35	-
NDFap	51.87	50.19	55.37	57.04	13.87	12.78	-
Acid detergent fiber	38.55	42.32	37.74	35.28	7.89	11.55	-
NDIN[2]	13.45	15.30	15.77	15.76	8.98	1.37	-
ADIN[2]	11.69	13.88	15.21	15.47	0.58	0.04	-
Lignin	7.44	8.68	7.76	7.18	6.39	2.68	-
NFC	41.57	40.25	34.48	33.13	82.68	11.86	-
Total carbohydrates	92.88	90.54	90.03	89.85	84.98	41.20	-
Total digestible nutrients	59.55	55.93	54.94	55.19	82.38	62.76	-

Sugarcane - fresh sugarcane (time zero); HS24, HS48 and HS72 - hydrolyzed sugarcane stored for 24, 48 and 72 hours, respectively; Corn - finely ground corn grain; Soybean - soybean meal; NDFap - neutral detergent fiber corrected for ash and protein; NDIN - nitrogen insoluble in neutral detergent; ADIN - acid detergent insoluble nitrogen; NFC - Non-fibrous carbohydrates.
[1] % of natural matter.
[2] % of total nitrogen (N).

Table 4 - Chemical composition of experimental diets

% of dry matter	Treatments			
	FS	HS24	HS48	HS72
Dry matter[1]	53.85	54.43	54.94	55.41
Mineral matter	5.64	6.93	7.18	7.28
Crude protein	16.07	16.13	16.14	16.11
Ether extract	1.53	1.54	1.57	1.59
Neutral detergent fiber	35.07	34.75	37.74	38.95
NDFap	33.17	32.03	35.25	36.19
Acid detergent fiber	25.70	27.65	25.32	23.94
Neutral detergent insoluble N[2]	3.65	3.91	3.99	3.97
Acid detergent insoluble N[2]	1.49	1.79	1.98	1.98
Lignin	5.98	6.65	6.16	5.84
Non-fibrous carbohydrates	38.78	37.97	34.76	33.73
Total carbohydrates	76.98	75.57	75.40	75.34
Total digestible nutrients	62.39	60.59	59.74	59.77

FS - diet on fresh sugarcane (time zero); HS24, HS48 and HS72 - diets on hydrolyzed sugarcane stored for 24, 48 and 72 hours, respectively; NDFap - neutral detergent fiber corrected for ash and protein.
[1] % of natural matter.
[2] % of total nitrogen (N).

a 30 mL balloon. A polyethylene hose was adapted to the free side of the catheter, through which the urine was led to a plastic container with a lid, containing 200 mL of H_2SO_4 at 20%. At the end of each 24 hour period, the urine was weighed and measured for volume, then homogenized, and had a 10 mL sample diluted in 40 mL of H_2SO_4 0.036 N and then stored in plastic bottles at -15 ºC. At the end of the experiment, the samples were unfrozen and analyzed for allantoine and uric acid contents by the colorimetric method (Fujihara et al., 1987).

The absorbed purine (X, mmol/d) was estimated from the excretion of purine derivatives (Y, mmol/d), by the equation $Y = 0.85X + 0.385BW^{0.75}$, in which 0.85 is the recuperation of absorbed purine as purine derivatives and $0.385BW^{0.75}$ is the endogenous contribution for purine excretion (Verbic et al., 1990). The ruminal synthesis of nitrogenized compounds (Y, g/d) was calculated from the absorbed purine (X, mmol/d) using the equation of Chen & Gomes (1992): $Y = 70X/(0.83 \times 0.134 \times 1000)$, in which 70 is the content of N in purine (mg/mmol); 0.134 is the purine N/total bacteria N ratio (Valadares et al., 1999); and 0.83 is the digestibility of microbial purine.

The total nitrogen content in urine samples was determined according to the AOAC (1990). The nitrogen balance was calculated by means of the following equations: Absorbed nitrogen (g/d) = consumed N – fecal N; Retained nitrogen (g/d) = absorbed N – urinary N; Biological value (% absorbed N) = (retained N/absorbed N) × 100; and Biological value (% ingested N) – (retained N/ingested N) × 100.

To determine the apparent digestibility in diets, total feces collection was performed manually and before its contact with the floor, between days 15 and 20 of each period. Feces were weighed, sampled (10% of total excreted daily), previously dried in forced ventilation oven at 55 ºC for 72 hours, and ground by a Willey mill with 1 mm sieves. In these samples, the contents of dry matter, as well as mineral matter, crude protein (AOAC, 1990), neutral detergent fiber and acid detergent fiber (Van Soest, 1973; Van Soest et al., 1991) were determined. Digestibility was calculated by the expression: apparent digestibility of nutrients, % = [(nutrient intake – nutrients excreted)/ nutrient intake] × 100.

On the last day of each period, manual collections of the ruminal content were performed in order to measure pH, ammoniacal nitrogen concentration ($N\text{-}NH_3$), before feeding (time zero) and 2, 4, 6, 8, 10 and 12 hours after the morning feed was provided; and to quantify ruminal protozoans. Measurements for pH values were performed in the ruminal

liquid filtered through double-layered gauze, immediately after collection, by using a digital pH-meter. Then, these samples were frozen at -15 ºC for analysis of the N-NH_3 concentration on the following day. The N-NH_3 concentration was determined by distillation with potassium hydroxide 2N, according to the methodology described by Fenner in 1965 and adapted by Vieira (1980).

To quantify the ruminal protozoans, a ruminal content collection was performed (solid + liquid) by ruminal fistula, before the first feeding of the day. A sub-sample was obtained from the material taken off the rumen, and a 10 mL/animal sample was stored in a screw-lidded bottle with a stopper containing 10 mL of formaldehyde 37%. At the end of the trial, the number of protozoans in the samples was determined by quantifying the cells in 50 reticular grids, observed in optical microscopy (100 times), in a Sedgewick-Rafter camera (Dehority, 1993).

The sugarcane pH was determined on the last day of each experimental period. Three forage samples were collected from each hydrolyzed and fresh sugarcane heap right after chopped, then processed in a blender according to the methodology described by Kung Jr. et al. (1984) in order to obtain aqueous extract used to determine pH in the digital pH-meter.

Data were submitted to a normality analysis and, when these presuppositions were met, they were also submitted to analysis of variance, polynomial regression, orthogonal contrast and multiple regression, when necessary. The contrast analysis compared averages between control diets (fresh sugarcane) and hydrolyzed sugarcane diets; the coefficients used for the decomposition of the sum of squares was represented by -3 for diet control (fresh sugarcane) and +1 for each one of the other diets. The value for the determination coefficient was calculated from the sum of squares of the regression considered significant, divided by the total sum of squares recalculated [sum of squares of the regression + sum of squares of the error in variance analysis (pure error)].

For the analysis, the statistical software SAS (Statistical Analysis System, version 8.02) was used, considering a 5% significance level. The general mathematical model was represented by $\gamma_{ij} = \mu + \tau_i + \alpha_j + (\tau^*\alpha)_{ij} + \varepsilon_{ij}$, in which γ_{ij} = dependent variable, μ = overall mean, τ_i = treatment effect, α_j = effect for period j, $(\tau^*\alpha)_{ij}$ = interaction between treatment i and period j; ε_{ij} = residual experimental error. For variables considered repeated measures along time (ruminal pH and N-NH_3), the PROC MIXED feature of SAS statistical software (version 8.02) was used, in which the mathematical model is represented by $\gamma_{ijk} = \mu + \tau_i + £_j(\tau_i) + \alpha_k + (\tau\alpha)_{ik} + \varepsilon_{ijk}$, in which γ_{ijk} = dependent variable, μ = overall mean, τ_i = treatment effect, $£_j(\tau_i)$ = effect of repetition j in treatment i, α_k = effect of period k, $(\tau^*\alpha)_{ik}$ = interaction between treatment i and period k, ε_{ijk} = residual experimental error. For the regression study, the following model was used: $\gamma_{ij} = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \beta_3 X_i^3 + \alpha_j + \varepsilon_{ij}$, where γ_{ij} = dependent variables, β's = regression coefficients, X_i = independent variables, α_j = deviations of regression, and ε_{ij} = residual random error.

Results and Discussion

Dry matter and feed fractions intake values were not changed (P>0.05) by supplying diets of hydrolyzed sugarcane stored for up to 72 hours (Table 5), which is in agreement with the results found by Teixeira Júnior (2008), Domingues (2009), Sforcini (2009), Pancoti (2009) and Alves (2010). However, Dias (2009) and Pina et al. (2010) found higher dry matter intake when supplying hydrolyzed sugarcane with different levels of added lime in diets for crossbred cows and Nellore heifers, respectively. It must be stressed that Moraes et al. (2008a) found lower dry matter intake for Nellore heifers and Holstein Nellore crossbreeds fed hydrolyzed sugarcane with 1% quicklime stored for 24 hours.

Divergence between the studies mentioned may be associated to differences of calcium oxide concentration in

Table 5 - Averages for dry matter intake and feed fractions according to the treatments

Intake	Treatments				CV (%)
	FS	HS24	HS48	HS72	
Dry matter, kg/d	6.25	6.25	6.27	6.02	6.95
Dry matter, % of body weight	1.99	2.03	2.04	2.00	6.49
Crude protein, kg/d	1.02	1.03	1.05	1.02	5.14
Crude protein, % of body weight	0.33	0.33	0.34	0.34	4.69
Total digestible nutrients, kg/d	3.96	3.89	3.84	3.71	6.42
Total digestible nutrients, % of body weight	1.27	1.26	1.25	1.23	6.33
Neutral detergent fiber, kg/d	2.54	2.49	2.69	2.61	9.30
Neutral detergent fiber, % of body weight	0.81	0.81	0.87	0.86	8.80

P>0.05; FS - diet with fresh sugarcane (time zero); HS24, HS48 and HS72 - diets with hydrolyzed sugarcane stored for 24, 48 and 72 hours, respectively; CV - coefficient of variation.

lime; amount of lime applied to sugarcane; conditions for sugarcane hydrolyzation, storage, growth and chopping; dietary roughage:concentrate ratio; breed, animal category and feeding history of the animals, which were not described most of the times. In accordance, Reis & Da Silva (2006) state that the intake of conserved forage is the result of complex interactions involving plant characteristics before processing, inherent factors to the process of forage conservation, changes in nutritional values of the forage during storage and supply to the animals, physical processing of conserved forage and characteristics of the animals fed with the forage.

The lack of variation for the results of the present study on dry matter intake and feeding fractions may be associated to the category used (pubescent heifers), as there was no rejection of lime-added forage, a fact that has been used in some studies (Moraes et al., 2008a) as an explanation (low acceptability of forage) for lower intake in 8 to 12-month-old heifers fed hydrolyzed sugarcane. Another factor relates to the action of lime and the storage of sugarcane, once these procedures do not cause enough difference between chemical characteristics of hydrolyzed and fresh sugarcane (Table 3), so the formulation with approximately 56% of hydrolyzed and fresh sugarcane determine diets whose nutritional characteristics are equivalent (Table 4) for animal intake. This statement was accepted as true, since the chemical characteristics in diets did not take part (P>0.05) in the multiple regression model for dry matter intake. If on the one hand what was exposed above is contrary to the benefit of alkalinizing agents, as they do not improve the forage quality, on the other hand, the conservation of nutritional characteristics of hydrolyzed sugarcane stored for up to 72 hours justifies the use of the technique of hydrolysis with lime, as storing this forage permits greater logistic flexibility, benefiting sugarcane cutting and animal feeding managements.

Another aspect contributing to the similarity between dry matter intake and feeding fractions was associated to the fact that hydrolyzed sugarcane storage did not substantially affect ruminal digestion in heifers, a circumstance confirmed by the similarity (P>0.05) between apparent digestibility of the diets (Table 6).

The similarity for the digestibility results may be attributed to the nutritional characteristics of the diets, the dry matter intake and the ruminal conditions generated by the consumed feed. Digestibility is clearly related to dry matter intake, once, in low quality diets (high fiber content), intake is limited by rumen fill (Van Soest, 1994), in which higher digestibility causes lower ruminal retention of fiber and higher feed intake (Oba & Allen, 1999). On the other hand, a raise in the intake level may increase the passage rate, causing the particles to stay in the rumen for a shorter time and lowering the feed digestibility.

In the literature, studies have reported divergent results for apparent digestibility on hydrolyzed sugarcane diets if compared with fresh sugarcane diets (Dias, 2009; Carvalho et al., 2010; Pina et al., 2010). A number of studies on *in vitro* methodology found an increased sugarcane digestibility by adding lime (Oliveira et al., 2007; Dias, 2009; Mota et al., 2010), though their results are contested due to possible bias associated to *in vitro* methodology, causing divergent results from those obtained by the *in vivo* method (Detmann et al., 2005; Carvalho et al., 2010). Different results may certainly be expected depending whether *in vitro* or *in vivo* methodology is preferred, as most studies *in vitro* evaluated the forage, while most studies *in vivo* evaluated the diets. However, the primary issue is still the search for feeding situations with bovines under hydrolyzed sugarcane diets producing similar results to those obtained in *in vitro* assays.

Because of the similarity between dry matter intake, digestibility and nutritional characteristics of diets, the nitrogen (N) ingested, excreted through feces and urine, absorbed and retained, were not influenced (P>0.05) by storage of hydrolyzed sugarcane (Table 7). Likewise, the biological value of nitrogen did not significantly vary with storage time of sugarcane under addition of 0.5% of hydrated lime. These results were different from those found by Moraes (2006), who found a lower nitrogen retention in heifers fed hydrolyzed sugarcane compared with those fed

Table 6 - Averages for the coefficients of apparent digestibility of the diets

Digestibility, %	Treatments				CV(%)
	FS	HS24	HS48	HS72	
Dry matter	70.37	68.77	67.76	67.03	5.22
Organic matter	71.67	70.27	69.38	69.00	4.96
Crude protein	74.85	76.23	74.95	73.78	5.09
Neutral detergent fiber	51.12	50.03	53.45	52.08	12.29
Acid detergent fiber	49.37	49.22	46.68	45.01	17.35

P>0.05; FS - diet with fresh sugarcane (time zero); HS24, HS48 and HS72 - diets with hydrolyzed sugarcane stored for 24, 48 and 72 hours, respectively; CV - coefficient of variation.

fresh sugarcane. Carvalho et al. (2011) attributes lower intake, balance and biological value of nitrogen to possible changes in ruminal fermentation patterns according to how much calcium oxide has been used (0; 0.75; 1.5; and 2.25%) in sugarcane treatment, a fact that was not detected in the present study.

The urinary volume was not changed (P>0.05) by storage time of hydrolyzed sugarcane (Table 8). However, the amounts of allantoine, uric acid, total purine and absorbed purine reduced linearly (P<0.05) as the storage time of hydrolyzed sugarcane increased. Because of this, the flow of microbial nitrogen compounds into the small intestine, determined by urinary excretion of purine derivatives, lowered linearly according to storage time of sugarcane under addition of 0.5% of hydrated lime. It should be noted that the coefficient of determination for the variables mentioned was low, so other unidentified factors may be associated to such variations.

In spite of the existing variation for purine derivatives and flow of nitrogen compounds into the small intestine, the efficiency of microbial synthesis was unchanged (P>0.05) by storage time of sugarcane under addition of 0.5% of hydrated lime (Table 8). According to Owens & Goetsch (1993), the total microbial production of the rumen, represented in this study by the microbial nitrogen compounds, usually increases with the amount of fermented organic matter in the rumen; the microbial efficiency is independent of microbial production, so these terms must not be confused. Regarding the statement of the authors above, one can deduce that the results on microbial synthesis efficiency are coherent, once there was no difference for digestibility and intake values between diets.

The number of total protozoans and protozoans of *Entodiniun*, *Diplodiniinae* and *Dasytricha* genera was not changed (P>0.05) by hydrolyzed sugarcane stored for up to 72 hours (Table 9). According to Moura Marinho (1982), the number and diversity of protozoans in the rumen is influenced by the type of diet, by ruminal pH and by the relationships established between the protozoans themselves and between them and the bacterial population. So, the results presented may be attributed to equivalence between nutritional characteristics of diets, as no correlation (P>0.05) was found between the number of total protozoans and the genera mentioned with ruminal liquid pH. It can be said that these results have contributed to the existing similarity between diets for digestibility and nutrient ingestion, once the protozoans are closely related to the ruminal digestive process (Van Soest 1994).

The number of protozoans of the genus *Isotricha* presented an adjustment (P<0.05) to the linear regression

Table 7 - Average values for nitrogen (N) intake, N in feces, N in urine, N absorbed, N retained and biological value of nitrogen according to treatments

Items	Treatments				CV (%)
	FS	HS24	HS48	HS72	
N intake, g/d	163.44	164.12	167.54	162.58	5.14
N in feces, g/d	41.02	38.71	42.46	42.19	15.93
N in urine, g/d	72.98	81.76	68.79	77.12	23.93
N absorbed, g/d	122.42	125.41	125.07	120.39	7.47
N retained, g/d	49.45	43.64	56.28	43.27	48.02
Biological value of nitrogen, %[1]	28.59	25.48	32.98	25.88	47.79
Biological value of nitrogen, %[2]	79.96	63.80	87.14	72.02	63.48

P>0.05; FS - diet with fresh sugarcane (time zero); HS24, HS48 and HS72 - diets with hydrolyzed sugarcane stored for 24, 48 and 72 hours, respectively; CV - coefficient of variation.
[1] % of intake N.
[2] % of absorbed N.

Table 8 - Average values for urinary volume, urinary excretion and absorption of purine derivatives, microbial nitrogen compounds (micN) and efficiency of microbial synthesis (Efficiency) according to treatments

Items	Treatments				CV (%)	R^2	Regression equation		
	FS	HS24	HS48	HS72			L	Q	C
Urine, kg	4.83	5.97	5.28	5.66	17.08	-	NS	NS	NS
Allantoine, mmol/d	91.39	85.06	74.61	73.20	11.80	0.23	*	NS	NS
Uric acid, mmol/d	6.94	6.63	6.03	5.30	6.87	0.18	*	NS	NS
Tpurine, mmol/d	98.33	91.69	80.63	78.51	11.15	0.25	*	NS	NS
Abs. purine, mmol/d	104.18	96.38	83.38	80.73	12.78	0.25	*	NS	NS
micN, mmol/d	409.81	379.10	327.98	317.54	12.77	0.25	*	NS	NS
Efficiency[1]	72.93	68.14	59.378	60.37	13.33	-	NS	NS	NS

FS - diet with fresh sugarcane (time zero); HS24, HS48 and HS72 - diets with hydrolyzed sugarcane stored for 24, 48 and 72 hours, respectively; Abs. purine - absorbed purine; Tpurine - total purine; CV - coefficient of variation; R^2 - coefficient of determination; L, Q and C - equation for linear, square and cubic regressions, respectively. *P<0.05; NS = P>0.05; Allantoine = 90.8149 - 0.3473x; micN = 65.24725 - 0.2186x.
[1] g of N/kg of rumen-fermented organic matter.

Table 9 - Averages for the most probable number of ruminal protozoans (cells/mL) according to treatments

Genera	Treatments				CV (%)	R^2	Regression equation		
	FS	HS24	HS48	HS72			L	Q	C
Entodiniun	81.25	91.12	128.50	75.00	5.97	-	NS	NS	NS
Diplodiniinae	1.88	2.25	3.38	2.25	11.81	-	NS	NS	NS
Isotricha	3.12	2.12	1.12	0.50	9.02	0.45	*	NS	NS
Dasytricha	1.12	1.50	2.62	0.50	17.39	-	NS	NS	NS
Total	87.38	97.00	135.62	78.25	5.76	-	NS	NS	NS

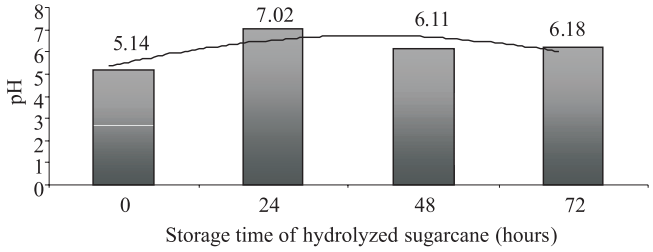
FS - diet with fresh sugarcane (time zero); HS24, HS48 and HS72 - diets with hydrolyzed sugarcane stored for 24, 48 and 72 hours, respectively.
Isotricha, cells/mL = 3.05 - 0.03698x; R^2 = 0.45; *P<0.0001.

equation, with a decrease of 0.04 cells/mL every extra hour during storage time of hydrolyzed sugarcane (Table 9). This result may indicate that protozoans of this genus are more sensitive to variations in the quantity of sugar, once *Isotricha* are associated to ruminal degradation of carbohydrates to fulfill their own energetic demands (Theodorow & France, 2005). Consequently, the quantity of soluble sugar in sugarcane diets may be changed by forage storage, as the saccharose content may be lowered by action of invertase enzymes degrading the saccharose in monosaccharides or by microorganisms (Leuconostoc spp.) which transform saccharose by producing metabolites with high molecular weight such as *dextrana* (Egan, 1969).

The genus *Entodinium* predominated among protozoans, varying from 93% to 96% of the total concentration (Table 6). According to Dehority (1991), 90% of the ruminant fauna are *Entodinium*. In sugarcane diets, the protozoan population is divided in *Holotrichos* (*Isotricha* and *Dasytricha*) and *Entodinium*. Concerning biomass, the *Holotrichos* predominate, with *Isotricha* outdoing *Dasytricha* (Minor et al., 1977; Valdez et al., 1977). On the other hand, no protozoans were found for the genera *Epidinium* and *Charonina* in the ruminal content of the experimental heifers.

Hydrolyzed sugarcane storage influenced (P<0.05) the forage pH quadratically, with highest values 24 hours after lime addition (Figure 1), fact associated with the addition of the alkaline substance in the forage.

As for pH of ruminal liquid, significant interaction was found between the diets and the moment when the ruminal content was collected (Figure 2). For heifers fed fresh sugarcane and hydrolyzed sugarcane stored for 24 and 48 hours, the pH of ruminal liquid varied quadratically (P<0.05) according to the moment of ruminal content collection, with minimum estimated after 8.3, 10.0 and 9.0 hours after the first feeding of the day, respectively. In heifers fed hydrolyzed sugarcane stored for 72 hours, the pH of ruminal fluid decreased linearly (P<0.05) according to the moment of ruminal content collection (Figure 2).

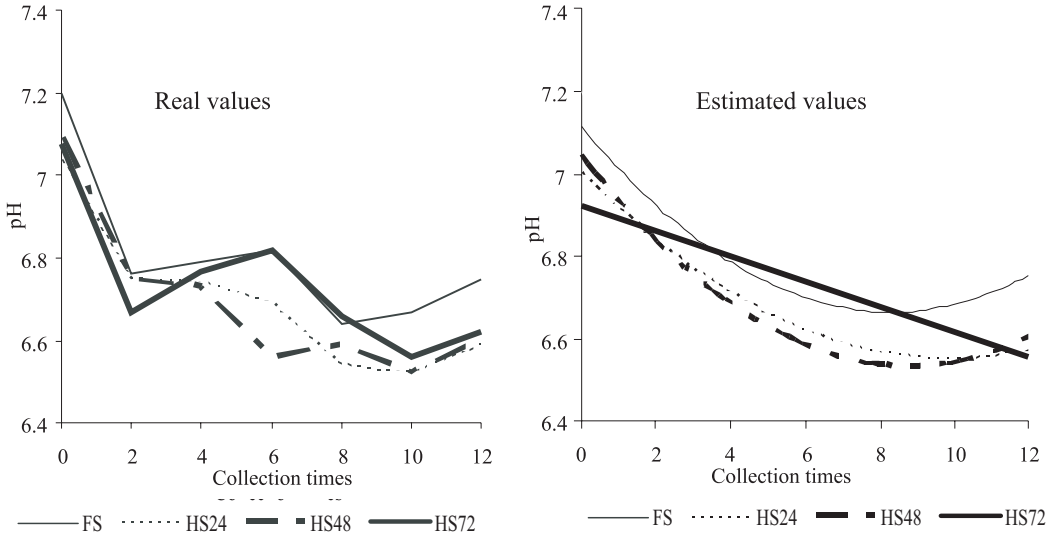


pH = 5.33 + 0.066x - 0.00078x^2; R^2 = 0.60; CV = 15.44%; P = 0.0003.
CV - coefficient of variation.

Figure 1 - Sugarcane pH right after chopping (time zero) and for hydrolyzed sugarcane stored for 24, 48 and 72 hours, respectively.

It must be highlighted that the average data for ruminal fluid pH, related to treatments, were not adjusted (P>0.05) to the tested regression models. The average values found were 6.80, 6.70, 6.69 and 6.74 for fresh and hydrolyzed sugarcane diets after 24, 48 and 72 hours of storage, respectively. However, when a contrast analysis was performed (-3 + 1 + 1 + 1), it was found that the ruminal fluid pH for heifers fed fresh sugarcane was higher (P<0.05) than in heifers fed hydrolyzed sugarcane, which was an unexpected result, once lime had been added to the forage. According to the multiple regression analysis, variations in ruminal fluid pH resulted from a combination of factors, so 86% of the variation in this variable was associated to dry matter digestibility, dry matter intake, ammoniacal nitrogen, ingested and absorbed nitrogen, microbial nitrogen and ruminal protozoans of the genus *Diplodiniinae* (Table 10).

Ruminal pH is associated with dry matter digestibility and intake, as higher ingestion and digestion increases the concentration of volatile fatty acids in the rumen, decreasing the pH level (Van Soest, 1994). An increase in the concentration of ruminal ammonia also increases the ruminal wall absorption rate, caused by a difference of ammonia concentration between the rumen and the bloodstream and also by the buffer effect of ammonia, which increases



FS - diets with fresh sugarcane (time zero); HS24, HS48 and HS72 - diets with hydrolyzed sugarcane stored for 24, 48, 72 hours, respectively; FS = 7.11 - 0.11x + 0.006x^2, R^2 = 0.76; HS24 = 7.00 - 0.09x + 0.005x^2, R^2 = 0.91; HS48 = 7.05 - 0.12x + 0.007x^2, R^2 = 0.93; HS72 = 6.92 - 0.031x, R^2 = 0.63.

Figure 2 - Interaction extension between hydrolyzed sugarcane storage and moment of collection of ruminal fluid for real and estimated ruminal liquid pH values.

ruminal pH, fostering its absorption (Fernandez et al., 1990). Ruminal protozoans influence ruminal pH, as they digest starch in a slower pace than bacteria and are lactate fermenters, decreasing depression of ruminal pH (Church, 1979).

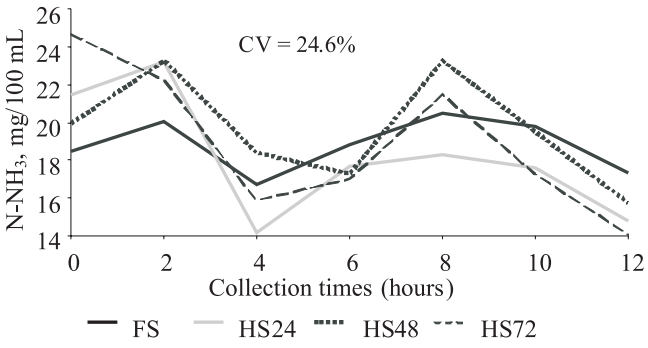
The content of ammoniacal nitrogen in different diets varied independently (P>0.05) at the moment of ruminal content collection (Figure 3). No significant difference was found between treatment averages. The average values observed were 19.09, 18.16, 19.62 and 18.92 mg of N-NH_3/100 mL for heifers fed fresh sugarcane, hydrolyzed sugarcane stored for 24, 48 and 72 hours, respectively. These values were higher than the minimum recommended (5 mg of N/100 mL to keep normal function of the rumen (Satter & Slyter, 1974), and than the value considered appropriate (10 mg of N/100 mL) for maximum ruminal fermentation (Van Soest, 1994). However, an optimal level of N-ammoniacal should not be considered as a fixed number, as the capacity of rumen bacteria to synthesize protein and use ammonia depends on the fermentation rate of carbohydrates and their synchronization with degradation proteins (Van Soest, 1994).

The results obtained in this study for N-NH_3 are in agreement with those found by Moraes (2006), who found changes in average values for this variable in heifers fed fresh sugarcane and hydrolyzed sugarcane with 1% of quicklime stored for 24 hours. For crossbreed cows fed fresh sugarcane, Dias (2009) found high values N-NH_3

Table 10 - Partial and total determination coefficients (R^2) for the main variables entered the multiple regression model for ruminal fluid pH

Variables	Partial R^2	Total R^2
Dry matter digestibility (DMD)	0.19	0.19
Absorbed nitrogen (AbsorN)	0.17	0.36
Dry matter intake (DMI)	0.15	0.51
Microbial nitrogen (MicN)	0.11	0.62
Ingested nitrogen (IngesN)	0.10	0.72
Number of ruminal protozoans of *Diplodiniinae* genus (Diplo)	0.08	0.80
Ammoniacal nitrogen (N-NH_3)	0.06	0.86

Ruminal liquid pH = -0.03DMD - 0.04AbsorN + 1.54DMI - 8.81MicN - 4.92IngesN + 0.08Diplo + 0.003N-NH_3; P<0.0001.

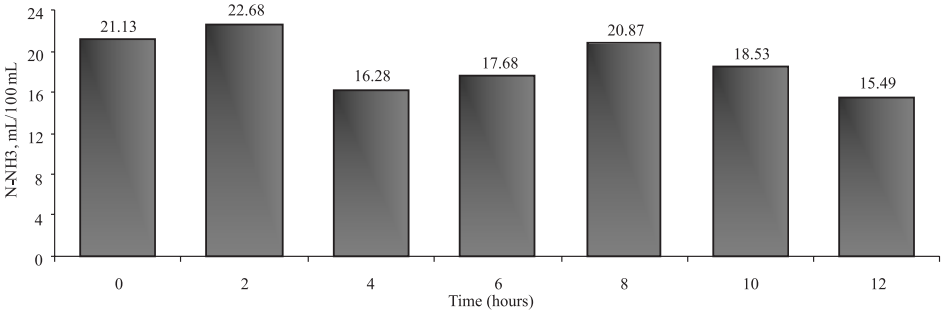


FS - diets with fresh sugarcane (time zero); HS24, HS48 and HS72 - diets with hydrolyzed sugarcane stored for 24, 48 and 72 hours, respectively; CV - coefficient of variation.

Figure 3 - Ammonical nitrogen according to diets and ruminal content collection moment.

(>25 mL) in a period of 3 hours after feeding, compared with diets of hydrolyzed sugarcane with hydrated lime. According to this author, the results indicate a loss of N (process not identified by the author) in diets with fresh sugarcane, suggesting that sugarcane treatment with crescent lime doses may potentialize ruminal fermentation and improve sugarcane use by the ruminant. In the present study, such a supposition cannot be made, once the N-NH_3 in diets with fresh sugarcane kept intermediate and more constant along time than the other diets (Figure 3).

The N-NH_3 content varied cubically for ruminal content collection time, with minimum and maximum for 4 and 8 hours after providing the first feeding of the day (Figure 4). According to Nolan & Dobos (2005), the concentrations of N-NH_3 in the ruminal content tend to increase between 2 and 4 hours after each feeding. However, microbial assimilation of peptides, amino acids and N-NH_3 reduces maximum potential concentrations of ammoniacal nitrogen; its concentration along time depends on the degradability of feed protein and the microbial growth.



$Y = 22.23 - 2.092x + 0.383x^2 - 0.021x^3$; $R^2 = 0.55$; $P<0.05$; CV = 22.90. CV - coefficient of variation.

Figure 4 - Average values for N-NH_3 for collection time.

Conclusions

Sugarcane treated with 0.5% of hydrated lime and stored for up to 72 hours does not change ruminal digestion to the point that the amount of feed consumed by a pubescent Nellore heifer is changed. Thus, lime is a viable technology, once it allows long storage time and bee control on treated forage, which contributes to animal feeding logistics.

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