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Effects of energy sources and inclusion levels of concentrate in sugarcane-silage-based diets of finishing Nellore young bulls: Nutrient digestibility, rumen metabolism and ecosystem

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Abstract

Intake in sugar-rich diets can be limited either via rumen fill or excessive rumen fermentation and source of non-fibre carbohydrate (NFC) in the diet can affect both factors. The aim of the current study was to quantify the effect of partially replacing ground maize (GM) with steam-rolled maize (SRM) or pelleted citrus pulp (PCP) at two concentrate levels in sugarcane-based diets on digestibility, rumen ecosystem and metabolism of Nellore steers. Six rumen-cannulated steers were assigned to a 6 × 6 Latin square, replicated in time, in a 2 × 3 factorial arrangement of treatments with two levels of concentrate (600 or 800 g concentrate/kg dry matter [DM]) and three NFC sources. Each steer within a period was considered an experimental unit. Feeding more concentrate increased total tract digestibility of organic matter and decreased fibre intake and passage rate. It also reduced rumen populations of *Fibrobacter succinogenes* and *Streptococcus bovis* and increased *Ruminococcus flavefaciens*. Substituting PCP for GM increased rumen pH, acetic acid and organic matter digestibility. Feeding PCP also reduced *R. flavefaciens* and *R. amylophilus* rumen populations. Substituting SRM for GM increased starch digestibility and rumen propionic acid, but decreased rumen ammonia concentration. Feeding SRM increased rumen populations of *Megasphaera elsdenii* with the high-concentrate diet but reduced *Ruminococcus albus* populations at both concentrate levels. In conclusion, partial replacement of GM by PCP decreased intake in sugar-rich diets, while increasing total tract neutral detergent fibre digestibility. Replacement of GM with SRM increases rumen fermentation and total tract digestibility of starch.

Introduction

Finishing cattle in feedlots requires the use of high concentrate diets to increase digestibility and improve cattle performance. Maize is the primary energy source in cattle diets and its processing can affect the rate and extent of ruminal degradation, leading to changes in the efficiency of energy and nitrogen utilization. Steam-rolling is a processing method that increases the surface area and accessibility of starch to amylolytic bacteria, accelerating rumen fermentation (Owens and Basalan, 2016). However, diets with more digestible starch that can be digested more quickly produce larger amounts of volatile fatty acids, changing the fermentation patterns which can reduce the population of fibrolytic bacteria such as *Fibrobacter succinogenes, Ruminococcus flavefaciens* and *Ruminococcus albus*, reducing fibre degradation (Forsberg *et al*., 1997). At the same time, faster starch degradation increases the population of amylolytic bacteria, such as *Streptococcus bovis* and *Ruminococcus amylophilus*, and of lactate utilizing bacteria, such as *Megasphaera elsdenii* (Tajima *et al*., 2001; Nagaraja and Titgemeyer, 2007; Petri *et al*., 2012).

Moreover, the excess of ruminal starch fermentation combined with higher concentrate inclusion in cattle diets can lead to metabolic disorders, decreasing dry matter intake and consequently, cattle performance (Gonzalez *et al*., 2012). Therefore, replacing starch sources with citrus pulp, a high-pectin by-product available in the dry season (Oni *et al*., 2008), tends to yield more rumen acetate and prevent the increase of lactate in the rumen (Hall and Eastridge, 2014) and is commonly included as replacement of rapid fermentable starchy feedstuffs in finishing diets to prevent acidosis.

Therefore, it is hypothesized that citrus pulp would increase acetate and steam-rolled maize would increase propionate concentration in the rumen, affecting intake when replacing ground

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maize. Moreover, different concentrate inclusion in sugarcane silage diets is also likely to alter rumen dynamics. Thus, the aim of the current study was to quantify the effect of partial replacement of ground maize with steam-rolled maize or pelleted citrus pulp in two concentrate levels in sugarcane-based diets on digestibility, rumen metabolism and ruminal ecosystem of Nellore rumen-cannulated steers.

Materials and methods

Six rumen-cannulated 20-month-old Nellore steers, with average initial body weight (BW) of 345 ± 14.6 kg, were assigned into two non-contemporary 6 × 6 Latin squares, replicated in time. The steers were kept in tie stalls with concrete floor and individual feed and water supplies. The six treatments were assigned randomly to the pens in a 2 × 3 factorial arrangement of treatments. The factors consisted of two levels of concentrate in the diet (CONC), either 600 or 800 g concentrate/kg dry matter (DM) and three non-fibre carbohydrate (NFC) sources: pelleted citrus pulp (PCP) and steam-rolled maize (SRM; density 415 g/l), partially replacing ground maize (GM).

Sugarcane silage was used as the roughage source and harvested at the maturity stage (above 180 g sucrose/l juice). Sugarcane (variety IAC-SP 93-3046) was harvested mechanically and chopped to obtain particle sizes of 8–10 mm. During chopping, sugarcane was inoculated with *Lactobacillus buchneri* (strain NCIMB 40788-LALSIL Cana; Lallemand Animal Nutrition, Aparecida de Goiânia, GO, Brazil) to prevent alcoholic fermentation, and ensiled in a surface silo for 32 days before the beginning of the experiment. Diets were formulated according to NRC (2000) to fulfil the maintenance requirements and promote an average daily gain of 1.4 kg/day. Diet composition is described in detail by Ferrari *et al.* (submitted).

For better adaptation to facilities, steers were placed into the stalls for 21 days before the beginning of the experiment and fed a sugarcane-based diet. The experiment consisted of six periods of 14 days, with 10 days for diet adaptation and the last 4 days for sample collections. The length of the adaptation period was chosen based on previous studies demonstrating that 5–10 days would be enough for microbial adaptation (Potter and Dehority, 1973; Marden *et al.*, 2008; Cantalapiedra-Hijar *et al.*, 2011).

Diets were fed at 08.00 h and, during the last 4 days of each period, samples of ingredients and orts were collected and analysed for DM and intake calculations following the same procedures described in Ferrari *et al.* (submitted). The DM and component intakes were calculated by subtracting the DM and components of orts from the DM and components of the offered diet.

Total tract digestibility

Total tract apparent digestibility of DM, neutral detergent fibre (NDF) and starch was calculated by spot faecal sample collections on days 11, 12 and 13 of each experimental period. Faeces were collected at 07.00 and at 14.00 h and analysed for indigestible NDF (iNDF) to estimate total faecal production. The iNDF was determined as the residual NDF after 288-h *in situ* incubation in a rumen-cannulated steer (Huhtanen *et al.*, 1994) with four replicates for each sample.

Rumen fibre kinetics

Entire ruminal contents of the steers were manually evacuated through the rumen cannula on day 12 at 10.00 h (2 h post-feeding) and on day 13 at 06.00 h (2 h before feeding) of each period to determine the solid and fluid digesta weights (Dado and Allen, 1995). Ruminal contents from both phases (solid and liquid) were mixed and sampled for analysis of DM and nutrient pool size. A separate aliquot of approximately 300 ml of each phase was also collected for quantification of ruminal bacterial population. The remaining digesta was then returned to the rumen of the same steer. The ruminal fibre kinetics was calculated based on iNDF rumen pool size (Dado and Allen, 1995). It is important to highlight that because of the rumen evacuation method used to estimate the NDF passage rate (*kp*), it is not possible to completely separate passage from digestion rate, because greater digestion can lead to a greater passage rate of forage particles, which is likely to be the case in the present study. The NDF disappearance rate was calculated as NDF intake divided by ruminal mass of NDF, and the NDF *kp* was calculated by dividing iNDF intake by total mass of ruminal iNDF.

Ruminal fermentation

Rumen fluid samples were collected at 0 (before feeding), 1, 3, 6, 9 and 12 h after feeding on day 14 of each period. Samples were taken from caudal, ventral and cranial areas of the rumen via cannula, mixed and filtered through a 1-mm nylon mesh (Albercan Group, Itajubá, Brazil). Approximately 100 ml of rumen fluid was divided into two aliquots: one aliquot for measuring rumen pH and another for short chain fatty acids (SCFA) and ruminal ammonia nitrogen ($N\text{-}NH_3$) analysis.

Rumen fluid pH was measured immediately with a portable pH-meter (Tec-3MP, Tecnal, Brazil). After centrifuging the remaining aliquot of rumen fluid at 6500 × ***g*** for 15 min, 1 ml of 1 *N* sulphuric acid was added to another 2 ml of the supernatant and frozen at −20 °C for ammonia-N determination according to the phenol-hypochlorite method (Broderick and Kang, 1980). Another 2-ml sub-sample of the supernatant was taken, mixed with 0.4 ml of formic acid and frozen at −20 °C for SCFA determination.

Rumen SCFA from fluid was measured by gas chromatography with a capillary column (Stabilwax; Restek – Bellefonte, USA), using the method described by Erwin *et al.* (1961) and adapted by Getachew *et al.* (2002). Acidified rumen fluid samples were centrifuged at 14 500 × ***g*** for 10 min then 1 ml of supernatant was transferred into a vial with 100 µl of internal standard (2-ethyl-butyric acid 100 mM; Chem Service, West Chester, PA, USA). Concentrations of SCFA were determined using gas chromatography (GM-2014; Shimadzu, Kyoto, Japan), with split injector and dual flame ionization detector temperatures at 250 °C and column temperature at 145 °C. External standards were prepared with the acetic, propionic and butyric acids (Chem Service). For calculations of SCFA, the software GMSolution (Shimadzu) was used for separation and integration of chromatographic peaks.

Ruminal bacterial populations

Three ruminal bacteria important for fibre degradation (*Fibrobacter succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens*), two ruminal bacteria important for starch digestion and lactic acid production (*Streptococcus bovis* and *Ruminobacter amylophilus*) and a lactate-fermenting microorganism (*Megasphaera elsdenii*) had their relative population quantified by quantitative real-time polymerase chain reaction (qPCR) for determining the

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Table 1. Real-time PCR primers used in quantification of rumen microorganisms

Species	Sequence (5′–3′)	Amplicon size (bp)	Reference
F. succinogenes	F: GGTATGGGATGAGMTTGM	445	Tajima *et al.* (2001)
	R: GMCTGMCCCTGAACTATC		
R. albus	F: CCCTAAAAGMAGTCTTAGTTCG	175	Wang *et al.* (1997)
	R: CCTCCTTGMGGTTAGAACA		
R. flavefaciens	F: TCTGGAAACGGATGGTA	295	Koike and Kobayashi (2001)
	R: CCTTTAAGACAGGAGTTTACA		
S. bovis	F: CTAATACCGMATAACAGMAT	127	Stevenson and Weimer (2007)
	R: AGAAACTTCCTATCTCTAGG		
R. amylophilus	F: CAACCAGTCGMATTCAGA	642	Tajima *et al.* (2001)
	R: CACTACTCATGGMAACAT		
M. elsdenii	F: GACCGAAACTGMGATGMTAGA	129	Ouwerkerk *et al.* (2002)
	R: CGMCTCAGMGTCAGTTGTC		
Eubacteria	F: CCTACGGGAGGMAGMAG	193	Muyzer *et al.* (1993)
	R: ATTACCGMGGMTGMTGG		

effect of diets on ruminal bacterial population. For each sample collected during rumen evacuation, 25 ml of fluid and 25 g of solids were processed as described by Stevenson and Weimer (2007) and the resulting bacteria pellet was dissolved in 700 µl of buffer (100 mM Tris(hydroxymethyl)aminomethane hydrochloride [Tris/HCl], 10 mM ethylenediaminetetraacetic acid [EDTA] and 0.15 M sodium chloride [NaCl], pH 8.0) and stored at −80 °C until DNA extraction. For each ruminal sample, 200 µl of the bacteria resuspension was submitted to DNA extraction with QIAamp DNA Stool Mini Kit (Qiagen®, Valencia, CA, USA), according to the manufacturer's instructions, and ethanol precipitated as described by Yu and Morrison (2004).

The qPCR reactions were performed in 96-well plates on a 7500 Real-Time PCR System, with a final volume of 20 µl per reaction, containing 10 µl of 2x SYBR Green Master Mix (Applied Biosystems®, Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA), 1.20 µM concentration of each primer (Table 1) and 1 µl of DNA template. A universal primer, *Eubacteria universal*, was used for quantification of total bacteria to standardize the amount of DNA added to the reactions. The qPCR reactions of all bacteria were run in duplicate. The qPCR amplification protocol consisted of an initial denaturation step at 95 °C for 10 min, followed by 44 cycles of heating and cooling at 95 °C for 15 s and 60 °C for 30 s, and extension at 72 °C for 30 s. At the end of the reactions, melting curves were analysed to verify the specificity of each amplification.

Statistical methods

The statistical analyses were conducted using the MIXED procedure of SAS version 9.1.2 for Windows (SAS Institute Cary, NC, USA). Data were analysed as two non-contemporary 6 × 6 Latin squares design with six experimental periods and six treatments. Each steer within an experimental period was considered an experimental unit. The model included the fixed effects of CONC, NFC, CONC × NFC, as well as the random effect of square, steer within square and periods. For rumen pH, SCFA and ammonia, data were analysed using repeated measures and time × treatments interactions were included as fixed effect in the model.

Denominator degrees of freedom were calculated using the Kenward–Roger approximation. Different error covariance structures were investigated and the one that best fit, according to the Bayesian information criterion, was selected. When there was a significant interaction, the effects of treatments were compared using the SLICE option of the MIXED procedure. Treatment effects were considered significant at $P \leqslant 0.05$.

Results

Feed intake, rumen mass and fibre kinetics

Intake was reduced ($P = 0.038$) by 7 and 11% when GM was replaced with SRM and PCP, respectively (Table 2). There was an NFC × CONC interaction ($P = 0.036$) on crude protein intake (CPI) and rumen mass of organic matter (OM) and NDF (Table 2). The SRM and PCP decreased CPI when animals were fed 800 g concentrate/kg, and PCP decreased the rumen mass of OM and NDF for animals fed 600 g concentrate/kg (Table 2). In addition, animals fed 800 g concentrate/kg had a decreased NDF intake (by 36%, $P < 0.001$), NDF disappearance rate (by 37%, $P < 0.01$), digestible NDF (dNDF) disappearance rate (by 38.5%, $P < 0.01$) and *kp* of NDF (by 40%, $P < 0.001$), compared with that for animals fed 600 g concentrate/kg. Also, the dNDF disappearance rate was affected by the source of NFC, where animals fed SRM decreased by 12%, and PCP increased dNDF disappearance rate by 36% compared with that for the GM (Table 2).

Rumen fermentation and total tract digestibility

Feeding PCP increased rumen pH (6.68 *v.* 6.34, $P < 0.001$) and the proportion of acetic acid (65.6 *v.* 61.0 mM/100 mM of total SCFA, $P < 0.001$) but reduced the proportion of propionic acid (18.43 *v.* 22.15 mM/100 mM of total SCFA, $P < 0.001$) in the rumen (Table 3). Consequently, PCP increased acetate to propionate (A : P) ratio (3.65 *v.* 2.80, $P < 0.001$) compared with that for

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Table 2. Intake, pool size, nutrient content and rumen kinetics of Nellore steers fed diets containing three sources of non-fibrous carbohydrate and two levels of concentrate

	600 g CON/kg			800 g CON/kg				*P*-value		
Item	GM	SRM	PCP	GM	SRM	PCP	S.E.M.	CON	NFC	*C* × *N*
	Intake (kg/day)									
DMI	10.7	10.2	9.7	10.4	9.4	9.0	0.87	0.093	0.038	0.843
CPI	1.1	1.2	1.2	1.3	1.1	1.1	0.10	0.805	0.135	0.036
NDFI	3.5	3.3	3.5	2.2	2.0	2.3	0.24	<0.001	0.213	0.845
	Rumen mass (kg)									
OM	43.0	42.8	39.9	41.8	43.7	44.4	2.12	0.683	0.487	0.029
DM	5.0	4.9	4.6	4.8	5.1	5.1	0.31	0.731	0.500	0.098
NDF	3.5	3.4	3.0	3.1	3.5	3.3	0.21	0.966	0.194	0.046
iNDF	2.0	2.1	2.0	2.0	2.1	2.1	0.14	0.926	0.923	0.618
	Fibre kinetics (/h)									
Disapp NDF	0.043	0.041	0.049	0.030	0.025	0.029	0.0031	<0.001	0.067	0.228
Disapp dNDF	0.047	0.047	0.070	0.034	0.025	0.041	0.0042	<0.001	<0.001	0.101
kp of NDF	0.043	0.040	0.040	0.027	0.024	0.023	0.0040	0.002	0.298	0.741

DMI, dry matter intake; CPI, crude protein intake; NDFI, neutral detergent fibre intake; OM, organic matter; DM, dry matter; NDF, neutral detergent fibre; iNDF, indigestible neutral detergent fibre; Disapp NDF, disappearance of neutral detergent fibre; Disapp dNDF, disappearance rate of digestible neutral detergent fibre; *kp*, calculated fractional passage rate; GM, ground maize; SRM, steam-rolled maize; PCP, pelleted citrus pulp; CON, concentrate inclusion effect; NFC, non-fibrous carbohydrate effect; *C* × *N*, CON × NFC interaction effect.

Table 3. Rumen parameters and total tract digestibility of Nellore steers fed diets containing three sources of non-fibrous carbohydrate and two levels of concentrate

	600 g CON/kg			800 g CON/kg				*P*-value		
Item	GM	SRM	PCP	GM	SRM	PCP	S.E.M.	CON	NFC	CON × NFC
Ruminal pH	6.4	6.3	6.7	6.2	6.4	6.6	0.08	0.453	<0.001	0.146
Ammonia, mg/dl	13.4	12.0	13.0	17.4	10.1	15.9	2.04	0.354	0.030	0.154
Total SCFA, mM	92	99	97	101	99	103	6.6	0.426	0.840	0.728
	mM/100 mM of total SCFA									
Acetic acid	60.9	59.1	64.9	61.1	58.2	66.3	1.94	0.822	<0.001	0.536
Propionic acid	22.4	23.3	18.8	21.9	25.1	18.1	1.10	0.803	<0.001	0.369
Butyric acid	12.4	12.2	12.8	11.5	11.0	12.6	1.15	0.188	0.257	0.762
A : P ratio	2.8	2.6	3.5	2.8	2.3	3.8	0.21	0.692	<0.001	0.307
	Total tract digestibility coefficient									
OM	0.60	0.63	0.68	0.72	0.63	0.76	0.040	0.009	0.007	0.131
DM	0.58	0.60	0.67	0.70	0.60	0.75	0.041	0.008	0.004	0.134
NDF	0.32	0.32	0.46	0.39	0.17	0.48	0.063	0.666	0.002	0.139
Starch	0.86	0.95	0.91	0.94	0.96	0.94	0.015	<0.001	<0.001	<0.001

SCFA, short chain fatty acid; A : P ratio, acetate : propionate ratio; OM, organic matter; DM, dry matter; NDF, neutral detergent fibre; GM, ground maize; SRM, steam-rolled maize; PCP, pelleted *citrus* pulp; CON, concentrate inclusion effect; NFC, non-fibrous carbohydrate effect; CON × NFC, CON and NFC interaction effect.

GM (Table 3). In contrast, partial replacement of GM by SRM increased propionic acid (24.2 *v.* 22.15 mM/100 mM of Total SCFA, $P < 0.001$), decreased acetic acid (58.65 *v.* 60.0 mM/100 mM of total SCFA, $P < 0.001$) and, consequently, decreased A : P ratio (2.51 *v.* 2.80, $P < 0.001$) compared with that for the GM diets (Table 3). The replacement of GM with SRM also decreased rumen ammonia concentration (11.08 *v.*15.37 mg/dl, $P = 0.030$; Table 3). Total SCFA concentration and indigestible NDF mass of rumen were not influenced by any treatment ($P > 0.05$).

For the measures taken over time, there was no interaction of Time × NFC ($P = 0.961$), Time × CON ($P = 0.269$), nor Time × NFC × CON ($P = 0.924$) on total SCFA. For acetic acid, there was a Time × NFC interaction ($P < 0.001$, Fig. 1(a)), in which animals fed PCP showed an increase in this acid at 0, 1, 6, 9 and 12 h

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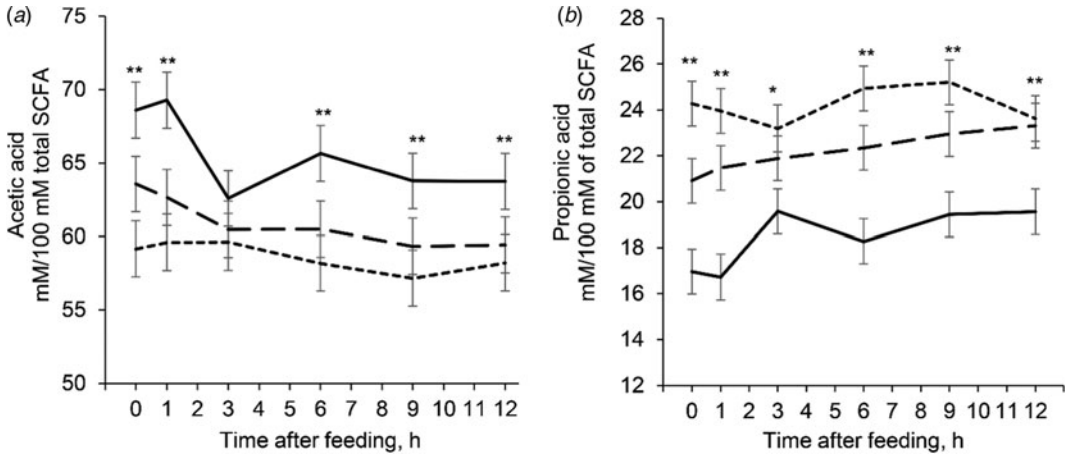

Fig. 1. Content (g/100 g of total short chain fatty acids (SCFA)) of acetic acid (a) and propionic acid (b) relative to time in Nellore steers fed diets containing three sources of non-fibrous carbohydrate (NFC). Sources of NFC were steam-flaked maize (– – – – SFM), ground maize (— — GM) and pelleted citrus pulp (——— PCP). $^{**}P < 0.001$ for NFC effect on a time point. $^{*}P < 0.05$ for NFC effect on a time point.

after feeding, with no effect at 3 h after feeding. There was a significant Time × NFC interaction ($P = 0.021$, Fig. 1(b)) on propionic acid in the rumen, in which animals fed PCP presented a lower propionic compared to GM and SRM at all times measured. There was also a Time × NFC interaction ($P < 0.001$) on A : P ratio, in which inclusion of PCP increased A : P ratio at all times measured compared to GM and SRM.

For butyric acid, there was a Time × CON interaction ($P = 0.012$, Fig. 2). In addition, there was a Time × CON interaction ($P = 0.010$, Fig. 3) on rumen pH, in which diets with 600 g concentrate/kg increased rumen pH 3 h post-feeding. There was no interaction of Time × NFC ($P = 0.189$), Time × CON ($P = 0.244$), or Time × NFC × CON ($P = 0.942$) on ammonia concentration in the rumen.

Replacing GM with PCP increased total tract digestibility of OM (0.720 *v.* 0.662, $P = 0.007$) and of DM (0.708 *v.* 0.639, $P = 0.004$), compared with that for the GM diets, and increased total tract digestibility of NDF (0.470 *v.* 0.242, $P = 0.002$; Table 3) compared for the SRM. There was an interaction of NFC × CONC on total tract apparent digestibility of starch ($P < 0.001$, Table 3), in which replacing GM with either PCP or with SRM increased starch digestibility in diets with 600 g concentrate/kg but not on diets with 800 g concentrate/kg. Feeding 800 g concentrate/kg increased total tract digestibility of OM (0.701 *v.* 0.639, $P = 0.009$) and DM (0.684 *v.* 0.616, $P = 0.008$), compared with that for the 600 g concentrate/kg (Table 3).

Rumen microbial populations

The increase of concentrate level in the diet resulted in decreases of 81 and 62.5% ($P < 0.001$) in *Fibrobacter succinogenes* and *Streptoccocus bovis* populations, respectively (Table 4). In addition, greater concentrate diets increased ($P = 0.048$) *Ruminoccocus flavefaciens* relative population, with no change on *Ruminoccocus albus* ($P = 0.629$) or *R. amylophilus* populations ($P = 0.321$; Table 4). The partial replacement of GM with PCP resulted in increased relative population of *S. bovis* ($P = 0.008$) but reduced *R. flavefaciens* ($P = 0.009$) and *R. amylophilus* ($P < 0.001$) populations, without changing *F. succinogenes* ($P = 0.872$). Moreover, the replacement of GM with SRM reduced *R. albus* ($P = 0.012$) relative population. There was a significant

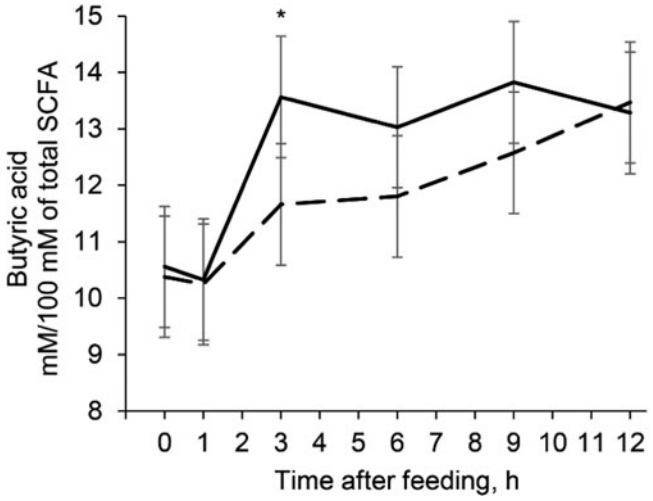

Fig. 2. Content (g/100 g of total short chain fatty acids (SCFA)) of butyric acid relative to time in Nellore steers fed diets containing either 600 (———) or 800 (— —) g concentrate per kg of dry matter. $^{*}P < 0.05$ for concentrate effect on a time point.

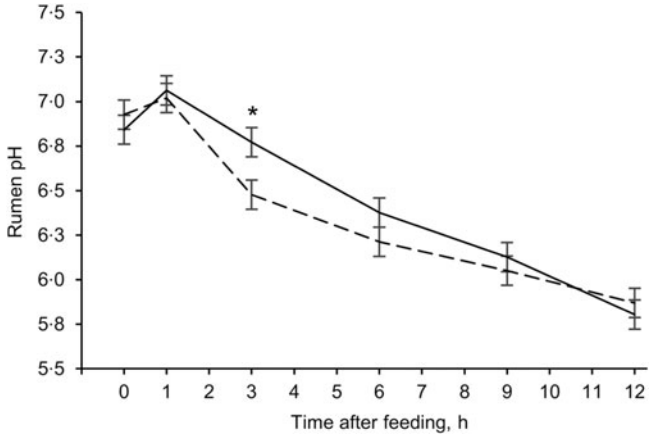

Fig. 3. Rumen pH relative to time in Nellore steers fed diets containing either 600 (———) or 800 (— —) g of concentrate per kg of dry matter. $^{*}P < 0.05$ for concentrate effect on a time point.

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Table 4. Relative population of rumen bacteria of Nellore steers fed diets containing three sources of non-fibrous carbohydrate and two levels of concentrate

	600 g CON/kg			800 g CON/kg				*P*-value		
Item[a]	GM	SRM	PCP	GM	SRM	PCP	S.E.M.	CON	NFC	CON × NFC
F. succinogenes	1.0	0.9	1.3	0.2	0.2	0.2	0.99	<0.001	0.872	0.839
R. albus	1.0	0.1	1.0	0.9	0.2	0.3	0.72	0.629	0.012	0.220
R. flavefaciens	1.0	0.7	0.4	2.4	1.0	0.6	0.58	0.048	0.009	0.715
S. bovis	1.0	0.6	1.6	0.4	0.3	0.5	0.43	<0.001	0.008	0.204
R. amylophilus	1.0	0.8	0.1	4.9	0.5	0.2	0.92	0.321	<0.001	0.373
M. elsdenii	1.0	1.0	0.8	1.5	14.7	0.6	1.01	0.037	0.014	0.019

GM, ground maize; SRM, steam-rolled maize; PCP, pelleted citrus pulp; CON, concentrate inclusion effect; NFC, non-fibrous carbohydrate effect; CON × NFC, CON and NFC interaction effect.
[a]Treatment 600 g concentrate/kg dry matter with GM was considered as the reference. All other means are expressed as fold-change in relation to the reference treatment.

NFC × CON interaction only for *M. elsdenii* ($P = 0.019$), where SRM increased *M. elsdenii* population only at the 800 g concentrate/kg diet (Table 4).

Discussion

Feed intake and rumen metabolism can change dramatically in response to changes in diet composition or metabolic state (Allen *et al.*, 2009). The partial replacement of GM with SRM decreased DMI by 7%. Because extensive grain processing exposes the starch matrix to rumen degradation, animals fed SRM had a greater component digestibility, including starch, increasing production of propionic acid and, probably, its flux to the liver. Propionate is probably a primary satiety signal because its flux to the liver increases gluconeogenesis and generation of ATP by hepatic oxidation of fuels (Allen *et al.*, 2009). Similarly, Oba and Allen (2003) also demonstrated that a more rapidly fermented starch source reduced cow meal size by 17%, causing an 8% reduction in feed intake.

The 11% reduction in feed intake observed when GM was replaced with PCP represents a distinct mechanism. Physical aspects, such as PCP density, texture, size of the pellet (approximately 1.8 cm length in this study) or taste differences among citrus sources (Cribbs *et al.*, 2015) are probably related to the reduced intake. The effect of PCP diet on the decreased OM and NDF rumen mass with 600 g concentrate/kg in diet can be explained by the lower intake and rapid degradation rate of citrus pulp, which appears to be associated with its large content of sugars and pectin (Bampidis and Robinson, 2006), which are quickly and extensively degraded in the rumen (Van Soest, 1994). The different rumen kinetics of citrus pulp was also expressed in the 45% faster dNDF disappearance rate and in the 57% greater NDF total tract digestibility, compared with that of other NFC sources. The faster NDF digestibility of citrus pulp is explained by the different profile of pectin fermentation, composed of galacturonic acid. This component is highly available in the rumen ecosystem and with different physicochemical parameters, such as high cation exchange capacity and high water retention (Van Soest, 1994).

Citrus pulp fermentation yields little lactate and more acetic acid than starch fermentation, causing less of a decline in rumen pH (Strobel and Russell, 1986) and better conditions for fibre fermentation (Bueno *et al.*, 2002). Also, PCP contains a readily digestible NDF fraction, contrary to the slowly degrading sugarcane NDF (Miron *et al.*, 2001). Additionally, the lower intake when steers were fed PCP may have contributed to a higher NDF total tract digestibility. These results are supported by previous research (Bampidis and Robinson, 2006) that showed a linear increase in apparent digestibility of NDF as levels of PCP increased in the diet. Similarly, Gouvêa *et al.* (2016) evaluated the replacement of 0, 250, 500, 750 and 1000 g flaked maize/kg DM with PCP and reported a linear increase of rumen acetate and A : P ratio, and decreased propionate. Similar changes in the SCFA profile have been reported by others (Van Soest, 1994; Broderick *et al.*, 2002; Gouvêa *et al.*, 2016) when pectin replaced starch in cattle diets.

Substituting SRM for GM increased total tract digestibility of starch in the present study. Starch granules are encapsulated in a protein matrix that acts as a primary barrier to starch digestibility. The steam-rolling of maize promotes the gelatinization of starch, which in the presence of water and heat, causes an irreversible disruption of the crystalline structure of the granule that increases the solubility of starch and improves its susceptibility to enzyme attack (Julliand *et al.*, 2006).

The greater rumen starch digestibility of SRM not only increases propionic acid production but can influence protein metabolism, as exemplified by the reduced rumen ammonia concentration in the present study. Ruminal microorganisms can use ammonia and when the utilization rate exceeds the production rate, ammonia concentration in rumen decreases (Russell *et al.*, 1992). Crocker *et al.* (1998) evaluated levels of replacement of dry-rolled maize with steam-flaked maize and reported decreased concentrations of ruminal ammonia N and a linear increase in total tract digestibility of starch as inclusion of steam-flaked maize increased. Therefore, the rate of starch digestion may influence the ammonia utilization by the microbes, which probably explains the lower ammonia concentration in the rumen when SFC replaced GM in the present study. This suggests that there was probably greater microbial protein synthesis when GM was replaced with SFC, as previously reported by Theurer *et al.* (1999).

As seen in the present study, increasing the rumen digestibility of starch increases propionate production and decreases acetate production. The same result was obtained by Simas *et al.* (2008) in a dairy cattle study comparing flaked maize with finely or coarsely ground maize. The increase in propionate production has been associated with lower methane energy loss (Beauchemin *et al.*, 2008) and greater NE value of maize (Zinn *et al.*, 2002). However, greater rumen starch digestibility can impact fibre digestion negatively. Beauchemin *et al.* (2001) evaluated four processing levels of barley and found no negative impact on ruminal,

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post-ruminal and total tract digestibility of NDF, or on the efficiency of microbial protein synthesis. Similarly, Santos *et al.* (2001) fed lactating cows with either coarsely ground maize, steam-flaked maize, or PCP and reported no effect by method of maize processing on total tract digestibility of fibre, although NDF digestibility was improved when maize was replaced with PCP.

The increased concentrate level in the diet affected all fibre kinetics parameters, decreasing the disappearance rate and *kp* of NDF, which is inversely proportional to rumen retention time. The lower disappearance rate of NDF in high-concentrate diets probably reflects changes in the rumen to a less favourable environment for fibrolytic bacteria. Changes in the diet, the level of concentrate and the type of NFC affect the pattern of rumen fermentation, and consequently the composition of rumen microbiota (Koike *et al.*, 2003; Mosoni *et al.*, 2007; Khafipour *et al.*, 2009). The fibre-digesting bacteria were represented in the current study by the species *F. succinogenes*, *R. albus* and *R. flavefaciens*. These fibrolytic bacteria are sensitive to low pH, especially when rumen pH is below 6.2 (Russell and Dombrowski, 1980; Weimer, 1993).

In the present study, the increase of concentrate in the diet reduced the population of the fibrolytic bacteria *F. succinogenes*, consistent with other studies (Tajima *et al.*, 2001; Wanapat and Cherdthong, 2009; Fernando *et al.*, 2010; Petri *et al.*, 2012). However, the population of *R. flavefaciens* was increased by greater concentrate inclusion and decreased by PCP in the present study. Singh *et al.* (2014) also reported greater population of *R. flavefaciens* in the rumen when concentrate was included in the diet.

Liu *et al.* (2014), evaluating different inclusion levels of pectin and starch in the diet of sheep, found that the abundance of typically amylolytic bacteria, such as *Succinovibrio dextrinosolvens*, *R. amylophilus* and *Succinomonas amylolytica*, was higher when starch was supplemented. This may explain the observation that partial replacement of GM by PCP reduced the population of *R. amylophilus* in the present study, because of reduced availability of starch in the rumen.

From the three fibrolytic species analysed, partial replacement of the GM by SRM resulted in reduction of *R. albus* population, despite no measurable changes in rumen pH. There was no effect on the other two species of fibrolytic bacteria. The maize flocculation results in the starch gelatinization, thereby increasing grain surface susceptible to the action of rumen microorganisms. Adhesion of bacteria to the fibre particle is essential for degradation (Koike and Kobayashi, 2009), and low pH and greater soluble starch may impair the attachment of microbes to cellulose (Owens and Goetsch, 1993).

Partial replacement of GM by SRM in the diet with 800 g concentrate/kg increased *M. elsdenii* population. According to Khafipour *et al.* (2009), in trials conducted to evaluate changes in the ruminal ecosystem during phases of acidosis, the *S. bovis* species predominated during severe acidosis and *M. elsdenii* was the dominant species in mild acidosis caused by grain intake. Long *et al.* (2014) reported that *M. elsdenii* (H6F32 strain) reduced the accumulation of lactate and increased pH *in vitro*, indicating that this microorganism has the potential to prevent ruminal acidosis. Therefore, the observed increase in *M. elsdenni* population could help explain the maintenance of rumen pH, even when the concentrate was increased to 800 g concentrate/kg and GM was replaced with SRM.

Conclusion

The partial replacement of GM with PCP in finishing cattle diets decreases feed intake while increasing total tract NDF digestibility. The use of SRM increases total tract digestibility of starch. Therefore, partial replacement of GM with SRM could be beneficial for sugarcane silage-based diets.

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Conflict of interest. None

Ethical standards. All experimental animal procedures were approved by the Animal Bioethics Committee from the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo.

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