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# Characteristics of forage and feeding behavior of Nellore heifers fed hydrolyzed sugarcane[1]

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**ABSTRACT** - The objective of this study was to evaluate the chemical characteristics of the forage and ingestive behavior of Nellore heifers fed hydrolyzed sugarcane in different periods of storage. Twenty-four heifers with initial body weight of 119.6±8.1 kg were utilized. The experimental design was completely randomized, in which the treatments were diets with fresh sugarcane and hydrolyzed sugarcane (5 g of lime $kg^{-1}$ of chopped sugarcane) stored for 24, 48 or 72 hours as the only roughage. The addition of lime to sugarcane associated with its storage up to 72 hours provided an increase of 20% of the potentially degradable cell wall of carbohydrates, from 382.4 to 458.8 g $kg^{-1}$ of total carbohydrates. The *in vitro* digestibility of dry matter was altered by the storage of hydrolyzed sugarcane, increasing 7.08% when the storage time was increased from 24 to 72 hours. Heifers fed fresh sugarcane remained more time consuming compared with heifers fed other diets. The time used for water intake was not influenced by the diet. The rumination time presented a quadratic variation in relation to storage time of the hydrolyzed sugarcane, with higher values for the of hydrolyzed sugarcane diets stored for 48 hours. Heifers fed hydrolyzed sugarcane spent more time on other activities than those fed fresh sugarcane. The supply of hydrolyzed sugarcane stored up to 72 hours in the proportion of 600 g $kg^{-1}$ of dry matter in the diet, alters the intake patterns, reducing the feed intake in Nellore heifers.

Key Words: digestibility, hydrated lime, idle, nonstructural carbohydrates, rumination

## Introduction

The intensification of studies with sugarcane treated with microprocessed lime is relatively recent (Anualpec, 2009) and has been associated with improvement in the forage nutritional value due to beneficial changes in the chemical composition and digestibility of the fiber (Oliveira et al. , 2007; Pires et al., 2010). The action of alkaline agents occurs in the fiber, promoting the rupture of hydrogen bonds, leading to expansion of the cellulose molecules and hemicellulose solubilization due to the rupture of bonds of esters with lignin, making these fractions more susceptible to the action of cellulase enzymes (Reis et al., 1993; Van Soest, 1994). Moreover, there is rupture of the bonds between carbohydrate and lignin cell wall and hydrolysis of cell wall polysaccharides, resulting in release of soluble sugars (Van Soest, 1994).

The treatment of sugarcane with lime also allows the storage of forage for relatively long periods, improving cutting logistics of forage. However, little is known about the effects of adding lime to the sugarcane and its storage on the ingestive behavior of cattle. The ingestive behavior of ruminants is characterized by uneven distribution of a succession of periods defined and discrete activities, classified as ingestion, rumination and idleness (Penning et al., 1991). Among them, the ingestion is the most important for ruminants, which respond differently to many kinds of feeds and diets, changing levels of production and their feeding behavior (Pires et al., 2001).

Ruminants, like other species, seek to adjust the feed intake to their nutritional requirements, especially energy (Arnold, 1985). Confined animals spend about one hour consuming feed rich in energy, or more than six hours, for sources with low energy (Van Soest, 1994). The increase in the consumption of forage changes the time spent ruminating, as this characteristic is influenced by the diet, and is proportional to the content of the cell wall of roughages (Van Soest, 1994). Therefore, the objective of this study was to evaluate the chemical characteristics of the forage and ingestive behavior of Nellore heifers fed hydrolyzed sugarcane at different periods of storage.

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## Material and Methods

The study was conducted between September 6nd and December 15th, 2008, at Faculdade de Ciências Agrárias e Veterinárias, in Jaboticabal, São Paulo, located at 21°14'05" South latitude and 48°17'09" West longitude, at an altitude of 613.98 m. The soil is classified as a clay Oxisol (Red Latosol) of medium fertility. According to the Köppen classification, the climate is Awa type, with hot summers and dry winters. The maximum, minimum and average temperature (ºC), relative humidity (%) and precipitation (mm) of experimental periods were of 31.22, 18.00, 23.65, 67.08 and 109.08, respectively.

Twenty-four Nellore heifers, weaned at seven months of age, were used. After weaning, heifers were maintained on *Brachiaria brizantha* cv. Marandu pasture with mineral supplementation; at the beginning of the experiment they were nine months old and had 119.6±8.1 kg of body weight. The heifers were confined in individual stalls (14 $m^2$) partly covered, with concrete floor, equipped with individual feeders and drinkers. Prior to the experimental period, heifers were distributed (six replicates) in the treatments composed of the experimental diets (Table 1) and adapted to the facilities, diets and artificial lighting at night for 17 days.

Table 1 - Composition of experimental diets

| g $kg^{-1}$ of dry matter | Fresh sugarcane (time zero) | Hydrolyzed sugarcane stored | | |
|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours |
| Sugarcane | 610.0 | 610.0 | 620.0 | 630.0 |
| Finely ground corn grain | 164.5 | 164.5 | 160.2 | 156.0 |
| Soybean meal | 200.6 | 200.6 | 195.4 | 190.3 |
| Mineral nucleus[1] | 3.2 | 3.2 | 3.1 | 3.0 |
| Calcitic limestone | 3.9 | 3.9 | 3.8 | 3.7 |
| Urea | 17.9 | 17.9 | 17.4 | 17.0 |

[1] Levels guaranteed: P - 40 g; Ca - 146 g; Na - 56 g; S - 40 g; Mg - 20 g; Cu - 350 mg; Zn -1300 mg; Mn - 900 mg; Fe - 1050 mg; Co - 10 mg; I - 24 mg; Se - 10 mg; F (max.) 400 mg; excipient q.s.: 1000 mg.

The sugarcane (IAC 86-2480) was grown in the Cattle Farming Department of Faculdade de Ciências Agrárias e Veterinárias, in Jaboticabal, SP, Brazil. On December 17th, 2007, it was subjected to cover fertilization (400 kg of nitrogen $ha^{-1}$) and plague control by a tractor-pulled cultivator. The sugarcane was cut at 10-12 months of age (3rd cut) and disintegrated to obtain a particle size of 2-3 cm. Once disintegrated, the sugarcane was spread in layers of about 20 cm onto a concrete floor in covered shed and added to the suspension of hydrated lime (partial composition: MgO = 1.5% total CaO = 72.5% ($Ca(OH)_2$ = 95.5%) with 500 g of lime in 2 liters of water per 100 kg of sugarcane. After the suspension homogenization of the lime with the sugarcane, the material was heaped (approximately 500 kg and 80 cm high), so that, from the same heap hydrolyzed sugarcane was obtained and stored for 24, 48 or 72 hours. For the diet containing fresh sugarcane, the forage was provided immediately after disintegration.

The diets were formulated for a body weight gain of 750 g $day^{-1}$, with an estimated intake of 2.4% of body weight according to the NRC (1996). The diets were formulated to have the same protein value (140 g crude protein $kg^{-1}$ dry matter). The feed intake was recorded daily by weighing the feed supplied (roughage and concentrate) and leftovers, and the supply was maintained at 10% above the intake and provided in two daily meals (at 8h00 and 14h00), mixing the roughage and concentrate inside the feeders. Feed and leftovers were sampled weekly, and samples were pre-dried in a forced-ventilation oven at 55 °C for 72 hours and processed in a knife mill (1 mm). After milling, the feedstuffs were analyzed for chemical composition (Tables 2 and 3).

The dry matter (DM), crude protein (CP), ether extract (EE) and mineral matter (MM) were determined by the AOAC (1990). The neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were

Table 2 - Chemical composition of roughages and ingredients of the concentrate

| g $kg^{-1}$ of dry matter | Fresh sugarcane (time zero) | Hydrolyzed sugarcane stored | | | Finely ground corn grain | Soybean meal | Urea |
|---|---|---|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours | | | |
| Dry matter, g $kg^{-1}$ of natural matter | 259.7 | 256.7 | 273.8 | 283.3 | 850.0 | 873.1 | 990.0 |
| Mineral matter | 37.3 | 58.4 | 58.8 | 59.4 | 11.6 | 57.1 | -- |
| Crude protein | 35.9 | 38.5 | 36.7 | 35.7 | 84.4 | 482.4 | 2810.0 |
| Ether extract | 12.2 | 12.7 | 12.9 | 13.1 | 41.8 | 21.0 | -- |
| Neutral detergent fiber | 534.3 | 550.8 | 548.9 | 574.7 | 178.5 | 184.3 | -- |
| NDFap | 505.6 | 511.4 | 505.0 | 545.9 | 148.3 | 165.2 | -- |
| Acid detergent fiber | 324.0 | 343.0 | 332.0 | 351.0 | 51.1 | 130.0 | -- |
| NDIN, % of total nitrogen | 384.9 | 409.1 | 423.0 | 455.3 | 6.3 | 5.4 | -- |
| ADIN, % of total nitrogen | 205.3 | 194.1 | 206.5 | 233.5 | 2.4 | 3.2 | -- |
| Lignin | 72.3 | 59.7 | 55.4 | 59.2 | 0.6 | 44.9 | -- |
| Non-fibrous carbohydrates | 363.0 | 319.5 | 330.2 | 298.3 | 583.7 | 200.6 | -- |
| Total carbohydrates | 914.6 | 890.4 | 891.6 | 891.8 | 862.2 | 384.9 | -- |
| Starch | 123.6 | 116.7 | 72.2 | 120.3 | 865.9 | 84.0 | -- |
| Total digestible nutrients | 582.4 | 583.9 | 590.7 | 576.4 | 831.9 | 699.6 | -- |

NDFap - neutral detergent fiber corrected for ash and protein; NDIN - neutral detergent insoluble nitrogen; ADIN - acid detergent insoluble nitrogen.

determined according to Licitra et al. (1996). The contents of neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin were determined using the procedures of Van Soest (1973) and Van Soest et al. (1991), and the NDF was corrected for ash and protein (NDFap). The carbohydrate fractions of the forage were obtained using the equations of Sniffen et al. (1992), in which: total carbohydrates (TC, g $kg^{-1}$ of DM) = 1000 - CP (g $kg^{-1}$ of DM) - EE (g $kg^{-1}$ of DM) - MM (g $kg^{-1}$ of DM); non-fibrous carbohydrates (NFC, g $kg^{-1}$ of DM) = 100 - NDFap (g $kg^{-1}$ of DM) - CP (g $kg^{-1}$ of DM) – EE (g $kg^{-1}$ of DM) - MM (g $kg^{-1}$ of DM); fraction C (g $kg^{-1}$ of TC) = 1000 * [NDFap (g $kg^{-1}$ of DM) * 0.001 * lignin (g $kg^{-1}$ of NDF) * 2.4]/TC; fraction B2 (g $kg^{-1}$ of TC) = 1000 * [NDFa (g $kg^{-1}$ of DM) - NDIP (g $kg^{-1}$ of CP) * 0.001 * CP (g $kg^{-1}$ of DM) - NDFap (g $kg^{-1}$ of DM) * 0.001 * lignin (g $kg^{-1}$ NDF) * 2.4]/TC, in which: NDFa = ash-free neutral detergent fiber; and NDIP = neutral detergent insoluble protein; fraction B1 (g $kg^{-1}$ of TC) = starch (g $kg^{-1}$ of NFC) * [1000 - B2 (g $kg^{-1}$ of TC) - C (g $kg^{-1}$ of TC)]/1000; fraction A (g $kg^{-1}$ of TC) = [1000 - starch (g $kg^{-1}$ of NFC)] * [1000 - B2 (g $kg^{-1}$ of TC) - C (g $kg^{-1}$ of TC)]/1000. The starch content was estimated using Hendrix methods (1993) for extraction, and dinitrosalicinic acid (DSA) for colorimetric reading (Miller, 1959). The content of total digestible nutrients was estimated according to the equations of Weiss (1993).

In the heaps of hydrolyzed sugarcane, the internal temperature was evaluated by the infrared thermometer fitted with metal rod, in three different points, before the first feeding of the day. The temperature of the fresh sugarcane was measured after the disintegration and the temperature of the diet was measured immediately after the supply of feed in the morning.

Data collection concerning the ingestive behavior occurred during the period of confinement of animals, in six days, in which, in each trial, 48 consecutive hours of visual evaluation were done, by the method of scan sampling (Martin & Bateson, 1986), at ten-minute intervals. The behavioral variables observed and recorded were composed by the time of feed and water consumption, ruminating and time in other activities. Chews per bolus and chewing time per bolus were also recorded by visual counting and digital timer, respectively..

From the consumption variables and ingestive behavior, the following ratios were determined (Polli et al., 1996; Burger et al., 2000): feeding efficiency (dry matter intake/feeding time), rumination efficiency of dry matter (dry matter intake/rumination time), rumination efficiency of neutral detergent fiber (neutral detergent fiber intake/rumination time), daily chewing time (time spent eating + rumination time); daily ruminated boluses (rumination time/chewing time per bolus); daily chews (chews per bolus * daily ruminated boluses). Feeding frequency was determined by the number of times the animals reached the feeders to eat and the number of daily meals was determined considering 20 minutes as the minimum time to perform this activity.

To estimate the *in vitro* digestibility, the rumen fermenter Daisy II methodology (Ankon®) described by Holden (1999) was used. To collect the ruminal fluid, two rumen-fistulated Nellore cattle were used, fed *ad libitum* with water and diet containing 300 g $kg^{-1}$ dry matter of the fresh sugarcane and 300 g $kg^{-1}$ dry matter of hydrolyzed sugarcane, and concentrated fraction (400 g $kg^{-1}$ dry matter) similar to the feedlot period.

The experimental design used was completely randomized with four treatments (diets) and six replicates (animals) and three periods (time repeated measures) for the ingestive behavior and diet temperature. For the fractionation of carbohydrates and *in vitro* digestibility, four replicates were used and for internal temperature of heaps, three replicates per period were used. The data were analyzed for normality and whenever necessary, transformed by $\log^2$. Once the assumptions for normality and homogeneity of variances were satisfied, data were submitted to variance analysis, orthogonal contrasts and Pearson correlation. For the contrast analysis, the sum of treatment squares was decomposed into three orthogonal contrasts: fresh versus hydrolyzed sugarcane (-3 1 1 1) and a linear (0 -1 1 0) and quadratic (0 -1 2 - 0) effects of the time of hydrolysis.

Table 3 - Chemical composition of experimental diets

| g $kg^{-1}$ of dry matter | Fresh sugarcane (time zero) | Hydrolyzed sugarcane stored | | |
|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours |
| Dry matter[1] | 515.0 | 513.2 | 517.1 | 516.8 |
| Mineral matter | 58.6 | 71.5 | 71.4 | 71.5 |
| Crude protein | 147.4 | 149.0 | 145.0 | 141.5 |
| Ether extract | 19.1 | 19.4 | 19.4 | 19.3 |
| Neutral detergent fiber | 413.1 | 423.1 | 425.2 | 444.8 |
| NDFap | 369.0 | 372.6 | 375.1 | 401.5 |
| Acid detergent fiber | 233.9 | 245.5 | 241.2 | 255.6 |
| N insoluble in neutral detergent[2] | 236.9 | 251.7 | 264.3 | 288.8 |
| N insoluble in acid detergent[2] | 126.3 | 119.4 | 129.0 | 148.1 |
| Lignin | 53.2 | 45.5 | 43.2 | 45.9 |
| Non-fibrous carbohydrates | 364.9 | 338.4 | 344.5 | 324.0 |
| Total carbohydrates | 788.5 | 773.8 | 777.5 | 780.6 |
| Starch | 243.1 | 238.9 | 208.2 | 234.9 |
| Total digestible nutrients | 647.1 | 648.0 | 650.6 | 640.0 |

NDFap - neutral detergent fiber corrected for ash and protein.
[1] g $kg^{-1}$ of natural matter.
[2] % of total nitrogen (N).

For the analysis, software SAS (Statistical Analysis System, version 8.02) was used adopting the significance level of 0.05. The general mathematical model was represented by: $\gamma_{ijkl} = \mu + \tau_i + £_j(\tau_i) + \alpha_k + (\tau\alpha)_{ik} + \varepsilon_{ijkl}$, in which: $\gamma_{ijkl}$ = dependent variable; $\mu$ = overall mean; $\tau_i$ = treatment effect i; $£_j(\tau_i)$ = effect of repetition j within treatment i; $\alpha_k$ = effect of period k; $(\tau\alpha)_{ik}$ = interaction between treatment i and period k; $\varepsilon_{ijkl}$ = experimental residual error.

## Results and Discussion

The temperature inside the heaps was increased (P<0.05) by adding hydrated lime to the fresh sugarcane, in which storage of hydrolyzed sugarcane had a quadratic effect of this heap feature, with maximum temperature 48 hours after the addition of lime (Table 4). Domingues (2009), evaluating different doses of quicklime (0, 5, 10, 15 and 20 g $kg^{-1}$ of sugarcane) and periods of the sugarcane storage (0, 24, 48, 72 and 96 hours), showed a maximum temperature of 41.3 ºC, 56 hours after the addition of lime, and after this period there was a decrease in the temperature, which tended to stabilize between 25 and 30 ºC. The differences between the results of this study and those of Domingues (2009) may be related to the size of heaps, since the maximum temperature in that study was measured in samples of two kilograms conditioned in a climatic chamber at 25 °C to evaluate the aerobic stability.

The temperature inside the heaps of the hydrolyzed sugarcane was largely related to environmental conditions, to the action of wind and the size of the heaps (approximately 500 kg). It should be noted that the factors that determine variations in the internal temperature inside heaps of hydrolyzed sugarcane are not well known, a fact to be investigated in future studies. However, it is believed that in large heaps (≥500 kg) the surface layer of sugarcane can act as insulating from the environmental factors previously mentioned, by reducing moisture and heat losses of forage located internally, providing conditions favorable to anaerobic fermentation and high internal temperature. On the other hand, due to the presence of $O_2$ in the outer part, part of the microbial activity can be represented by yeasts and fungi, which obtain energy by the oxidation with concomitant heat production. In addition, the temperature of the hydrolyzed sugarcane may be modified by addition of lime suspension, due to the heat release at the time of mixing of the lime with water (exothermic reaction).

The diet temperature immediately after the supply was lower (P<0.05) for diets with fresh sugarcane, not modified by the storage of hydrolyzed sugarcane (Table 4). According to Moraes et al. (2008), the high temperature of the sugarcane treated with quicklime, when compared with the fresh, contributed to the lower intake of nutrients in heifers. However, this explanation cannot be considered in this study, because, after weighing the forage and mixing it with the concentrate, the diets with hydrolyzed sugarcane showed an average reduction of temperature of 67.03%. Moreover, it can be noted that no visual change was observed in relation to the rejection of the diet the animals immediately after the supply. It is worth stressing that the temperature of the diet in the feeders, at least initially, tends to quickly approach the ambient temperature, which was benefited by the manipulation of feed during the supply and feed intake of the animals.

For the fractionation of carbohydrates, it was found that the fraction A, represented by the water soluble carbohydrates, particularly sucrose and glucose and fraction B1, composed by starch and pectin rapidly fermented by ruminal bacteria (Sniffen et al., 1992) were not changed (P>0.05) by the addition of hydrated lime to fresh sugarcane and the hydrolysis time (Table 5).

The lack of variation for the carbohydrate fraction was not expected, because decrease in soluble carbohydrates during the storage time of the hydrolyzed sugarcane was expected, due to its use by yeasts and fungi present in the heaps. These results may be indicating that the addition of lime to sugarcane allowed the reduction of microorganisms in heaps of hydrolyzed sugarcane, agreeing with the results obtained by Domingues (2009).

Table 4 - Variables related to the internal temperature of the diets and internal temperature of heaps of fresh sugarcane (FS) and of hydrolyzed sugarcane stored for different periods

| Temperature | Fresh sugarcane (time zero) | Hydrolyzed sugarcane stored | | | CV (%) | Orthogonal contrasts[1] | | |
|---|---|---|---|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours | | FS vs HS | Linear | Quadratic |
| Internal temperature of heaps, ºC | 22.00c | 48.33b | 55.66a | 48.00b | 8.63 | 0.0001 | 0.5415 | 0.0487 |
| Internal temperature of the diets, ºC | 22.67b | 33.17a | 34.50a | 33.83a | 6.56 | 0.0001 | 0.4553 | 0.4987 |

Means followed by different lowercase letters in the row differ (P<0.05) by analysis of orthogonal contrasts.
CV - coefficient of variation.
[1] Probability for the contrasts: FS vs HS = fresh versus hydrolyzed sugarcane (-3 1 1 1) and linear (0 -1 1 0) and quadratic (0 -1 2 - 0) effects of the time of hydrolysis.

The B2 fraction represented by the portion of digestible cell walls (cellulose and hemicellulose), with slower degradation rate compared with the fraction B1 (Sniffen et al., 1992), was affected (P<0.05) by the addition of hydrated lime to the fresh sugarcane, increasing linearly (P<0.05) with the increasing storage time of hydrolyzed sugarcane (Table 5). These results indicate that the addition of hydrated lime to the sugarcane promoted hydrolysis of the chemical bonds (hydrogen bonds and ester bonds) between the cell wall constituents of the forage, increasing the amount of structural carbohydrates which can be degraded, a fact that has been associated with studies about hydrolyzing agents (Jackson, 1977; Klopfenstein, 1978; Reis et al., 1993).

Carbohydrate fraction C, however, was not affected (P>0.05) by the addition of hydrated lime and the storage time of hydrolyzed sugarcane (Table 5). According to Sniffen et al. (1992), fraction C is the indigestible carbohydrates portion in the gastrointestinal tract of animals, obtained from the lignin content of the feed, which, according Klopfenstein (1978), is not altered by the process of hydrolysis.

The *in vitro* digestibility of dry matter and neutral detergent fiber was not affected (P>0.05) by adding lime to fresh sugarcane; however, the *in vitro* digestibility of dry matter increased as a function of the time of storage of hydrolyzed sugarcane (Table 6). These results differ from those found by Domingues (2009), who evaluated the *in vitro* digestibility of hydrolyzed sugarcane (5 g lime $kg^{-1}$ of sugarcane) at different storage times (0, 24, 48, 72 and 96 hours) and found no significant change for the same variables mentioned. However, with respect to *in vitro* digestibility, most studies have demonstrated improvement in the digestibility of sugarcane with the addition of lime (Oliveira et al., 2007; Pires et al. 2010; Dias, 2009).

An important aspect about the digestibility of feeds is associated to the dry matter intake. Thus, in situations of feeding with low quality diets (high fiber), intake is limited by rumen fill (Van Soest, 1994), in which the increase in digestibility promotes a lower retention time of ruminal fiber and increased feed intake (Oba & Allen, 1999). On the other hand, in situations in which the rumen fill is not limiting, the increase in digestibility negatively affects the intake, due to higher release of energy per kilogram of dry matter of fermented feed, promoting satiety (chemiostatic control). In this study, these aspects were not observed, since there was a significant correlation between the *in vitro* digestibility of sugarcane with the dry matter intake (Table 9).

The times of feed and water intake were not affected by the storage time of hydrolyzed sugarcane (Table 7). On the other hand, it was found that heifers fed fresh sugarcane remained (P<0.05) feeding longer than the heifers fed the other diets. Dias (2009) evaluated the *in vivo* digestibility of diets, feed intake and feeding behavior of crossbred cows fed hydrolyzed sugarcane with different levels of lime (0, 8, 16 and 24 g lime $kg^{-1}$ of sugarcane) applied before the supply, and did not find changes in the time that the animals remained eating, but found a quadratic response of the dry matter intake according to the levels of lime, which was associated with improvement in diet digestibility and efficiency of rumination.

Table 5 - Variables related to the carbohydrate fractions of fresh sugarcane and the hydrolyzed sugarcane stored for different periods

| Fractions[2] | Fresh sugarcane (time zero) | Hydrolyzed sugarcane stored | | | CV (%) | Orthogonal contrasts[1] | | |
|---|---|---|---|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours | | FS vs HS | Linear | Quadratic |
| A | 305.60 | 286.80 | 306.40 | 245.00 | 8.63 | 0.3426 | 0.4859 | 0.1871 |
| B1 | 137.70 | 132.10 | 121.30 | 136.30 | 6.56 | 0.0843 | 0.8750 | 0.1125 |
| B2 | 382.40d | 393.00c | 413.70b | 458.80a | 9.90 | 0.0405 | 0.0430 | 0.6590 |
| C | 174.30 | 188.10 | 158.60 | 159.90 | 23.27 | 0.1578 | 0.9424 | 0.2289 |

Means followed by different lowercase letters in the row differ (P<0.05) by analysis of orthogonal contrasts.
CV - coefficient of variation.
[1] Probability for the contrasts: FS vs HS = fresh versus hydrolyzed sugarcane (-3 1 1 1) and linear effect (0 -1 1 0) and quadratic (0 -1 2 - 0) effects of the time of hydrolysis.
[2] kg of dry matter $kg^{-1}$ of total carbohydrate.

Table 6 - Variables related to *in vitro* digestibility of fresh sugarcane and hydrolyzed sugarcane stored for different periods

| Digestibility | Fresh sugarcane (time zero) | Hydrolyzed sugarcane stored | | | CV (%) | Orthogonal contrasts[1] | | |
|---|---|---|---|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours | | FS vs HS | Linear | Quadratic |
| Dry matter | 632.49 | 622.74c | 633.44b | 666.80a | 8.63 | 0.3575 | 0.0194 | 0.4234 |
| Neutral detergent fiber | 367.10 | 375.74 | 361.51 | 365.50 | 6.56 | 0.9589 | 0.4126 | 0.5279 |

Means followed by different lowercase letters in the row differ (P<0.05) by analysis of orthogonal contrasts. DM - *in vitro* dry matter digestibility (g $kg^{-1}$ of dry matter); NDF - *in vitro* neutral detergent fiber digestibility (g $kg^{-1}$ of dry matter); CV - coefficient of variation.
[1] Probability for the contrasts: FS vs HS = fresh versus hydrolyzed sugarcane (-3 1 1 1) and linear effect (0 -1 1 0) and quadratic (0 -1 2 - 0) effects of the time of hydrolysis.

Feeding time can be considered the main activity responsible for the amount of feed ingested; however, in this study, no significant correlation of the feeding time with the dry matter intake and neutral detergent fiber intake was found (Table 9). Otherwise, it was found that feeding time was correlated (P<0.05) with the supply frequency (r = 0.85) and feed efficiency (r = 0.57), indicating that the intake of nutrients was associated with the presence of the heifers in the feeders and the time of feed selection by the animals. These results infer that the storage of sugarcane with hydrated lime limited the intake, which was probably related to the forage acceptability by the animals (psychogenic mechanism of intake control). According to Mertens (1994) this mechanism involves the responses of animal behavior to inhibitor factors or stimulators of feed (taste, odor, texture, visual appearance) or environmental factors (emotional status of the animal, social interaction and learning) not related to diet energy or filling power. It is believed that the ingestion of diets consisting of hydrolyzed sugarcane hydrolyzed with lime may be associated with this roughage acceptability by young Zebu cattle, since these restrictions were not observed in the literature when the experiments were conducted with heifers or cows of dairy breeds (Carvalho, 2008; Sforcini, 2008; Teixeira Junior, 2008) or cross-European breeds (Dias, 2009; Domingues, 2009; Alves, 2010).

Another aspect to be highlighted is the lack of correlation (P>0.05) between feeding time with the digestibility and the fraction B1 of the sugarcane carbohydrates (Table 9), demonstrating that changes in *in vitro* digestibility of dry matter and chemical composition of the hydrolyzed sugarcane were not enough to encourage heifers to remain feeding. According to Allen (2000) increased digestibility results in reduced rumen pH, increased osmolarity and greater absorption of volatile fatty acids, causing faster satiety to the animal. This fact, according to the author, can cause shorter meal time, and consequently less time feeding.

The time used for other activities was higher (P<0.05) for heifers fed fresh sugarcane compared with those fed hydrolyzed sugarcane; there was no effect of storage time of hydrolyzed sugarcane on ingestive behavior (Table 7). Among the correlations concerning ingestive behavior, nutrient intake and characteristics of forage (Table 9), the correlation (P<0.05) of time in other activities with the dry matter intake (r = -0.56), neutral detergent fiber intake (r = -60) and feeding time (r = -0.44) stand out. In part, these correlations explain the lower (P<0.05) feed intake for heifers fed hydrolyzed sugarcane, since these animals spent less time eating feed and more time resting, and were less efficient to feed. According to Hodgson (1990) the daily activities of animals are mutually exclusive, in which the increase of rumination and idleness implies a decrease in feeding time.

The time at rumination was changed (P<0.05) by adding hydrated lime to sugarcane and by the hydrolysis time, in which the longest periods used to this activity were observed for diets with fresh sugarcane and hydrolyzed sugarcane stored for 48 hours, which were similar (Table 7). According to Van Soest et al. (1991) rumination time is influenced by the nature of the diet and is proportional to the cell wall content of roughages, which was not observed in this study. Rumination time was significantly correlated (Table 9) with dry matter intake (r = 0.57), neutral detergent fiber intake (r = 0.52), feed efficiency (r = 0.52) and dry matter *in vitro* digestibility (r = -0.45). These results indicate that rumination time was the result of feed intake and digestibility of ingested feed, since the animals that consumed more spent more time for rumination and remained less time idle (r = -0.74).

Feeding frequency was changed (P<0.05) by adding lime to the sugarcane, but not by the hydrolysis time, and the highest values were observed for heifers fed fresh sugarcane (Table 8). However, the number of daily meals was changed (P<0.05) by adding lime to the sugarcane

Tabela 7 - Variables related to feed intake and ingestive behavior of heifers fed fresh sugarcane and hydrolyzed sugarcane stored for different periods

| Items | Fresh sugarcane (time zero) | Hydrolyzed sugarcane stored | | | CV (%) | Orthogonal contrasts[1] | | |
|---|---|---|---|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours | | FS vs HS | Linear | Quadratic |
| DM intake (g kg$^{-1}$ of body weight) | 24.90a | 20.20b | 21.40b | 19.80b | 11.15 | 0.0001 | 0.2499 | 0.0913 |
| NDF intake (g kg$^{-1}$ of body weight) | 9.10a | 7.00b | 7.50b | 7.00b | 14.13 | 0.0001 | 0.6875 | 0.5066 |
| Feeding time (hours day$^{-1}$) | 4.53a | 3.92b | 3.90b | 3.86b | 18.37 | 0.0025 | 0.7794 | 0.8500 |
| Time allocated for water intake (hours day$^{-1}$) | 0.18 | 0.18 | 0.23 | 0.18 | 61.19 | 0.1719 | 0.8008 | 0.6286 |
| Time allocated for other activities (hours day$^{-1}$) | 11.36b | 12.86a | 12.06a | 12.96a | 7.55 | 0.0001 | 0.3473 | 0.0612 |
| Time allocated for rumination (hours day$^{-1}$) | 7.94a | 7.05b | 7.81a | 6.99b | 10.28 | 0.0001 | 0.3611 | 0.0109 |

Means followed by different lowercase letters in the row differ (P<0.05) by analysis of orthogonal contrasts.
CV - coefficient of variation.
[1] Probability for the contrasts: FS vs HS = fresh versus hydrolyzed sugarcane (-3 1 1 1) and linear (0 -1 1 0) and quadratic (0 -1 2 - 0) effects of the time of hydrolysis.

and by the hydrolysis time, and the highest values were observed for heifers fed hydrolyzed sugarcane stored for 72 hours and fresh sugarcane. The feeding frequency was significantly correlated (Table 9) with the number of daily meals (r = 0.54), neutral detergent fiber intake (r = 0.41), efficiency of rumination (r = 0.41) and time spent on other activities (r = -0.49), indicating that the greater presence of animals in the feeders was indirectly associated with feed intake. Between frequency of feed supply and the number of daily meals, the latter seems to be the most important variable in feed intake, since it was the best correlated with dry matter intake (r = 0.40), neutral detergent fiber intake (r = 50) and rumination efficiency (r = 0.45).

The number of daily ruminated boluses was higher (P<0.05) for heifers fed fresh sugarcane in relation to those fed hydrolyzed sugarcane (Table 8). Quantitatively, the number of daily ruminated boluses is close to those found in the literature, since 360-790 amounts of feed are typically ruminated, with size ranging from 80 to 120 grams (Furlan, 2006). Similarly, the daily mastication time was higher (P<0.05) for heifers fed fresh sugarcane compared with those fed hydrolyzed sugarcane, resulting in higher dry matter intake (r = 0.52) and neutral detergent fiber intake (r = 0.78). Allen (1997), reviewing the literature, reported for the results of 132 treatments, average of 32 experiments for daily mastication time and mentioned mean value of 11.13 hours day$^{-1}$. The values for daily mastication time, in this study, for heifers fed hydrolyzed sugarcane, were close to the value cited by the author mentioned above; however, for heifers fed fresh sugarcane, the value was higher. These results are associated with the dry matter intake and the type of roughage in the diet.

The number of chews per bolus was higher (P<0.05) for heifers fed diets containing hydrolyzed sugarcane in relation to those fed fresh sugarcane, which may be a consequence of the lower palatability and acceptability of hydrolyzed sugarcane used by the animal category in the experiment (Table 8). Dias (2009) found a quadratic variation of this variable and chewing time per bolus (seconds) as a function of elevation of the levels of hydrated lime (0, 8, 16 and 24 g of lime kg$^{-1}$ of sugarcane) for the hydrolysis of sugarcane, showing that the improvement in digestibility of hydrolyzed sugarcane facilitates chewing. It should be noted that the values for this variable are similar to those found in the literature, since, usually, there can be from 40 to 70 jaw movements (chews) during rumination (Furlan, 2006), and around 55 chews per bolus for confined cattle fed diets containing sugarcane, according to a study conducted by Polli et al. (1996).

The number of daily chews was changed only (P<0.05) by the sugarcane hydrolysis, and the highest values were recorded for animals fed diets with fresh sugarcane (Table 8). This variable was significantly correlated with dry matter intake (r = 0.46) and neutral detergent fiber intake (r = 0.42), demonstrating the need for greater chewing activity for higher consumption of feed.

Feeding efficiency and rumination efficiency were not affected (P>0.05) by storage time of hydrolyzed sugarcane (Table 8). However, heifers fed fresh sugarcane were more efficient for both the characteristics in relation to those fed hydrolyzed sugarcane, explaining the higher dry matter intake. This fact was evinced by the significant correlation (r = 0.70) between feeding efficiency and dry matter intake (Table 9).

Table 8 - Variables related with the other characteristics of ingestive behavior of heifers fed fresh sugarcane and hydrolyzed sugarcane stored for different periods

| Items | Fresh sugarcane (time zero) | Hydrolyzed sugarcane stored | | | CV (%) | Orthogonal contrasts[1] | | |
|---|---|---|---|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours | | FS vs HS | Linear | Quadratic |
| Feeding frequency | 12.61a | 10.72b | 9.78b | 10.11b | 20.32 | 0.0001 | 0.5626 | 0.4684 |
| Daily meals | 5.88a | 3.82b | 4.89b | 5.67a | 28.96 | 0.0085 | 0.0018 | 0.8798 |
| Daily ruminated boluses | 603.17a | 488.80b | 516.09b | 453.13b | 17.42 | 0.0002 | 0.2907 | 0.9112 |
| Chews per bolus | 50.00b | 52.20a | 53.59a | 54.47a | 13.47 | 0.0458 | 0.0937 | 0.7440 |
| Daily chews | 29643a | 25221b | 27553b | 24469b | 15.34 | 0.0012 | 0.2715 | 0.4267 |
| Chewing time per bolus (seconds) | 48.56c | 52.35c | 55.72b | 55.88a | 13.50 | 0.0012 | 0.0435 | 0.9971 |
| Daily mastication time (hours) | 12.46a | 10.96b | 11.70b | 10.85b | 8.04 | 0.0001 | 0.8730 | 0.4435 |
| Feeding efficiency (kg DM hours$^{-1}$) | 0.63a | 0.53b | 0.57b | 0.52b | 22.17 | 0.0022 | 0.5095 | 0.2380 |
| Rumination efficiency (kg DM hours$^{-1}$) | 0.35a | 0.27b | 0.29b | 0.28b | 14.47 | 0.0001 | 0.7064 | 0.8630 |
| Rumination efficiency of NDF (kg hours$^{-1}$) | 0.12a | 0.08b | 0.09b | 0.10b | 15.70 | 0.0001 | 0.6994 | 0.9999 |

Means followed by different lowercase letters in the row differ (P<0.05) by analysis of orthogonal contrasts.
CV - coefficient of variation.
[1] Probability for the contrasts: FS vs HS = fresh versus hydrolyzed sugarcane (-3 1 1 1) and linear (0 -1 1 0) and quadratic (0 -1 2 - 0) effects of the time of hydrolysis.

Table 9 - Correlation matrix for main variables related to ingestive behavior, nutrient intake and characteristics of forage

| | | F | OA | R | DM | FF | FE | DC | RE | DMI | NDFI | IVDDM | IVDNDF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OA | r | -0.44 | | | | | | | | | | | |
| | P | 0.0001 | | | | | | | | | | | |
| R | r | -0.18 | -0.74 | | | | | | | | | | |
| | P | 0.4972 | 0.0001 | | | | | | | | | | |
| DM | r | 0.57 | -0.50 | 0.07 | | | | | | | | | |
| | P | 0.0001 | 0.0001 | 0.1006 | | | | | | | | | |
| FF | r | 0.85 | -0.49 | -0.17 | 0.54 | | | | | | | | |
| | P | 0.0001 | 0.0001 | 0.1564 | 0.0001 | | | | | | | | |
| FE | r | 0.57 | 0.02 | 0.52 | -0.13 | -0.36 | | | | | | | |
| | P | 0.0001 | 0.8465 | 0.0001 | 0.1902 | 0.0001 | | | | | | | |
| DC | r | -0.01 | -0.68 | 0.84 | 0.13 | -0.01 | 0.32 | | | | | | |
| | P | 0.9552 | 0.0001 | 0.0001 | 0.2151 | 0.9297 | 0.0018 | | | | | | |
| RE | r | 0.14 | -0.28 | 0.16 | 0.45 | 0.41 | 0.55 | 0.05 | | | | | |
| | P | 0.1890 | 0.0061 | 0.1052 | 0.0001 | 0.0001 | 0.0001 | 0.6307 | | | | | |
| DMI | r | 0.04 | -0.56 | 0.57 | 0.40 | 0.31 | 0.70 | 0.46 | 0.82 | | | | |
| | P | 0.6253 | 0.0001 | 0.0001 | 0.0001 | 0.0006 | 0.0001 | 0.0001 | 0.0001 | | | | |
| NDFI | r | 0.03 | -0.60 | 0.52 | 0.50 | 0.41 | 0.61 | 0.42 | 0.88 | 0.89 | | | |
| | P | 0.3812 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | | | |
| IVDDM | r | 0.15 | 0.24 | -0.45 | -0.01 | 0.18 | -0.30 | -0.19 | -0.01 | 0.18 | -0.05 | | |
| | P | 0.2178 | 0.1007 | 0.0001 | 0.9343 | 0.1004 | 0.0120 | 0.1133 | 0.9074 | 0.1246 | 0.6098 | | |
| IVDNDF | r | 0.14 | 0.06 | 0.01 | 0.15 | 0.03 | 0.02 | 0.17 | 0.11 | 0.11 | 0.18 | 0.33 | |
| | P | 0.2225 | 0.5130 | 0.8712 | 0.2892 | 0.4785 | 0.8700 | 0.1588 | 0.3716 | 0.3877 | 0.1239 | 0.0048 | |
| FB1C | r | 0.27 | -0.20 | 0.14 | 0.07 | 0.03 | 0.19 | 0.13 | 0.40 | 0.44 | 0.48 | 0.04 | -0.08 |
| | P | 0.0651 | 0.1227 | 0.1246 | 0.8163 | 0.9175 | 0.2765 | 0.4403 | 0.0177 | 0.0079 | 0.0034 | 0.9648 | 0.1792 |

F, OA and R - hours per day allocated for feeding, other activities and rumination, respectively; DM - daily meals; FF - feeding frequency; FE - feeding efficiency (kg of DM hour$^{-1}$); DC - daily chews; RE - rumination efficiency (kg of DM hour-1) - DMI and NDFI - dry mater (DM) and neutral detergent fiber (NDF) intake, respectively; IVDDM and IVDNDF - *in vitro* digestibility of DM and NDF, respectively; FB1C- fraction B1 of carbohydrates, r - correlation coefficient, P - probability.

## Conclusions

The storage of sugarcane with the addition of 5 g of hydrated lime kg$^{-1}$ natural matter for up to 72 hours alters the chemical composition of sugarcane by increasing the potentially digestible carbohydrate fraction of the cell wall. Nevertheless, the supply of hydrolyzed sugarcane stored for up to 72 hours at a ratio of about 600 g kg$^{-1}$ of dry matter of diet affects the intake patterns, reducing the feed intake in Nellore heifers.

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