Revista Brasileira de Zootecnia
© 2012 Sociedade Brasileira de Zootecnia
ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., v.41, n.4, p.963-969, 2012

Ruminal parameters of bovines fed diets based on sugar cane with doses of calcium hydroxide

Alexandre Menezes Dias[1], Luís Carlos Vinhas Ítavo[1,5], Julio Cesar Damasceno[2,5], Geraldo Tadeu dos Santos[2,5], Ériklis Nogueira[3], Camila Celeste Brandão Ferreira Ítavo[4]

[1] Universidade Católica Dom Bosco - UCDB, Av. Tamandaré, 6000. Jardim Seminário, 79117-900, Campo Grande-MS.
[2] Universidade Estadual de Maringá - UEM, Maringá-PR.
[3] Embrapa Pantanal, Corumbá-MS.
[4] Universidade Federal de Mato Grosso do Sul - UFMS, Campo Grande-MS.
[5] Productivity scholar from CNPq.

ABSTRACT - The objective of this study was to evaluate the administration of different doses of calcium hydroxide mixed with sugar cane fed to cows by the pH, ammonia nitrogen and volatile fatty acids concentration in ruminal content. Four cows with fistulated rumen were distributed in a Latin square (4 × 4) in split plot. The treatments involved adding doses of 0, 8, 16 and 24 g/kg of calcium hydroxide to sugar cane *in natura*. Samples of rumen fluid were collected and the pH and concentration of N-NH_3 was determined before (time zero) and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hours after the feeding time. The concentration of volatile fatty acids at 0, 3, 6, 9 and 12 hours was also determined. The calcium hydroxide contributed to maintain the pH of rumen fluid close to neutral. The average concentrations of N-NH_3 in the ruminal fluid was 20.59, 20.49, 17.28 and 18.22 mg/100 mL for samples with calcium hydroxide at 0, 8, 16, 24 g/kg, respectively. There was an effect of the addition of calcium hydroxide on the volatile fatty acids concentration from before feeding until 12 hours after feeding time. There was an effect on doses tested for the concentration of volatile fatty acids. The calcium hydroxide added to the sugar cane influences on ruminal parameters. The dose of approximately 10 g of calcium hydroxide per kg sugar cane *in natura* is recommended.

Key Words: ammonia nitrogen, ruminal pH, *Saccharum officinarum*, VFA

Introduction

Sugar cane *in natura* presents high lignocellulose content, so the feasibility of its use requires the development of treatment methods that promote the breakdown of the structure of the fiber fraction to make it more digestible, thus favoring better performance. Ítavo & Ítavo (2005) suggested that ruminal parameters can directly influence the performance of animals; this way, knowing of these events and seeking to improve these parameters are necessary to achieve maximum animal performance.

Alkalizing agents such as calcium oxide (CaO) and calcium hydroxide ($Ca(OH)_2$) can be used for this purpose, since they promote the alkaline hydrolysis of the cell wall and consequently improve the digestibility. The ruminal pH is directly related to the final products of fermentation and with the rate of the growth of rumen microorganisms (Kozloski, 2002).

The ruminal N-NH_3 concentration is a consequence of the balance between production, absorption and utilization by microorganisms (Silveira et al., 2009). In order to have maximum rumen microbial fermentation, Van Soest (1994) mentioned that the optimum level is 10 mg N-NH_3/100 mL.

The molar proportion of acetate:propionate:butyrate vary widely, with values from 75:15:10 found in diets rich in fiber, to 40:40:20, in diets rich in non-fibrous carbohydrates (NFC), with the total volatile fatty acids (VFA) between 60 and 150 mmol/mL in the rumen liquid (Bergman, 1990). The intake of rapidly fermentable food, for example, increases microbial activity rapidly, causing fluctuations in the final products of fermentation and in the pH of the rumen contents, which may reflect in the use of dietary nutrients.

Thus, the objective was to evaluate the pH, ammonia nitrogen (N-NH_3) and the concentration of volatile fatty acids (VFA) in rumen fluid from cattle receiving diets based on sugar cane *in natura* treated with increasing doses of calcium hydroxide ($Ca(OH)_2$).

Material and Methods

The study was conducted at the Fazenda Escola São Vicente at Universidade Católica Dom Bosco - UCDB, in Campo Grande, Mato Grosso do Sul state, Brazil.

Received August 20, 2010 and accepted October 20, 2011.
Corresponding author: alexandre.dias@ucdb.br

A forage harvester was used to harvest and to chop the sugar cane (VAR. RB7515), which was harvested 14 months after the last cut and chopped into 4-mm pieces. Four crossbred (Angus × Nellore) cows with rumen cannula and with an average body weight (BW) of 412.06±37.3 kg were confined in individual stalls with a concrete area covering 5 m^2 with roof, provided with concrete feeders and drinkers. The cows were fed once daily at 8 a.m., to keep the leftovers that were supplied around 5-10%. The quantity of sugar cane, concentrate and leftovers were all recorded daily. The animals had free access to water and minerals.

The animals were distributed into a Latin square (4 × 4) design with four doses of calcium hydroxide ($Ca(OH)_2$) (hydrated lime - CH I) and four experimental periods of 21 days: 14 days for adaptation of the animal to the diets and seven days for data collection. The animals underwent a pre-trial period of 30 days before the start of the experimental phase, to adapt to the diet and management. The animals were weighed at the beginning and end of each experimental period.

Calcium hydroxide was added at doses of 0, 8, 16 and 24 g per kg natural matter of fresh sugar cane; the calcium hydroxide was mixed to the sugar cane before being mixed in concentrate and then supplied to the animals, with no removal of dry straw. The roughage:concentrate ratio of diets (Table 2) was 65:35 on a dry matter (DM) basis. Sugar cane was provided after being cut and doses of calcium hydroxide in powder form were added, without diluting with water, according to the treatment, until completely homogenized. Then, roughage was mixed with concentrate and given to the animals (Table 1).

Samples of sugar cane, concentrate and feed leftovers from each animal were collected daily. Composite samples were made per period. Samples of each period were oven-dried (55 ºC, 72 hours) and ground to pass through a 1-mm mesh sieve.

Sugar cane samples with and without calcium hydroxide, concentrate, feces and leftovers were composed proportionately to each animal and contents of dry matter (DM), mineral matter (MM) , crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed as described by Silva & Queiroz (2002) (Tables 1 and 2).

Non-fiber carbohydrates (NFC) were calculated through the equation NFC - 100 - (% CP +% EE +% MM% + NDF), in which CP - crude protein; EE - ether extract; MM - mineral mixture; NDF - neutral detergent fiber. The values are presented in g/kg DM.

In each experimental period, rumen contents were collected to obtain the ruminal fluid, in order to quantify the pH quantification carried out before supply of the diet at time zero and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hours after supply of the diet. A plastic container with a capacity of 250 mL, where the ruminal content was collected through the ruminal fistula, was used.

The rumen fluid samples were collected at the liquid/solid interface region of the rumen environment and filtered through a triple gauze layer in order to obtain 100 mL of ruminal fluid and proceed with the immediate determination of pH with the digital potentiometer. To assess the concentration of ammonia, the samples were kept in plastic container with 1 mL of hydrochloric acid (1:1), identified and frozen (-20 °C).

To evaluate the concentrations of volatile fatty acids (VFA), starting earlier than the food supply taking as time zero (0), 3, 6, 9 and 12 hours after feeding, the rumen content collected was filtered through gauze layers in order to obtain 100 mL of ruminal fluid and then 1 mL of hydrochloric acid (HCl) 1:1 was added. The rumen fluid samples were stored at -5 °C.

Table 1 - Chemical composition of sugar cane *in natura* and concentrate in experimental diets

Item	Sugar cane	Concentrate[1]
Dry matter (g/kg of natural matter)	274.3	837.0
Organic matter[2]	960.7	911.0
Crude protein[2]	32.8	159.8
Ether extract[2]	5.00	48.4
Neutral detergent fiber[2]	489.2	311.5
Acid detergent fiber[2]	377.8	186.7
Non-fibrous carbohydrate[2]	433.7	391.3
Total digestible nutrients[2,3]	532.0	Nd

[1] Concentrate: 27% soybean meal, 20% ground corn, 20% ground sorghum, 20% soybean hulls, 9% protected fat, 2% urea and 2% mixed mineral; nd: not determined.
[2] g/kg of dry matter.
[3] Total digestible nutrients estimated according Capelle et al. (2001).

Table 2 - Chemical composition of diets based on sugar cane with increasing doses of calcium hydroxide

Item	Calcium hydroxide (g/kg)			
	0	8	16	24
Dry matter (g/kg of natural matter)	471.20	478.20	480.38	488.70
Organic matter[1]	943.30	932.30	912.90	866.40
Crude protein[1]	77.30	75.80	75.60	75.80
Ether extract[1]	20.20	20.10	20.30	20.30
Neutral detergent fiber[1]	427.00	425.90	421.70	412.00
Acid detergent fiber[1]	310.90	275.10	269.10	261.20
Hemicelullose[1]	116.00	150.80	152.60	150.80
Non-fibrous carbohydrate[1]	418.91	410.55	395.33	392.72

[1]g/kg of dry matter.

For quantification of short-chain fatty acids (SCFA), the samples were centrifuged at 15,000 x g (4 °C) for 25 minutes, then analyzed by gas-liquid chromatography (Hewlett Packard 5890 Series II GC column packed cabopack, 3 m), with oven temperature at 120 °C, equipped with integrator (Hewlett Packard 3396 Series II Integrator) and automatic injector (Hewlett Packard 6890 Series injector) temperature of 106 °C, and FID-type detector at 190 ºC. The carrier gas used was nitrogen, with no ramp heating. The internal standard used was 2-methylbutyric acid, with 100 µl of internal standard, 800 µl from the sample and 200 µl of formic acid added to each tube for reading in chromatograph. A mixture of volatile fatty acids with known concentration was used as external standard for calibration of the integrator (Campos et al., 2004).

The results were evaluated by statistical program SAEG (Sistema para Análises Estatísticas e Genéticas, version 9.1) through variance and regression analysis. The analysis of pH, N-NH_3 and VFA in rumen fluid were performed in a split-plot, with treatments in the plot and the collection time in the subplot.

Results and Discussion

The highest values of ruminal pH were found in treatments with higher doses of calcium hydroxide (16 and 24 g/kg), due to the alkalinizing power of calcium hydroxide used for the alkaline hydrolysis of sugar cane (Table 3). The higher ruminal pH, between 7.16 and 7.20, allows growth of cellulolytic bacteria (Ítavo & Ítavo, 2005).

The pH data suggest that the experimental diets had ruminal fermentation pattern appropriate to the action of cellulolytic bacteria. The values found in this study are in the range considered adequate for diets with a higher percentage of roughage.

Table 3 - Means and regression equation of pH of the rumen fluid of cattle fed diets based on sugar cane with doses of calcium hydroxide, at different times

Times (hours)	Calcium hydroxide (g/kg)			
	0	8	16	24
0	7.08	6.89	7.16	7.22
1	6.86	6.77	7.00	7.00
2	6.62	6.62	6.90	6.91
3	6.55	6.56	6.82	6.93
4	6.51	6.65	6.78	6.91
5	6.58	6.63	6.83	6.97
6	6.58	6.74	6.84	7.02
7	6.66	6.81	6.94	7.00
8	6.69	6.80	7.00	7.06
9	6.71	6.84	6.97	7.03
10	6.63	6.85	6.94	7.00
11	6.75	6.85	7.04	7.05
12	6.71	6.80	7.02	7.02

$\hat{Y} = 6.89769 - 0.202080 \times hour + 0.0353822 \times hour^2 - 0.00166461 \times hour^3 + 0.0014380 \times dose$ ($R^2 = 0.89$).

Solving the equation (Table 3), pH values of 6.89, 6.90, 6.92 and 6.93 were observed before supply of the diet for treatments 0, 8, 16 and 24 g/kg, respectively. In the first hour after diet supply, there was a reduction in pH values , and the lower pH values were observed four hours after supply of the diet: 6.54, 6.56, 6.57 and 6.58 for doses 0, 8, 16 and 24 g calcium hydroxide per kg of sugar cane, respectively.

An important fact to consider is the alkalinizing power that the calcium hydroxide provides to the rumen environment, from the supply of the diet until the next supply, which can promote the degradation of NDF.

In a study evaluating the pH of the rumen fluid of cattle fed diets with sugar cane as forage, Silveira et al. (2009) found values of ruminal pH of 6.87 in the animals fed with sugar cane exclusively; 6.58 fed sugar cane + urea; 6.58 in the animals fed with cane sugar + gluten corn-60; and 6.55 in those fed sugar cane + soybean meal, which can be attributed to the fact that the diet is composed exclusively of roughage, leading to greater rumination and chewing by the animal and, consequently, increased production of saliva, similarly to the values from this experiment.

The treatment with higher dose of calcium hydroxide showed the higher pH of the rumen fluid at all collection times. From the results of pH, it is observed that only the treatments 0 and 8 g/kg remained between 6.5 and 7.0, whereas treatment with 24 g/kg showed values above 6.9. This may influence the growth of cellulolytic bacteria and therefore the degradation of the fibrous portion of the diet (Ítavo & Ítavo, 2005).

It is observed that the doses 8 and16 g calcium hydroxide per kg of sugar cane showed average pH between 6.68 and 6.75, which could favor the development of cellulolytic bacteria and consequently improve the digestibility of NDF (Dias et al., 2011), and, according to Vanzant et al. (1990), ruminal pH below 6.7 would reduce fiber digestion. In this sense, Oliveira et al. (2008) emphasized improvement in digestibility of sugar cane treated with alkaline hydrolysis, highlighting the property buffering that the roughage acquires with treatment, which provides security and stability in feed for ruminants.

Regardless of the treatment, the minimum values were reached four hours after feeding, indicating that treatment with an alkalizing agent had no negative effect on ruminal fermentation. According to Van Soest (1994), the breaking of ester bonds between lignin and complex structural carbohydrates, due to the increase of pH proportionate by alkaline hydrolysis, allows more action of microbial enzymes,

due to the higher production of VFA (Table 5), which may be related to improvement in the digestibility of the diet.

The pH is an important factor in ruminal activity, and its value is typically between 5.5 and 7.0 (Van Soest, 1994), depending on the nature of the diet, time after ingestion of food, feeding frequency and time and sampling the rumen fluid, and the decrease in rumen pH causes reduction of appetite, rumen motility, microbial growth and, consequently, digestion, especially of fibrous fractions of feed.

Dias et al. (2000), however, observed a linear reduction of the pH of the feeding time until 8 hours, probably due to the intensification of the post-prandial process and increase in VFA concentrations in diets with different concentrate levels.

When the rumen pH was maintained between 6.5 and 6.6, the concentrations of N-NH_3 decreased, possibly by the use of N by ruminal bacteria, once the cellulolytic bacteria have a preference for available N in the rumen, since there is availability of carbon skeleton for the synthesis of amino acids. Prior to delivery (time 0 hours) of experimental diets, the values found for N-NH_3 were 25.28, 22.94, 25.28 and 21.07 mg/100 mL of rumen fluid for the 0, 8, 16 and 24-g/kg doses of hydroxide calcium, respectively (Table 4).

According to Silveira et al. (2009) the concentration of N-NH_3 in rumen fluid is consequence of the balance of its production, use by microorganisms and absorption through the rumen wall, and the use by microorganisms depends on the amount of available energy.

Goularte et al. (2010) found average concentrations of N-NH_3 of 37.40, 39.62, 35.59 and 34.13 mg/100 mL of rumen fluid for concentrate levels of 30, 40, 50 and 60%, respectively. It is noteworthy that high levels of N-NH_3 can mean bigger losses of nitrogen in the system, since, in order for there to be synthesis microbial protein and, consequently, use of N-NH_3, there should be availability of carbon skeletons in synchrony with degradation protein in the rumen, which does not occur in diets with reduced starch and non-structural carbohydrates and/or sources of energy from fat, which should lead to loss of energy in the form of ATP, for processing into urea by the liver to urinary excretion, which probably did not occur in the present experiment, due to the lower concentrations of N-NH_3.

Martins et al. (2006), evaluating the ruminal parameters (pH and N-NH_3) in cattle supplemented with fibrolytic enzymes consuming diets containing corn silage and Tifton 85 hay, found that in diets composed of Tifton hay with and without the addition of fibrolytic enzymes, variations were from 9.68 to 16.89 mg/100 mL and 9.30 to 15.86 mg/100 mL, respectively. For both roughages, the maximum concentration of N-NH_3 was obtained two hours after feeding. The average concentration of ammonia was 10.25 mg/100 mL for corn silage and 11.86 mg/100 mL for the Tifton hay.

Table 4 - Means and regression equation of N-NH_3 (mg/100mL) of the rumen fluid of cattle fed diets based on sugar cane with doses of calcium hydroxide, at different times

Times (hours)	Calcium hydroxide (g/kg)			
	0	8	16	24
0	25.28	22.94	25.28	21.07
1	35.58	29.02	26.21	27.15
2	28.09	23.41	21.07	16.85
3	24.81	16.38	15.92	19.66
4	21.07	16.85	13.10	15.45
5	15.45	17.32	14.04	12.64
6	13.58	22.94	17.32	18.72
7	19.19	19.19	15.45	17.32
8	14.51	17.87	17.79	18.72
9	20.60	20.21	17.32	18.26
10	16.85	20.94	16.85	17.79
11	18.72	21.51	12.64	14.98
12	14.04	17.82	11.70	18.26

$\hat{Y} = 30.8409 - 0.291505 \times dose - 4.19002 \times hour + 0.480161 \times hour^2 - 0.023364 \times hour^3 + 0.0296939 \times (dose \times hour)$ (R^2=0.87).

According to Satter & Slyter (1974), the minimum concentration of N-NH_3 necessary not to limit microbial synthesis would be 5 mg/100 mL of rumen fluid. However, Van Soest (1994) cited as an optimal level of 10 mg/100 mL rumen fluid, a value that should not be considered fixed, for it varies depending on the microbial protein synthesis capacity, the availability of substrate and the rate of fermentation of carbohydrates. The ruminal ammonia concentrations obtained with the experimental diets in this study were close to the value quoted by Van Soest (1994) and probably did not limit the efficiency of microbial synthesis.

Pina et al. (2010), studying the effects of inclusion (0, 0.5 and 1.0% in the natural matter of sugar cane) and exposure times of sugar cane in calcium oxide (0 to 3 days) found that the rumen pH of Nellore heifers was influenced by the time of sample collection, by the addition of lime and the storage time of the cane. Significant interactions between inclusion of lime and storage time of sugar cane, and between times of sample collection and inclusion level of lime are also observed. The ruminal pH values stayed in the range of 6.18 to 6.81, similarly to those found in this study.

One must regard that as time went by, the values of N-NH_3 reduced within the treatments. When the dose of calcium hydroxide in the sugar cane was increased, the concentrations of N-NH_3 reduced, providing lower losses of ammonia nitrogen in the rumen, probably by the greater use by rumen microorganisms.

The level considered optimal for ruminal ammonia concentration (10 mg/100 mL) cannot be considered static, since the ability of bacteria to synthesize protein and capture ammonia depends on the fermentation rate of carbohydrates (Van Soest, 1994). In this sense, Ítavo et al. (2002) estimated maximum concentrations of $N\text{-}NH_3$ at 22.93 mg/100 mL of ruminal fluid, with 14.14% of concentrate and 1.89 hours after the feed intake. The values found for the maximum concentrations of $N\text{-}NH_3$ of diets, in function of the time of collection, are in agreement with Mehrez et al. (1977), who suggested that the maximal fermentative activity would occur when the concentrations of $N\text{-}NH_3$ are between 19 and 23 mg/100 mL of ruminal fluid.

It can be observed that the values of $N\text{-}NH_3$ in treatments with calcium hydroxide two hours post-feeding showed values close to those reported for the fermentation maximum. This suggests that treatment with calcium hydroxide in increasing doses could enhance ruminal fermentation of sugar cane, improving the utilization by ruminants.

The maximum concentrations of $N\text{-}NH_3$ were observed soon after the diet intake, where the pH tended to decline, indicating an increase in VFA production (Table 5). Four hours after supply of the experimental diets was the moment when the lowest pH values were observed, probably due to the greater microbial digestion, since this time the $N\text{-}NH_3$ values were 21.07, 16, 85, 13.10 and 15.45 mg/100 ml for the doses of 0, 8, 16 and 24 g/kg. Solving the equation (Table 4), in 12 hours, the lowest values of $N\text{-}NH_3$, 9.33, 9.84, 10.37 and 10.88 mg/100 mL for the doses of 0, 8, 16 and 24 g/kg, are verified.

According to Moraes et al. (2008), treatment of sugar cane with calcium oxide (CaO) in three doses (0, 0.5 and 1.0%) did not influence the concentrations of $N\text{-}NH_3$ in the rumen, but the levels of concentrate influenced the concentration of ammonia nitrogen in the rumen fluid, with the highest concentrations of ammonia nitrogen occurring with the supply of concentrate at 1% of body weight.

The mean values of $N\text{-}NH_3$ three hours after feeding were within the range of 15.0 to 20.0 mg/100 mL, which is in accordance with Mehrez et al. (1977), who suggested that the maximum fermentative activity would occur between 19 and 23 mg of $N\text{-}NH_3$/100 mL of rumen fluid.

Pina et al (2010) found that concentrations of $N\text{-}NH_3$ ranged from 8.19 to 21.5 mg/100 mL in the range 0 to 6 hours after feeding with sugar cane treated with calcium oxide. The concentration corresponding to the second hour (Table 4) is consistent with the range proposed by Mehrez et al. (1977), so, the maximum fermentative activity is achieved when the ruminal $N\text{-}NH_3$ reaches values between 19 and 23 mg/mL in the rumen fluid.

The digestion of ruminants involves constant symbiotic activity of rumen microorganisms with the hosts, which are highly susceptible to changes in the environment, affecting not only the extent of degradation of feed components, but also the amounts and proportions of the products resulting from the their action (Van Soest, 1994). The main limiting factor for fiber digestion is the low content of $N\text{-}NH_3$ due to lower bacterial activity (Hoover & Stokes, 1991). According to Church (1990), most bacteria are able to use the $N\text{-}NH_3$ as the sole nitrogen source, and therefore, the diet contains adequate concentrations in the rumen, maximizing microbial activity.

The cellulolytic bacteria use almost exclusively $N\text{-}NH_3$ as N source and their fermentative capacity is lower in the absence of $N\text{-}NH_3$, since their ability to use N as amino acids and peptides is greatly reduced. One factor to consider would be the low-CP diet, due to the low proportion of the concentrate, which may have favored increased recycling of nitrogen.

Silveira et al. (2009) found influence of the shape of rumen degradable nitrogen in diets of sugar cane on the values of $N\text{-}NH_3$, with mean concentrations of ruminal $N\text{-}NH_3$ of 18.09, 66.86, 27.90 and 39.24 mg/100 mL of ruminal fluid in animals maintained on diets sugar cane, sugar cane + urea, sugar cane + corn gluten and sugar cane + soybean meal, respectively. The authors found a peak production of ruminal $N\text{-}NH_3$ two hours after feeding the animals that received sugar cane and urea. The concentrations of ruminal $N\text{-}NH_3$ were sufficient for bacterial growth, like the minimum value quoted by Preston (1986), 5 mg $N\text{-}NH_3$/100 mL. However, the ammonia level should be higher than 10 mg/100 mL so there must be increased ruminal digestion of DM and over than 20 mg/100 mL for there to be an increased DM intake (Leng, 1990).

There was effect of addition calcium hydroxide in the acetic acid concentrations and the acetic acid:propionic acid ratio after supply of experimental diets (Table 5). The molar acetic acid ratio produced in the rumen and the relative acetic acid:propionic acid ratio are favorable for comparison and prediction of the nutritional value of the diet. In general, when the roughage:concentrate decreases, the acetic acid:propionic acid ratios also decreases, whereas the dose of 9.6 g of calcium hydroxide in seven hours produced 52.40 μmol/mL acetic acid, which probably is related to increased digestibility of the fibrous fraction of sugar cane provided by calcium hydroxide.

There was no significant effect on the concentrations of propionic acid, with an average concentration of 13.65 μmol/mL (Table 5). There was no interaction between dose and time of collection of rumen fluid for the acetic acid:propionic acid ratio. The acetic acid:propionic acid ratio

occurred in quadratic form, presenting a value of 3.3:1 for the dose of 12.10 g of calcium hydroxide and is within the standards presented by Teixeira & Teixeira (2001), who indicated that the ideal acetic acid:propionic acid ratio would be between 2:1 and 4:1. Decreasing the proportion of roughage from 70% to 40% in diets of dry cows, Rodrigues et al. (2000) observed decrease of 4.9%in the acetic acid:propionic acid ratio, showing no significant effect.

The fermented carbohydrates of diets were converted to 57.51, 58.15, 58.00 and 58.32% acetic acid, 19.36, 18.68, 18.10 and 18.39% propionic acid, 16, 62, 16.80, 18.11 and 16.49% of butyric acid and 2.56, 2.34, 2.29 and 2.87% for valeric acid doses of 0, 8, 16 and 24 g/kg, respectively, similarly to those shown by Teixeira & Teixeira (2001), for ruminants fed with diets rich in forage, where rumen microbial population usually converts carbohydrates fermented at 60 to 70% acetic acid, 18 to 22% propionic acid, 13 to 16% butyric acid and 2 to 4% valeric acid.

There was no significant effect on the concentrations of butyric, isobutyric, valeric and isovaleric acids (Table 6).

The means were 12.01, 1.02, 1.63 and 1.49 µmol/mL of ruminal fluid, for concentrations of butyric, isobutyric, valeric and isovaleric acids, respectively. Ruminal concentrations of isobutyrate and isovalerate are indicative of amino acid fermentation, which, in high concentrations, accumulate VFA, the main factor reducing the pH (Vargas et al., 2002).

Table 5 - Means of concentrations (µmol/mL) of acetic and propionic acids and acetic acid:propionic acid ratio of the rumen fluid of cattle fed diets based on sugar cane with doses of calcium hydroxide, at different times

Times (hours)	Calcium hydroxide (g/kg)			
	0	8	16	24
	Acetic acid[1]			
0	40.36	44.21	36.61	36.78
3	37.44	47.02	45.38	21.42
6	45.28	66.32	47.93	25.64
9	51.10	49.32	51.36	39.23
12	37.08	32.79	47.66	27.49
	Propionic acid[2]			
0	12.10	13.40	10.47	9.87
3	12.57	11.26	18.92	6.27
6	13.87	14.29	12.27	7.41
9	12.11	14.39	19.07	13.72
12	10.79	8.28	15.61	14.52
	Acetic acid:propionic acid ratio[3]			
0	3.03	3.15	3.49	3.70
3	2.97	4.18	2.39	3.17
6	3.01	4.46	4.67	4.27
9	4.21	4.06	2.71	2.97
12	3.41	3.96	3.77	2.15

[1] $Y = 33.3883 + 1.67161 \times dose - 0.0874658 \times dose^2 + 3.14783 \times hour - 0.224648 \times hour^2$ ($R^2 = 0.99$).
[2] $Y = 13.65$.
[3] $Y = 1.65693 + 0.372558 \times dose - 0.0154109 \times dose^2$ ($R^2 = 0.87$).

There was no interaction of dose and time for the total concentration of VFA (Table 7). Maximum point for total concentration of VFA of 9.20 g of calcium hydroxide was found, presenting value of 71.92 µmol/mL of ruminal fluid, which may be related to dry matter digestibility and feed intake (Dias et al., 2011).

Table 6 - Means of concentrations (µmol/mL) of butyric, isobutyric, valeric and isovaleric acids of the rumen fluid of cattle fed diets based on sugar cane with doses of calcium hydroxide, at different times

Times (hours)	Calcium hydroxide (g/kg)			
	0	8	16	24
	Butyric acid[1]			
0	13.97	9.45	8.67	10.12
3	14.39	10.27	9.74	5.16
6	15.75	17.68	15.26	7.41
9	10.03	18.16	18.64	16.05
12	9.75	6.78	12.67	9.31
	Isobutyric acid[2]			
0	2.06	1.26	0.93	1.32
3	1.48	1.92	0.38	0.39
6	0.82	1.12	0.73	0.96
9	0.61	1.39	1.19	1.15
12	0.37	0.57	0.85	1.08
	Valeric acid[3]			
0	1.22	1.62	0.90	1.32
3	1.92	1.74	1.01	0.58
6	2.75	2.56	2.13	1.27
9	1.09	2.65	2.39	3.33
12	1.17	0.72	1.25	0.74
	Isovaleric acid[4]			
0	3.18	2.31	1.53	2.14
3	1.99	1.09	0.71	0.55
6	1.45	2.15	1.15	1.23
9	0.74	2.53	1.92	1.73
12	0.64	0.90	1.05	1.06

[1] $Y = 12.00897$.
[2] $Y = 1.02024$.
[3] $Y = 1.63761$.
[4] $Y = 1.49296$.

Table 7 - Means of concentrations (µmol/mL) of total volatile fatty acids (VFA) in the rumen fluid of cattle fed diets based on sugar cane with doses of calcium hydroxide, at different times

Times (hours)	Calcium hydroxide (g/kg)			
	0	8	16	24
	VFA total concentration (µmol/mL)			
0	72.91	72.93	49.10	61.57
3	69.81	72.52	76.15	34.39
6	79.94	94.15	84.49	43.96
9	75.69	88.47	88.60	75.23
12	59.82	70.07	79.12	54.22

$Y = 70.8763 + 2.27012 \times dose - 0.0122948 \times dose^2$ ($R^2 = 0.96$).

Considering the concentration of acetic acid (9.60 g), butyric acid (9.60 g) total volatile fatty acids concentration (9.20 g) in the rumen fluid, the average value of 9.46 g/kg is found. These values can be correlated with NDF digestibility (Dias et al., 2011), characterizing the effect of alkaline hydrolysis on the cell wall of sugar cane.

Conclusions

Calcium hydroxide added to the sugar cane provides higher values of pH, N-NH_3 and the concentration of volatile fatty acids in the rumen fluid. The dose of approximately 10 g of calcium hydroxide per kg sugar cane *in natura* is recommended.

References

BERGMAN, E.N. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. **Physiology Review**, v.10, n.2, p.567-589, 1990.

CAMPOS, F.P.; NUSSIO, C.M.B.; NUSSIO, L.G. **Métodos de análises de alimentos**. Piracicaba: FEALQ, 2004. 135p.

CAPELLE, E.R.; VALADARES FILHO, S.C.; SILVA, J.F.C. et al. Estimativas do valor energético a partir de características químicas e bromatológicas dos alimentos. **Revista Brasileira de Zootecnia**, v.30, n.6, p.1837-1856, 2001.

DIAS, A.M.; ÍTAVO, L.C.V.; DAMASCENO, J.C. et al. Sugar cane treated with calcium hydroxide in diet for cattle: intake, digestibility of nutrients and ingestive behaviour. **Revista Brasileira de Zootecnia**, v.40, n.8, p.1799-1806, 2011.

DIAS, H.L.C.; VALADARES FILHO, S.C.; SILVA, J.F.C. et al. Eficiência de síntese microbiana, pH e concentrações ruminais de amônia em novilhos F1 Limousin x Nelore alimentados com dietas contendo cinco níveis de concentrado. **Revista Brasileira de Zootecnia**, v.29, n.2, p.555-563, 2000.

GOULARTE, S.R.; ÍTAVO, L.C.V.; MORAIS, M.G. et al. Consumo de nutrientes e parâmetros ruminais de vacas alimentadas com diferentes níveis de energia na dieta. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.62, n.2, p.357-364, 2010.

HOOVER, W.H.; STOKES, S.R. Balancing carbohydrates and proteins for optimum rumen microbial yield. **Journal of Dairy Science**, v.74, n.10, p.3630-3644, 1991.

ÍTAVO, L.C.V.; VALADARES FILHO, S.C.; SILVA, F.F. et al. Produção microbiana e parâmetros ruminais de novilhos alimentados com dietas contendo vários níveis de concentrado. **Revista Brasileira de Zootecnia**, v.31, n.3, p.1553-1561, 2002.

ÍTAVO, L.C.V.; ÍTAVO, C.C.B.F. Parâmetros ruminais e suas correlações com desempenho, consumo e digestibilidade em ruminantes. In: ÍTAVO, L.C.V.; ÍTAVO, C.C.B.F. (Orgs.) **Nutrição e ruminantes**: aspectos relacionados à digestibilidade e ao aproveitamento de nutrientes. Campo Grande: Editora UCDB, 2005. p.49-72.

KOZLOSKI, G.V. **Bioquímica dos ruminantes**. Santa Maria: UFSM, 2002. 140p.

LENG, R.A. Factors affecting the utilization of "poor-quality" forages by ruminants particularly under tropical conditions. **Nutrition Research and Review**, v.3, p.277-303, 1990.

MARTINS, A.S.; VIEIRA, P.F.; BERCHIELLI, T.T. et al. Taxa de passagem e parâmetros ruminais em bovinos suplementados com enzimas fibrolíticas. **Revista Brasileira de Zootecnia**, v.35, n.3, p.1186-1193, 2006.

MEHREZ, A.Z; ØRSKOV, E. R.; McDONALD, I. Rates of rumen fermentation in relation to ammonia concentration. **Bristish Journal of Nutrition**, v.38, n.3, p.437-443, 1977.

MORAES, K.A.K.; VALADARES FILHO, S.C.; MORAES, H.B.K. et al. Cana-de-açúcar tratada com óxido de cálcio fornecida com diferentes níveis de concentrado para novilhas de corte em confinamento. **Revista Brasileira de Zootecnia**, v.37, n.7, p.1293-1300, 2008.

OLIVEIRA, M.D.S.; SANTOS, J.; DOMINGUES, F.N. et al. Avaliação da cal hidratada como agente hidrolisante de cana-de-açúcar. **Veterinária Notícias**, v.14, n.1, p.9-17, 2008.

PINA, D.S.; VALADARES FILHO, S.C.; AZEVÊDO, J.A.G. et al. Efeitos da inclusão e dos tempos de exposição da cana-de-açúcar ao óxido de cálcio sobre os parâmetros digestivos e fisiológicos de novilhas nelores. **Revista Brasileira de Zootecnia**, v.39, n.7, p.1579-1586, 2010.

PRESTON, T.R. Analytical methods for characterizing In: Feed resources for ruminants. **Better utilization of crop residues and by products in animal feeding**: research guidelines. 2. A practical manual for research workers. Rome: FAO, 1986. p.106.

RODRIGUES, P.H.M.; LUCCI, C.S.; CASTRO, A.L. Efeitos da lasalocida sódica e proporção volumoso/concentrados sobre a fermentação ruminal em vacas secas. **Brazilian Journal of Veterinary Research and Animal Science**, v.37 n.3, p.253-258, 2000.

SATTER, L.D.; SLYTER, L.L. Effect of ammonia concentration on rumen microbial protein production in vitro. **Bristish Journal of Nutrition**, v.32, n.2, p.199-208, 1974.

SILVA, D.J.; QUEIROZ, A.C. **Análise de alimentos**: métodos químicos e biológicos. Viçosa, MG: UFV, 2002. 235p.

SILVEIRA, R.N.; BERCHIELLI, T.T.; CANESIN, R.C. et al. Influência do nitrogênio degradável no rúmen sobre a degradabilidade *in situ*, os parâmetros ruminais e a eficiência de síntese microbiana em novilhos alimentados com cana-de-açúcar. **Revista Brasileira de Zootecnia**, v.38, n.3, p.570-579, 2009.

TEIXEIRA, J.C.; TEIXEIRA, L.F.A.C. **Princípios de nutrição de bovinos leiteiros**. Textos acadêmicos. Lavras: UFLA/FAEP, 2001. 245p.

VANZANT, E.S.; COCHRAN R.C.; JACQUES, K.A. et al. Influence of level of supplementation and type of grain in supplements on intake and utilization of harvested, early-growing-season bluestem-range forage by beef steers. **Journal of Animal Science**, v.68, p.1457-1464, 1990.

VAN SOEST, P.J. **Nutritional ecology of the ruminant**. 2.ed. Ithaca: Cornell University Press, 1994. 476p.

VARGAS, L.H.; LANA, R.P.; JHAM, G.N. et al. Adição de lipídios na ração de vacas leiteiras: parâmetros fermentativos ruminais, produção e composição do leite. **Revista Brasileira de Zootecnia**, v.31, n.1, p.522-529, 2002.