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Rumen parameters and passage rate in cattle fed diets based on sugarcane hydrolyzed with calcium oxide

Parâmetros ruminais e taxa de passagem em bovinos alimentados com rações baseadas em cana de açúcar hidrolisada com óxido de cálcio

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Abstract

Nine non-lactating Holstein x Gyr cattle were fed with rations based on chopped sugarcane supplied *in natura* (control) or hydrolyzed (fresh matter basis) for 24 h with 1% or 2% calcium oxide (CaO), which corresponds to 3.1% or 6.2% CaO on a dry matter (DM) basis, respectively. Ruminal parameters (pH and ammonia N, acetate, propionate and butyrate concentrations) as well as the kinetics of fluid and particulate passage in the gastrointestinal tract from cattle were evaluated. A design using three 3 x 3 contemporaneous Latin Squares (LS) was adopted. The studies of kinetics of fluid and particulate passage were performed using the external markers cobalt-EDTA and chromium-mordanted-NDF, respectively. The ruminal parameters were analyzed according to a 3 x 3 LS replicated three times with repeated measures in time using mixed models that included the fixed effects of treatment (level of CaO), time of sampling and their interaction and the following random effects: LS, animal(LS), period of LS and period*animal (LS). The kinetic parameters of fluid and particulate passage were analyzed using mixed models with treatment as a fixed effect and period of LS, animal(LS) and LS as random effects. The linear and quadratic effects of the treatments were analyzed using orthogonal contrasts. Significant differences were declared at $P \leq 0.05$. No treatment*time interaction was observed ($P > 0.05$) for any ruminal parameter. A linear effect ($P = 0.0279$) of CaO inclusion on ruminal pH was observed, but there were no effects ($P > 0.05$) of the treatments on the ammonia N, acetate, butyrate and total volatile fatty acid ruminal concentrations. There was a quadratic effect ($P = 0.05$) of the addition of CaO on the ruminal propionate concentration. There was no effect ($P > 0.05$) of the treatments on the particulate rate of passage in the rumen as well as on the mean retention time in this compartment and in the total gastrointestinal tract. On the other hand, the addition of CaO to sugarcane promoted a linear increase ($P = 0.0258$) in the particulate post-ruminal passage rate and, consequently, a linear reduction ($P = 0.0363$) of the mean retention time in the cecum-proximal colon. There was no effect ($P > 0.05$) of the sugarcane hydrolysis with CaO on the ruminal parameters of the kinetics of fluid passage (dilution rate, retention time and turnover rate). The addition of 3.1% or 6.2% CaO on a DM basis (1%

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or 2% CaO on a fresh matter basis, respectively) of sugarcane does not improve ruminal fermentation, nor does it increase the fluid or particulate passage in the rumen of non-lactating Holstein x Gyr cattle.
Key words: Alcali. Bovine. Lime. pH. *Saccharum officinarum*. Volatile fatty acids.

Resumo

Nove fêmeas Holândês x Gir não lactantes, alocadas em três Quadrados Latinos (QL) 3 x 3 contemporâneos, foram alimentadas com rações à base de cana de açúcar picada, fornecida *in natura* (Controle) ou hidrolisada (base da matéria fresca) por 24 h com 1% ou 2% de óxido de cálcio (CaO), o que correspondeu, respectivamente, a 3,1% ou 6,2% de CaO com base na matéria seca (MS). O pH e as concentrações ruminais de N amoniacal, acetato, propionato e butirato foram avaliados. As cinéticas de passagem de fluidos e de partículas no trato gastrointestinal (TGI) dos animais foram estudadas utilizando, respectivamente, os indicadores externos Cobalto-EDTA e fibra em detergente neutro mordantada com Cromo. As análises estatísticas foram realizadas utilizando modelos mistos. Os parâmetros de fermentação ruminal foram analisados como medidas repetidas no tempo, sendo considerados efeitos fixos: o tratamento (nível de adição de CaO), o tempo de amostragem e sua interação, e efeitos aleatórios: QL, animal(QL), período do QL e período*animal(QL). Os parâmetros de cinética de fluidos e de partículas no TGI foram analisados considerando o tratamento como efeito fixo, e período do QL, animal(QL) e QL como efeitos aleatórios. Diferenças foram consideradas significativas quando $P \leq 0,05$. Não houve interação tratamento*tempo ($P > 0,05$) para nenhum parâmetro ruminal. A adição de CaO promoveu incremento linear ($P = 0,0279$) no pH do rúmen, mas não houve efeito ($P > 0,05$) dos tratamentos sobre as concentrações ruminais de N amoniacal, acetato, butirato e ácidos graxos voláteis totais. Foi observado efeito quadrático ($P = 0,05$) da adição de CaO sobre a concentração ruminal de propionato. Não houve efeito ($P > 0,05$) dos tratamentos na taxa de passagem de partículas no rúmen, bem como no tempo médio de retenção neste compartimento e no TGI. Por outro lado, a adição de CaO à cana de açúcar promoveu incremento linear ($P = 0,0258$) na taxa de passagem pós-ruminal de partículas e, consequentemente, houve redução linear ($P = 0,0363$) no tempo médio de retenção no ceco-cólon proximal. Não houve efeito ($P > 0,05$) da hidrólise da cana de açúcar com CaO sobre os parâmetros da cinética da passagem de fluidos no rúmen (taxa de diluição, tempo de retenção e taxa de reciclagem). A adição de 1% ou 2% de CaO na matéria fresca (respectivamente, 3,1% ou 6,2% na matéria seca) da cana de açúcar não melhora a fermentação ruminal nem aumenta as taxas de passagem de fluidos e de partículas no rúmen de bovinos Holandês x Gir não lactantes.
Palavras-chave: Ácidos graxos voláteis. Álcali. Bovino. Cal. pH. *Saccharum officinarum*.

Introduction

Sugarcane (*Saccharum officinarum* L.) is a source of roughage traditionally used in the seasonal period of tropical grass production in milk production systems of Brazil (CAMPOS et al., 2017).

However, despite the high production of forage with a high energy value, due to the high content of soluble carbohydrates (sucrose) in the stalks, sugarcane presents nutritional limitations that restrict its use as an exclusive ingredient in the ruminant diet (CAMPOS et al., 2017). For cows with a milk yield of ~21 kg day^{-1}, the limitation of the low crude protein content of sugarcane forage can be corrected by the inclusion of non-protein N sources, such as urea, in diets with 50% of concentrates (SOUZA et al., 2015).

However, perhaps the most challenging nutritional limitation of sugarcane for its inclusion in the diet of high-producing ruminants is the low digestibility of its fiber, a consequence of the high rumen-undegradable neutral detergent fiber (NDF) content of its forage combined with the low ruminal digestion rate of the potentially degradable NDF (SANTOS et al., 2011; RIBEIRO et al., 2015; CAMPOS et al., 2017). These fractions have a

high effect of rumen fill and are responsible for the reduction in the rate of passage of the digesta (RIBEIRO et al., 2015) and voluntary consumption of sugarcane-based diets, with a negative impact on animal performance (SANTOS et al., 2011).

The effect of alkalinizing agents, such as sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$) and, more recently, calcium oxide (CaO), on the digestibility of the fibrous fraction of sugarcane has been evaluated in studies conducted *in vitro* (MOTA et al., 2010; DOMINGUES et al., 2015) and *in vivo* in cattle (EZEQUIEL et al., 2005; MORAES et al., 2008; PINA et al., 2010; CAMPOS et al., 2011; FREITAS et al., 2011; PINA et al., 2011; MISSIO et al., 2012; DANIEL et al., 2013). In theory, these alkalis promote the breakdown of the cell wall and the partial solubilization of the hemicelluloses, lignin and silica, through the hydrolysis of uronic esters and acetic acid and the swelling of cellulose (JACKSON, 1977). Potentially, this may promote an increase in the ruminal passage rate of the digesta and consequently in the voluntary consumption of diets based on sugarcane, resulting in a greater availability of nutrients for maintenance and production.

There are few studies on ruminal fermentation parameters of digesta and the kinetics of fluid and particulate passage in the gastrointestinal tract of cattle fed with rations based on hydrolyzed sugarcane. As summarized by Seo et al. (2006), the rate of passage of fluids in the rumen may affect the digestion of soluble nutrients (*e.g.*, sucrose), outflow of the end products of fermentation (*e.g.*, volatile fatty acids - VFAs), peptide escape and microbial growth. In turn, the particulate rate of passage in the rumen is related to voluntary feed intake, extent of digestion of the diet, the site of protein digestion, efficiency of microbial growth, etc. Therefore, in models designed to evaluate diets and cattle performance based on relationships between the nutritional components of the digesta and the nutrient supply, information on the kinetics of fluid and particulate passage is fundamental to allow the modeling of rumen function and metabolism using mathematical equations and quantitative representations (SEO et al., 2006; TYLUTKI et al., 2008; VAN AMBURGH et al., 2015).

The results of pH and ammonia N concentration in the rumen of cattle were presented in studies performed with sugarcane hydrolyzed with calcium hydroxide (DIAS et al., 2012; MISSIO et al., 2012) or calcium oxide (MORAES et al., 2008; PINA et al., 2010; SILVA JÚNIOR, 2013). However, VFA concentrations in the rumen of cattle fed hydrolyzed sugarcane-based diets were evaluated only in the studies of Dias et al. (2012) and Silva Júnior (2013), which were carried out using calcium hydroxide and CaO as alkalizing agents, respectively. The article by Ezequiel et al. (2005), which used sodium hydroxide, was the only work that evaluated the effect of alkaline hydrolysis of sugarcane on the kinetics of particulate passage in the gastrointestinal tract of cattle. On the other hand, Silva Júnior (2013) did not observe the effect of the sugarcane hydrolysis with 1% CaO (fresh matter basis) on the kinetics of fluid passage in the rumen of cattle. However, there is no work of this type where a higher level of inclusion of CaO has been studied.

The aim of this study was to evaluate the ruminal fermentation parameters and the kinetics of fluid and particulate passage in the gastrointestinal tract of non-lactating Holstein x Gyr cattle fed with rations based on chopped sugarcane supplied *in natura* or hydrolyzed (fresh matter basis) with 1% or 2% CaO (3.1% or 6.2% CaO on a dry matter basis, respectively).

Material and Methods

The study was carried out at Embrapa Dairy Cattle in Valença (RJ), Brazil. All experimental procedures with animals were carried out according to the Embrapa Dairy Cattle guidelines for animal care and use in research.

Nine non-lactating Holstein x Gyr cattle were used (body weight = 440 ± 96 kg), each provided with ruminal cannula with an internal opening diameter of 110 mm. The animals were kept in a *tie-stall* type confinement system, each equipped with an individual trough and automatic drinking fountain.

A design using three 3 x 3 contemporaneous Latin Squares (LS) was adopted. Each period consisted of 21 days, with 14 and seven days for adaptation to the rations and the collections, respectively. The animals were homogeneously allocated to the LS based on body weight.

Three rations based on chopped sugarcane as the only roughage were evaluated (Table 1). The sugarcane was supplied *in natura* (control) or hydrolyzed (fresh matter basis) with 1% or 2% CaO, which corresponded to 3.1% or 6.2% CaO on a DM basis, respectively. The CaO was supplied in a microprocessed form with low levels of dioxins, furans and magnesium. The treatment of the sugarcane with CaO was always performed 24 h prior to feeding the animals.

Table 1. Chemical composition of the diets expressed as a percentage of dry matter (% DM).

Nutrient	% CaO added to sugarcane (DM basis)[1]		
(% DM)	0	3.1	6.2
Dry matter (%, fresh basis)	33.18	34.52	35.34
Organic matter	88.63	83.29	80.10
Ether extract	1.40	1.13	0.98
Non-fibrous carbohydrates	46.13	42.91	41.22
Neutral detergent fiber (NDF)	33.00	32.84	32.51
NDF corrected for ash and protein	31.75	30.95	29.92
Acid detergent fiber	23.27	20.77	19.84
Lignin	4.71	4.03	3.62
Cellulose	18.57	16.74	16.22
Hemicelluloses	9.72	12.07	12.67
Crude protein	9.35	8.30	7.99
Ash	11.37	16.71	19.90
Calcium (Ca)	0.30	2.01	3.55
Phosphorous (P)	0.19	0.20	0.20
Ratio Ca: P	1.55	10.70	19.77

[1]Corresponds to 0%, 1% or 2% CaO in the fresh matter of sugarcane, respectively.

The roughage: concentrate ratio of the rations (DM basis) was 75%:25%. The rations were provided to the animals twice a day, at 8h00 and 14h00, in sufficient amounts to allow for 10% of leftovers. The concentrate supplied had the following ingredient composition (as fed): 50% soybean meal, 42% corn meal, 5% urea: ammonium sulphate mixture (9:1), and 3% mineral supplement. The macro- and micromineral concentrations of the mineral supplement and of CaO are shown in Table 2.

The RB-73-9735 variety of sugarcane was used, which has a productivity estimated at 150 t ha^{-1}, medium maturation, rare flowering, and a harvest period between June and October. The sugarcane presented a mean Brix value of 22°. The formulation of the experimental rations was based on the nutritional requirements of the animals, determined according to the NRC (2001).

Table 2. Mineral composition of the mineral supplement and calcium oxide (CaO).

	Macromineral (% of DM)				Micromineral (ppm)			
Item	Ca	Mg	P	K	Cu	Zn	Fe	Mn
Mineral supplement	9.6	1.0	8.7	0.08	1,254	4,228	5,258	427
CaO	44.5	0.3	2.9	0.05	41	73	1,388	128

Ca = Calcium; Mg = Magnesium; P = Phosphorous; K = Potassium; Cu = Copper; Mn = Manganese; Fe = Iron and Zn = Zinc.

On the 15th day of each period of the LS, samples of ruminal fluid were collected from the ventral portion of the rumen prior to the morning feeding, and this was considered as time 0 (zero). Additional samples were obtained at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h after the first collection. The samples were filtered immediately using double gauze, and the pH was measured using a portable digital potentiometer. Two 50-mL aliquots were then pipetted, added to flasks with 1 mL of H_2SO_4 50% v/v (subsample 1) or 10 mL of metaphosphoric acid 25% v/v (subsample 2), and then frozen. After thawing, subsample 1 was analyzed for the ammonia N concentration according to the INCT-CA N-007/1 method (DETMANN et al., 2012). Subsample 2 was centrifuged (17,000 x *g* for 10 min) and analyzed for the molar concentration (μmol mL^{-1}) of the acetate, propionate and butyrate using a gas chromatographer model 7820A (Agilent Technologies Inc., Santa Clara, USA) provided with a Nukol capillary column (30 m x 22 mm x 0.25 μm) connected to free fatty acids (SUPELCO, Bellefonte, PA, USA) and equipped with a flame ionization detector.

Fresh and hydrolyzed sugarcane samples were collected and submitted to hot extraction using a neutral commercial detergent obtaining, on average, 84% NDF. Those materials were subsequently complexed with sodium dichromate ($Na_2Cr_2O_7.2H_2O$) according to the procedures reported by Udén et al. (1980), resulting in mordants with 3% Cr (DM basis). In each period of LS before the animals were fed their meal, 100 g of Cr-mordanted NDF was intra-ruminally administered in a single dose to each animal and fecal samples were collected directly from the rectum at 0, 6, 12, 18, 24, 36, 48, 60, 72, 84 and 96 h after dosing with the marker. Fecal samples were dried in a forced-air oven at 55°C (72 h), ground to pass through a 1-mm screen and stored for analysis for Cr content using atomic absorption spectrophotometry according to the INCT-CA M-005/1 method (DETMANN et al., 2012).

The study of the kinetics of the fluids in the rumen was performed using the external marker cobalt-EDTA (17.2% of cobalt (Co), DM basis), obtained according to the procedures reported by Udén et al. (1980). In each period of LS before the animals were fed their meal, each animal was intra-ruminally pulse-dosed with 10 g of Co-EDTA in a 200 mL aqueous solution (w/v). Subsequently, fecal samples were collected directly from the rectum at the same times as previously described. These samples were dried in a forced-air oven at 55°C (72 h), ground to pass through a 1-mm screen and stored for analysis for Co content using atomic absorption spectrophotometry (UDÉN et al., 1980).

Individual fecal Cr and Co excretion curves were fitted to the multi-compartmental model proposed by Dhanoa et al. (1985) and to the two-compartment biexponential time-independent model described by Grovum and Williams (1973), respectively, using the procedure for non-linear models of SAS version 9.0.

The multi-compartmental model proposed by Dhanoa et al. (1985) was of the form: $Y = A*\exp(-k_1*T)*\exp\{-(n-2)\exp[-(k_2-k_1))T]\}$, where Y (mg kg^{-1} DM) is the fecal marker concentration at time t (h); T = t – TT; TT is the transit time or time delay

(h) between the marker administration and its first appearance in the feces; k_1 (% h^{-1}) is the passage rate from the reticulorumen; k_2 (% h^{-1}) is the passage rate from the cecum-proximal colon; A is a scale parameter dependent on k_1; and *n* is the number of compartments in the model (2 in this case, the reticulorumen and the cecum-proximal colon). Mean retention times in the reticulorumen (RMRT, h) and the cecum-proximal colon (CMRT, h) were calculated as $1/k_1$ and $1/k_2$, respectively. Total mean retention time (TMRT, h) was calculated as TMRT = RMRT + CMRT + TT (MENDOZA et al., 2016).

The ruminal fluid passage rate or dilution rate (k, % h^{-1}) was estimated from the Grovum and Williams (1973) model. The rumen-reticulum retention time (RT, h) was calculated as the reciprocal of the ruminal fluid passage rate (*i.e.*, 1/k), and the turnover rate (*i.e.*, the number of times that the pool is completely recycled every 24 h) was calculated as 24/RT (times day^{-1}) as described by Silva et al. (2011).

The ruminal parameters were analyzed according to a 3 x 3 LS replicated three times with repeated measures in time using the MIXED procedure of SAS version 9.0. The model included the fixed effects of treatment (level of CaO), time of sampling and their interaction as well as the following random effects: LS, animal(LS), period of LS and period*animal (LS). Ten covariance structures of residues were compared, and the choice of matrix was made based on the Akaike information criterion. The kinetic parameters of fluid and particulate passage were analyzed using mixed models considering treatment as a fixed effect and period of LS, animal(LS) and LS as random effects. The linear and quadratic effects of the treatments were analyzed using orthogonal contrasts. The results are reported as LS means, and effects were considered significant when $P \leq 0.05$. Regression analyses of parameters of ruminal fermentation were performed (REG procedure of SAS) as a function of sampling time as well as treatment.

Results and Discussion

No treatment*time interaction was observed ($P > 0.05$) for any ruminal parameter. In a statistical analysis of data from 20 published studies that included 55 treatment means conducted to evaluate the effect of the addition of CaO to fresh sugarcane, Daniel et al. (2013) observed that the alkaline nature of CaO linearly raised the sugarcane pH. Similarly, the addition of the alkaline agent CaO to the sugarcane in the present study caused a linear increase ($P = 0.0279$) in the pH ruminal values (Table 3). In a study carried out with Angus x Nellore crossbred cows fed sugarcane-based diets hydrolyzed (fresh basis) with 0.8%, 1.6% and 2.4% calcium hydroxide, Dias et al. (2012) also observed this effect, *i.e.*, an increase in the pH ruminal values due to the alkalinizing power of calcium hydroxide. It should be noted that the DM content of sugarcane (27.43%) used in the work of Dias et al. (2012) was similar to that of the present study, which was on average 27.6%.

Regardless of the treatment, there was an effect of the sampling time on the ruminal pH of the animals ($\hat{y} = 6.80839 - 0.03923*time + 0.00220*time^2$; $R^2 = 0.59$; $P = 0.02$). Throughout the day, a minimum value of 6.6 was estimated, which is higher than the value of 6.0 that is considered the critical limit below which degradation of cellulose is inhibited (VALADARES FILHO; PINA, 2011). The ruminal pH values observed in the present experiment (Table 3) were in the range of 6.51 to 7.22 obtained by Dias et al. (2012) in the rumen of Angus x Nellore crossbred cows fed sugarcane-based diets that were either hydrolyzed (0.8%, 1.6% and 2.4%; fresh matter basis) or not with calcium hydroxide. In a study performed with Nellore heifers that were fed a diet with 20% concentrate and 80% sugarcane with or without treatment of 0.5% and 1.0% CaO (fresh matter basis), Pina et al. (2010) observed ruminal pH values ranging from 6.18 to 6.81, which can be considered close to those presented in Table 3. On the other hand, higher ruminal pH values (6.82 to

6.96) were observed by Silva Júnior (2013) in Jersey steers that were fed a diet with 30% concentrate and 70% chopped sugarcane supplied *in natura* or hydrolyzed for 24 h with 1% CaO (fresh matter basis).

Table 3. Parameters of ruminal metabolism from non-lactating Holstein x Gyr cattle fed sugarcane-based diets hydrolyzed with calcium oxide (CaO).

Parameter	% CaO added to sugarcane (dry matter basis)[1]			Standard error of the mean	P-value	
	0	3.1	6.2		Linear	Quadratic
pH	6.63	6.82	6.79	0.062	0.0279	0.0785
Ammonia N (mg dL^{-1})	12.04	12.04	11.70	0.967	0.8752	0.8657
Acetate (μmol mL^{-1})	31.63	33.83	31.76	4.309	0.7221	0.3137
Propionate (μmol mL^{-1})	11.36	9.14	9.93	1.458	0.1405	0.0500
Butyrate (μmol mL^{-1})	8.06	10.16	8.18	1.202	0.9983	0.1090
Volatile fatty acids[2] (μmol mL^{-1})	50.88	53.59	50.23	7.060	0.9719	0.3567
Acetate: propionate ratio	2.92	4.09	3.37	0.151	0.0019	<0.0001
Molar proportions (%)						
Acetate	61.55	65.01	63.66	1.390	0.2323	0.1547
Propionate	22.16	16.57	19.54	0.9126	0.2370	<0.0001
Butyrate	16.19	18.67	16.73	1.717	0.9315	0.2635

[1]Corresponds to 0%, 1% or 2% CaO in the fresh matter of sugarcane, respectively.
[2]Volatile fatty acids = Σ concentrations of acetate + propionate + butyrate.

No effect on the ruminal concentration of ammonia N was observed (P>0.05) with the inclusion of CaO in the diets. The mean values obtained (Table 3) were greater than 10 mg dL^{-1}, considered adequate to meet the net requirement of the microbiota that ferment fibrous carbohydrates in the rumen and preferably use ammonia N as a nitrogenous substrate for protein synthesis (VALADARES FILHO; PINA, 2011). The lack of an effect of sugarcane hydrolysis with 1% CaO on the ruminal concentration of ammonia N was also observed in two other studies, with Nellore heifers (PINA et al., 2010) and Jersey steers (SILVA JÚNIOR, 2013) fed diets containing 11.0% to 12.1% crude protein (DM basis). The ruminal ammonia N concentrations observed in the present experiment (Table 3) are within the range of the 8.03 to 13.4 mg dL^{-1} obtained by Pina et al. (2010) and Silva Júnior (2013).

There was no effect (P>0.05) of the inclusion of CaO on the concentration and molar proportions of acetate in the rumen of animals (Table 3). In a companion study performed concomitantly with the present study that used the same treatments and animals, Campos et al. (2011) did not observe an effect on the parameters of *in situ* ruminal degradability of the NDF of the sugarcane when adding CaO. This explains, at least in part, the similarity in the ruminal concentrations of acetate observed in the present study (Table 3).

There was a quadratic effect of the addition of CaO on the concentration (P = 0.05) and molar proportion (P<0.0001) of propionate in the rumen of the animals (Table 3). Throughout the day, the minimum ruminal concentration of propionate was 9.07 μmol mL^{-1}, estimated when 1.27% of CaO was added to sugarcane ($\hat{y}$ = 1.48*time^2 – 3.75*time + 11.45; R^2 = 0.40; P = 0.0044). In a statistical analysis of 20 published studies conducted to evaluate the addition of 0-3% of CaO to fresh sugarcane, Daniel et al. (2013) observed a quadratic effect (P<0.01) on the concentration of non-fibrous

carbohydrates (NFCs). After chopping and storing sugarcane under aerobic conditions, sugarcane soluble carbohydrates can be extensively oxidized by epiphytic microorganisms, as well as by plant cell metabolism itself (DANIEL et al., 2013).

The heat resulting from the CaO hydration reaction in the forage mass submitted to the hydrolysis can accelerate the sugarcane fermentation process. This promotes the reduction of the soluble carbohydrate content and, consequently, the NFCs. The quadratic effect observed in the NFC content in response to the addition of CaO to sugarcane can be explained as a function of the CaO dose used in the sugarcane hydrolysis (DANIEL et al., 2013). Thus, while using lower doses of CaO may promote a reduction in the NFC content, the addition of higher concentrations of CaO could inhibit microbial growth and metabolism of plant cells, thereby preserving the NFCs in sugarcane.

In this sense, in relation to chopped sugarcane *in natura*, Romão et al. (2013) observed a reduction in the content of the A + B1 fractions of carbohydrates when the sugarcane was hydrolyzed with 0.75% CaO. In addition to this, increases were observed in these contents when 2.25-4.0% of CaO was used in the hydrolysis. Using the equation $\hat{y} = 421 – 6.13x + 0.227x^2$ ($P<0.01$) obtained by Daniel et al. (2013), the lowest NFC content was estimated in the present study when ~1.4% of CaO was added to the fresh matter of sugarcane. This corroborates the study of Romão et al. (2013), who reported that the preservation of NFC in hydrolyzed sugarcane occurred with the addition of 1.5% of CaO.

In the present study, it is possible to suggest that the CaO dose used in the hydrolysis of sugarcane (DANIEL et al., 2013; ROMÃO et al., 2013) may be related to the quadratic effect observed in the concentration and the molar proportion of propionate in the rumen (Table 3). As shown in Table 1, in relation to the diet in which sugarcane *in natura* was supplied, cattle fed with hydrolyzed sugarcane inclusion had lower NFC contents and, consequently, less amount of substrate for fermentation to propionic acid in the rumen.

There was no effect ($P>0.05$) of the inclusion of CaO on the concentration and molar proportions of butyrate or in the total concentration of VFAs in the rumen of the animals (Table 3). The absence of an effect ($P>0.05$) on the total concentration of VFAs with the inclusion of CaO in sugarcane can be attributed to the similarity observed between treatments for the concentration of acetate, which is the major VFA in the rumen. The quadratic effect ($P<0.0001$) that the inclusion of CaO promoted in the acetate: propionate ratio (Table 3) was a consequence of the observed similarity between the treatments for acetate concentration ($P>0.05$) but was mainly modulated by the quadratic effect ($P = 0.05$) that the CaO inclusion promoted in the ruminal concentration of propionate.

In general, the ruminal concentrations of acetate, propionate, butyrate and VFAs observed in the present study (Table 3) can be considered low when compared to those obtained in studies for the evaluation of alkalizing agents in sugarcane-based diets supplied to cattle (DIAS et al., 2012; SILVA JÚNIOR, 2013). These reduced ruminal concentrations of VFAs are probably a reflection of the low availability of fermentable substrate, represented by the low quality of the sugarcane fiber (SANTOS et al., 2011; RIBEIRO et al., 2015), as well as the reduction of the NFC content promoted by the hydrolysis with CaO (Table 1).

There was no effect ($P>0.05$) of the treatments on the particulate rate of passage in the rumen as well as on the mean retention time in this compartment (RMRT). In a companion paper performed concomitantly with the present study that used the same treatments and animals, Campos et al. (2011) did not observe an effect on the parameters of *in situ* ruminal degradability of the fibrous fractions (NDF and ADF) of the sugarcane when adding CaO. This explains, at least in part, the similarity between the treatments regarding the particulate rate of passage

in the rumen (Table 4). Also, this shows that the treatment with CaO did not provide the expected benefits, mainly in relation to the mitigation of the negative impact of the typically low digestibility of the fibrous fraction of sugarcane on the particulate rate of passage in the rumen. In addition, this corroborates the results of the study by Daniel et al. (2013), where it was shown that the net hydrolysis of sugarcane fiber was not achieved with treatment of up to 3% CaO.

Table 4. Parameters of the kinetics of particulate passage in the gastrointestinal tract of non-lactating Holstein x Gyr cattle fed sugarcane-based diets hydrolyzed with increasing amounts of calcium oxide (CaO).

Parameter[1]	% CaO added to sugarcane (dry matter basis)[2]			Standard error of the mean	P-value	
	0	3.1	6.2		Linear	Quadratic
k_1 (% h^{-1})	3.82	3.56	3.46	0.4974	0.1816	0.7268
k_2 (% h^{-1})	6.45	6.22	7.95	0.6270	0.0258	0.0701
TT (h)	11.74	11.71	8.84	1.8680	0.0416	0.2347
RMRT (h)	27.09	31.26	30.98	5.1510	0.2012	0.5506
CMRT (h)	15.83	16.66	13.23	1.3479	0.0363	0.0559
TMRT (h)	54.66	58.51	53.06	3.4718	0.5879	0.0842

[1]Parameters estimated from the adjustment of the data of fecal excretion of Cr to the model proposed by Dhanoa et al. (1985): k_1 = rate of passage in the reticulorumen; k_2 = rate of passage in the cecum-proximal colon; TT = transit time or time delay (h) between marker administration and its first appearance in the feces; RMRT = mean retention time in the reticulorumen ($1/k_1$), CMRT = mean retention time in the cecum-proximal colon ($1/k_2$); and TMRT (Total mean retention time) = RMRT + CMRT + TT.
[2]Corresponds to 0%, 1% or 2% CaO in the fresh matter of sugarcane, respectively.

The addition of CaO promoted a linear increase ($P = 0.0258$; $\hat{y} = 0.75x + 6.12$) on the particulate post-ruminal passage rate, which in comparison to the rumen passage rate, presents less impact and importance considering sugarcane-based diets. In addition, the consequent linear reduction ($P = 0.0363$; $\hat{y} = -1.30x + 16.54$) observed for the CMRT was not sufficient to promote any change in the TMRT (Table 4), whose values were within normal range considering the literature for non-lactating cattle fed with sugarcane-based diets (AROEIRA et al., 1993; FIGUEIRA et al., 1993).

There was no effect ($P>0.05$) of the sugarcane hydrolysis with CaO on the ruminal parameters of the kinetics of fluid passage (Table 5), as also observed by Silva Júnior (2013), who worked with Jersey steers fed with *in natura* or hydrolyzed sugarcane-based diets. The parameters obtained in the present study (Table 5) are within the normal range considering the literature for non-lactating cattle fed with sugarcane-based diets (SILVEIRA et al., 2009; SILVA JÚNIOR, 2013). As summarized by Seo et al. (2006), the rate of passage of fluids in the rumen may affect digestion of soluble nutrients (*e.g.*, sucrose), outflow of the end products of fermentation (*e.g.*, VFAs), peptide escape and microbial growth. In this way, one can expect that the metabolism and the supply of these nutrients were similar among the evaluated diets and therefore had little impact on animal performance.

Table 5. Parameters of the kinetics of fluid passage in the rumen of non-lactating Holstein x Gyr cattle fed sugarcane-based diets hydrolyzed with increasing amounts of calcium oxide (CaO).

Parameter	% CaO added to sugarcane (dry matter basis)[1]			Standard error of the mean	P-value	
	0	3.1	6.2		Linear	Quadratic
Ruminal fluid rate passage (% h^{-1})	6.45	6.45	7.76	0.8066	0.1519	0.3715
Rumen-reticulum retention time (h)	18.35	16.25	13.17	2.2111	0.1021	0.5894
Turnover rate (times day^{-1})	1.55	1.55	1.86	0.1936	0.1518	0.3733

[1]Corresponds to 0%, 1% or 2% CaO in the fresh matter of sugarcane, respectively.

Conclusion

The addition of 3.1% or 6.2% CaO on a DM basis (1% or 2% CaO on a fresh matter basis, respectively) of sugarcane did not improve ruminal fermentation, nor did it increase the fluid or particulate passage in the rumen of non-lactating Holstein x Gyr cattle.

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