

Effect of different sources of non-fiber carbohydrate on ruminal pH and *in vitro* digestibility of tropical forage

Efeito de diferentes fontes de carboidratos não-fibrosos sobre o pH ruminal e digestibilidade in vitro de forragens tropicais

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Abstract

The present study aimed to evaluate non-fiber carbohydrates (NFC) in sugarcane-based diets on rumen pH, and forage digestibility, and to describe NFC degradation curves. The study consisted of two trials. For the first trial, three rumen cannulated steers, BW of 350 ± 15 kg (mean \pm SE), were assigned in a 3 \times 3 Latin Square (LS) design. They were fed diets containing finely-ground (0.9 mm average particle size) corn (GC), steam-rolled corn (SRC), or pelleted citrus pulp (PCP). Each period had 14 d, with the first 12 for adaptation. The 13th d was for serial measurement of rumen pH, and the 14th for rumen fluid collection and *in vitro* incubation for DM and NDF digestibility (IVDMD and IVNDFD) of *bermudagrass* hay (Hay), corn (CS), and sugarcane (SS) silages. In the second trial, rumen fluid of a cannulated bull, fed corn silage and a regular concentrate, was collected for *in vitro* digestion of NFC for multiple time points. The incubation results were used to adjust the NFC degradation curves, and calculate lag-time, feed fractions, and degradation rate. Data from first trial was analyzed in a 3 \times 3 LS. The model for the digestibility parameters included fixed effects of forage (Feed), diets with NFC (Diet), and their interaction (Feed \times Diet), and random effect of animal and period. The model for rumen pH included fixed effect of diet, time as repeated measures, animal and period as random effects. The significance was considered at probability $\leq 5\%$ ($\alpha = 0.05$). The NFC degradation curves were adjusted using the PROC NLIN procedure from SAS, and equation parameters compared using confidence intervals. There was a Diet \times Time interaction on rumen pH ($P = 0.04$), where SRC decreased pH compared to PCP and GC diets at the time 6 h, only. There was no Feed \times Diet interaction effect ($P > 0.05$) for any digestibility parameter. There was a Feed effect on both IVDMD and IVNDFD, either after 30 or 48 h incubation ($P < 0.01$). The CS had the greatest IVDMD, followed by SS and Hay, after 30 and 48 h of incubation. The CS had the greatest IVNDFD after 30 h, compared to SS and Hay. However, for IVNDFD after 48 h, CS presented the greatest mean, followed by SS and Hay. The rumen fluid from animals fed SRC decreased both IVDMD and IVNDFD ($P < 0.05$) of all roughages after 48 h. Results from the second trial showed that the PCP had lower Lag Time, B fraction and greater k_d compared to both corn sources, and SRC had greater k_d than GC. In conclusion, the SRC diet decreased rumen pH 6 h after feeding and, consequently, decreased fiber digestibility of the tropical forage sources evaluated. Although the PCP had lower lag time, and faster rate of degradation of B fraction, it did not negatively affect rumen pH or fiber digestibility of forage.

Keywords: Corn processing. Citrus Pulp. Fiber digestibility. Rumen pH. Forage sources.

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COBE

O presente estudo teve como objetivo avaliar os carboidratos não-fibrosos (CNF) em dietas à base de cana-de-açúcar sobre o pH ruminal e digestibilidade da forragem, e descrever as curvas de degradação dos CNF. O estudo foi composto de dois ensaios. No primeiro, três novilhos canulados no rúmen, com peso vivo de 350 ± 15 kg (Média \pm DP), foram alocados em um quadrado latino (QL) 3 \times 3, e alimentados com dietas contendo: milho moído (MM, tamanho de partículas 0,9 mm), laminado a vapor (MLV) ou polpa cítrica peletizada (PCP). Cada período tinha 14 d, sendo os primeiros 12 para adaptação e o 13^o para a medição seriada do pH e o 14^o para a coleta de líquido ruminal e incubação *in vitro* para digestibilidade da MS e FDN (DIVMS e DIVFDN) de feno de *bermudagrass* (Feno) e silagens de milho (SM) e cana (SC). No segundo ensaio, coletou-se fluido ruminal de um touro canulado, alimentado com silagem de milho e concentrado padrão, para digestão *in vitro* dos CNF em vários tempos. Esses resultados foram utilizados para ajustar as curvas de degradação dos CNF e calcular o tempo de colonização, frações alimentares e taxa de degradação. Os resultados do primeiro ensaio foram analisados em um QL 3 \times 3. O modelo dos parâmetros de digestibilidade incluiu efeito fixo de forragem (Alimento), dieta com CNF (Dieta) e interação (Alimento \times Dieta),

e efeito aleatório de animal e período. O modelo para pH incluiu efeito fixo de Dieta, Tempo como medida repetida, animal e período como aleatórios. Foi considerada a probabilidade significativa de $\leq 5\%$ ($\alpha = 0,05$). As curvas de degradação dos CNF foram ajustadas pelo PROC NLIN do SAS, e parâmetros de equação comparados por intervalo de confiança. Houve interação Dieta \times Tempo no pH ruminal ($P = 0,04$), onde o MLV diminuiu o pH comparado com PCP e MM apenas no tempo 6 h. Não houve interação Alimento \times Dieta ($P > 0,05$) para nenhum parâmetro de digestibilidade. Houve efeito de Alimento sobre a DIVMS e DIVFDN, após 30 e 48 h de incubação ($P < 0,01$). A SM teve a maior DIVMS, seguido por SC e Feno, após 30 e 48 h de incubação. A SM teve a maior DIVFDN após 30 h, comparado com SC e Feno. No entanto, para DIVFDN após 48 h, a SM teve maior média, seguida da SC e Feno. O fluido ruminal de animais alimentados com MLV diminuiu a DIVMS e DIVFDN ($P < 0,05$) de todas as forragens, após 48 h. Resultados do segundo ensaio mostram que PCP diminuiu o tempo de colonização, fração B e aumentou a k_d comparado com os dois milhos, e MLV apresentou maior k_d que o MM. Em conclusão, a dieta com MLV diminuiu o pH ruminal no tempo 6 h e, conseqüentemente, diminuiu a DIVFDN das forragens avaliadas. Embora PCP tenha apresentado menor tempo de colonização e maior taxa de degradação da fração B, não afetou negativamente o pH do rúmen nem a digestibilidade da fibra das forragens.

Palavras-chave: Processamento do milho. Polpa cítrica. Digestibilidade da fibra. PH de rúmen. Fontes de forragem.

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Introduction

Animal performance is directly related to nutrient intake, which, in turn, depends on the physical and chemical nature of the feed and diet digestibility. Highly digestible carbohydrates are widely used to achieve greater digestibility and performance. Dry milling processing of cereal grains provides greater surface area for microbial attachment and partial disruption of the protein matrix surrounding starch (OWENS; BASALAN, 2016), and wet and heat processing can also gelatinize the starch, breaking the intermolecular hydrogen bonds within the starch granule (NOCEK; TAMMINGA, 1991). With greater digestibility, the ruminal fermentation capacity is maximized, resulting in increased microbial protein synthesis and short-chain fatty acids (SCFA), particularly propionic acid, resulting in greater energy flow to the portal vein (THEURER et al., 1999; HUNTINGTON et al., 2006). However, the increase of rumen starch digestibility can cause a sudden drop in rumen pH, with possible reduction in fiber digestibility

(VAN SOEST, 1994) and in dry matter intake (BENGOCHEA et al., 2005).

Pelleted citrus pulp (PCP), similar to corn, is a non-fiber carbohydrate (NFC) feedstuff used as prompt energy for ruminant fermentation, with a fast and extensive rumen degradability (GOUVÊA et al., 2016; KIM et al., 2007), but with high pectin concentration (22-40% DM; ARTHINGTON et al., 2002; BAMPIDIS; ROBINSON, 2006). However, unlike cereal grains, it does not contain significant amounts of starch, thus preventing rumen acidosis because it tends to yield more acetate and little lactate. Therefore, PCP is usually included in replacement of rapidly fermentable starchy feedstuffs (HALL; EASTRIDGE, 2014).

Regarding forage source for this experiment, corn silage is reportedly one of the most common roughage sources used at cattle feedlots in Brazil, followed by grass silage, and sugarcane, provided either fresh as silage or as bagasse (MILLEN et al., 2009; OLIVEIRA; MILLEN, 2014). However, there is little information about the effect of different NFC sources in sugarcane silage diets and their effect on digestibility rates, although it is a very common crop grown in Southeastern Brazil. In this sense, we hypothesized that different NFC sources could alter rumen pH, affecting digestibility of tropical forages, due to changes in degradation rate and lag time, compared to the traditional ground corn and citrus pulp, rich in pectin. In this context, the present study aimed to evaluate the

effect of diets containing three NFC sources (ground, or steam-rolled corn, and citrus pulp) in sugarcane silage-based diets on rumen pH and *in vitro* DM and NDF digestibility of different forage sources.

Materials and Methods

The study consisted of two complementary trials, both conducted at the Beef Cattle Research Laboratory, University of São Paulo, located in the city of Pirassununga, state of São Paulo, in southeast Brazil. All experimental procedures were in agreement with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999), with all animal procedures approved by the University of São Paulo Animal Bioethics Committee (CEUA n° 2784310715).

Ruminal pH and digestibility of forage

For the first trial, three rumen cannulated Nellore steers with average BW of 350 ± 15 kg (mean \pm SE)

and 18-mo were housed in individual concrete pens (3.0 m wide \times 9.0 m deep; 3.0 m of linear bunk space) with *ad libitum* access to feed and water. The steers were fed twice daily, at 0800 h and 1400 h, three sugarcane silage-based diets, with a 40:60 forage:concentrate ratio (DM basis; Table 1). The diets were formulated to be iso-nitrogenous, to meet animal requirements and allowing for an ADG of $1.4 \text{ kg}\cdot\text{day}^{-1}$ (NRC, 2000). The amount of feed offered was adjusted daily allowing for 5% of orts during the experiment. The animals were assigned to treatments in a non-replicated, 3 \times 3 Latin Square design. The tested diets were: diet composed of finely-ground (0.9 mm particle size) corn (GC), and 70% of the ground corn replaced by either steam-rolled corn (SRC - density of $415 \text{ kg}\cdot\text{m}^{-3}$), or pelleted citrus pulp (PCP). Each period had 14 d, with the first 12 d for diet adaptation and the last two days for serial measurement of pH and collection of rumen fluid for *in vitro* digestibility of tropical forage.

Table 1 – Composition of the experimental diets with three sources of non-fiber carbohydrate on a DM basis fed to steers for the tropical forage digestibility trial – Pirassununga, SP, Brazil – Feb. 2018

Item ¹	GC	SRC	PCP
Sugarcane silage	40.0	40.0	39.9
Ground corn	51.8	15.6	15.5
Steam-rolled corn	-	36.3	-
Pelleted citrus pulp	-	-	36.3
Soybean meal	5.0	5.0	5.0
Urea	1.1	1.1	1.6
Limestone	0.8	0.8	-
Mineral mixture ²	1.1	1.1	1.1
Salt	0.2	0.2	0.2
Dicalcium phosphate	-	-	0.4
<i>Analyzed composition,</i>			
DM	64.5	64.9	64.8
CP	12.2	11.8	11.9
NDF	31.6	32.0	33.5
Forage NDF	25.7	25.7	25.6
ADF	23.0	22.6	27.8
ADL	3.8	4.1	3.8
Starch	35.4	35.73	12.22
NE _m ³	1.63	1.69	1.56
NE _g ³	1.03	1.08	0.96

¹Diets containing: GC: ground corn, SRC: steam-rolled corn, and PCP: pelleted citrus pulp. ²Guaranty levels per kg: Calcium 210 g; Cobalt 24 mg; Copper

720 mg; Sulfur 74 g; Fluorine 240 mg; Phosphorus 24 g; Iodine 40 mg; Magnesium 30 g; Manganese 1500 mg; Selenium 8 mg; Sodium 60 g; Zinc 2080 mg; Monensin 1830 mg. ³ Calculated based on the net energy (NE) of maintenance and gain equations of NRC (2000)

On the 13th d of each experimental period, samples from the rumen of each animal were taken from the cranial, ventral and caudal areas via cannula, mixed and filtered through four layers of a 1-mm nylon mesh (Albercan Group, Itajubá, Brazil). Then, the pH of rumen liquid was measured with portable pH-meter (Tec-3MP, Tecnal, Brazil) immediately before the first meal, considered time 0, and after 1, 3, 6, 9, and 12 h post feeding. First meal was between 07:00 and 08:00.

Approximately 0.5 g of Bermuda-grass (*Cynodon dactylon* cv. Coast-cross) hay (Hay), corn silage (CS), and sugarcane silage (SS) samples were weighed inside F-57 bags in triplicate for both dry matter and neutral detergent fiber digestibility (IVDMD and IVNDFD), and for 30 and 48 h incubation. On the 14th day of each experimental period, approximately 4 kg of rumen content of each animal were collected from caudal, ventral, and cranial areas via cannula, mixed and filtered using a 1-mm nylon mesh (Albercan Group, Itajubá, Brazil). Approximately 1.5 L of the collected liquid was transported in insulated bottles preheated with water at 37°C for *in vitro* analysis. Rumen fluid from each animal was added to one fermenter of the Daisy II incubator (ANKOM Technology Corp., Fairport, NY, USA). The anaerobiosis was maintained by gassing with CO₂, to displace dissolved O₂, and measured by infusing resazurin (RNO; 7-hydroxy-3Hphenoxazin-3-one-10-oxide) as an indicator of anaerobiosis. The *in vitro* digestibility of roughage was performed following the methodology proposed by Holden (1999). After 30 or 48 h of fermentation, half of the bags were analyzed for NDF and the other half dried in an oven at 105°C for 12 h, for NDF and DM *in vitro* digestibility determination, respectively.

Degradation curve of carbohydrate sources

The second trial was conducted to better describe the fermentation pattern of the three NFC sources used in the first trial by *in vitro* digestion of the feedstuffs for 0, 6, 12, 18, 24, 30, 36, 42, and 48 h of incubation as in Lei et al. (2018). The rumen fluid was collected from a rumen cannulated young bull fed a diet with 60% corn silage and 40% concentrate consisting of ground corn, soybean meal, urea and mineral mixture, and processed as previously described. Approximately 0.75 g of the GC, SRC and PCP samples was weighed in F-57 bags in triplicate per time of incubation. As the Daisy II incubator device (ANKOM Technology Corp., Fairport, NY, USA) consists of four fermenter bottles, two rounds were made. In the first round, samples were fermented during 6, 12, 18 and 24 h, while samples in the second round were fermented at 30, 36, 42 and 48 h. Thus, each fermenter was intended for each fermentation time. The IVDMD analysis was performed following the same methodology previously described. Time 0 hour of incubation was done by immersing the bags in distilled water at 37°C for 10 min. Then, after the incubation periods, the bags were dried in oven at 105°C for 12 h and weighed again. The results were used to adjust the DM degradation curve according to the model proposed by McDonald (1981), described as follows:

Deg = $a + [b \times (1 - e^{-kd \times t})]$, where:

Deg = degradation in time t (%)
 parameter equation; intersection of the exponential model when time t = 0 hour, corresponding to immediately soluble fraction if there was no lag-time
 a =
 parameter equation; would be potentially degradable fraction if there was no lag-time
 b =
 incubation time (h)
 t =

$e =$ represents the base of the natural logarithms
 $k_d =$ rate of degradation of the fraction B (%.h⁻¹)

Moreover, B fraction and Lag time were calculated as:

$B =$ potentially degradable insoluble fraction = $[(a + b) - A]$
 $A =$ soluble fraction (%)
 $Lag =$ "Lag-time" or colonization time = $[\ln (b/B)] / k_d$

Feed Samples

Samples from the tropical forage and the NFC sources were collected, dried for 72 h in a forced-ventilation oven at 55°C and ground to pass a 1-mm screen at a Willey mill (Tecnal, Piracicaba, Brazil). The ash and DM were analyzed according to AOAC (2000) methods 942.05 and 930.15, respectively. The

N was determined by combustion (Leco protein/N analyzer, model FP-528; Leco Corp., St. Joseph, MI, USA) and CP determined by multiplying N content by 6.25, and concentration of NDF determined according to Van Soest et al. (1991) using heat stable α -amylase (Sigma A3306; Sigma Chemical Co., St. Louis, MO, USA) using the ANKOM A200 Fiber Analyzer (ANKOM Technology Corp.). The ether extract (EE) content was analyzed using the traditional Soxhlet extraction, according to AOAC (2000) methods 920.39. The ADF and ADL were analyzed according to (VAN SOEST; ROBERTSON, 1985). Starch was determined colorimetrically using an ethanol extraction technique, followed by α -amylase and amyloglucosidase preparation to hydrolyze starch to glucose as described by Hendrix (1993). The non-fiber carbohydrate (NFC) content was calculated according to NRC (2016) equation. Chemical composition of the feedstuffs is presented in Table 2.

Table 2 – Chemical composition of feedstuffs – Pirassununga, SP, Brazil – Feb. 2018

Item	Chemical composition (%DM) ¹							
	DM	MM	CP	EE	NDF	ADF	ADL	NFC ²
<i>Roughage samples</i>								
Corn silage	33.0	4.1	7.2	2.23	59.0	33.5	7.5	27.5
Sugarcane silage	35.7	5.5	3.2	1.23	75.4	48.0	10.6	14.7
Coast-cross hay	91.5	4.7	6.4	1.06	83.6	39.4	7.2	4.2
<i>Concentrate samples</i>								
Ground corn	88.3	1.4	9.9	2.03	13.7	3.5	5.8	73.0
Steam-rolled corn	93.9	0.9	8.4	2.1	15.5	4.2	2.3	73.1
Pelleted citrus pulp	94.1	7.2	7.7	1.79	21.4	14.8	10.9	61.9

¹DM = dry matter, MM = mineral matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin, NFC = non-fiber carbohydrate. ² Calculated according to the NRC (2016) equation

Statistical analysis

The data from first trial was analyzed in a 3×3 Latin Square design using the MIXED procedure of the SAS version 9.2 for Windows (SAS Institute, Cary, NC, USA). The parameters of IVDMD and IVNDFD, both after 30 and 48 h of incubation, were considered dependent variables, and their model included fixed effects incubated tropical forage (Feed), NFC source in

the diet (Diet), and their interaction (Feed × Diet), and the random effects of animal and period. The random effects of animal × period × Trt and animal × period × Time × Trt were used to test the effect of Trt and Trt × Time. Degrees of freedom and tests were adjusted using the Kenward-Roger method and means were compared by the Fisher's protected t-test of LSMEANS.

The model for the rumen pH data also included fixed effect of Diet, time of sample collection (Time) was included as repeated measures with AR(1) as the covariance structure, and animal and period included as random effects. Means were compared by the Fisher's protected t-test of LSMEANS, and significance was considered at $\leq 5\%$ probability level. When the interaction between the main effects was significant, the interaction was decomposed, and treatment effects were analyzed using the SLICE option of the PROC MIXED. The degradation curve of the NFC sources was adjusted using the procedure PROC NLIN of the statistical package of SAS, using the secant method (DUD). The equation parameters were compared using confidence interval at 95% confidence level.

Results

There was a Diet \times Time interaction on rumen pH ($P = 0.04$, Figure 1) in which the diet with SRC decreased rumen pH compared to PCP and GC diets only at the time 6 h. The main effect of Diet on rumen pH was not significant ($P = 0.09$). There was no Feed \times Diet interaction effect ($P > 0.05$) for any parameter of digestibility (Table 3). There was a Feed effect on both DM and NDF digestibility either after 30 or 48 h of incubation ($P < 0.01$, Table 3). The CS had the greatest mean, followed by SS and, lastly, by Hay for IVDMD, both after 30 and 48 h of incubation. For the IVNDFD after 30 h, the CS had the greatest mean compared to SS and Hay, with no differences between them. However, when IVNDFD was measured after 48 h of incubation, CS presented the greatest mean, followed by SS and Hay (Table 3).

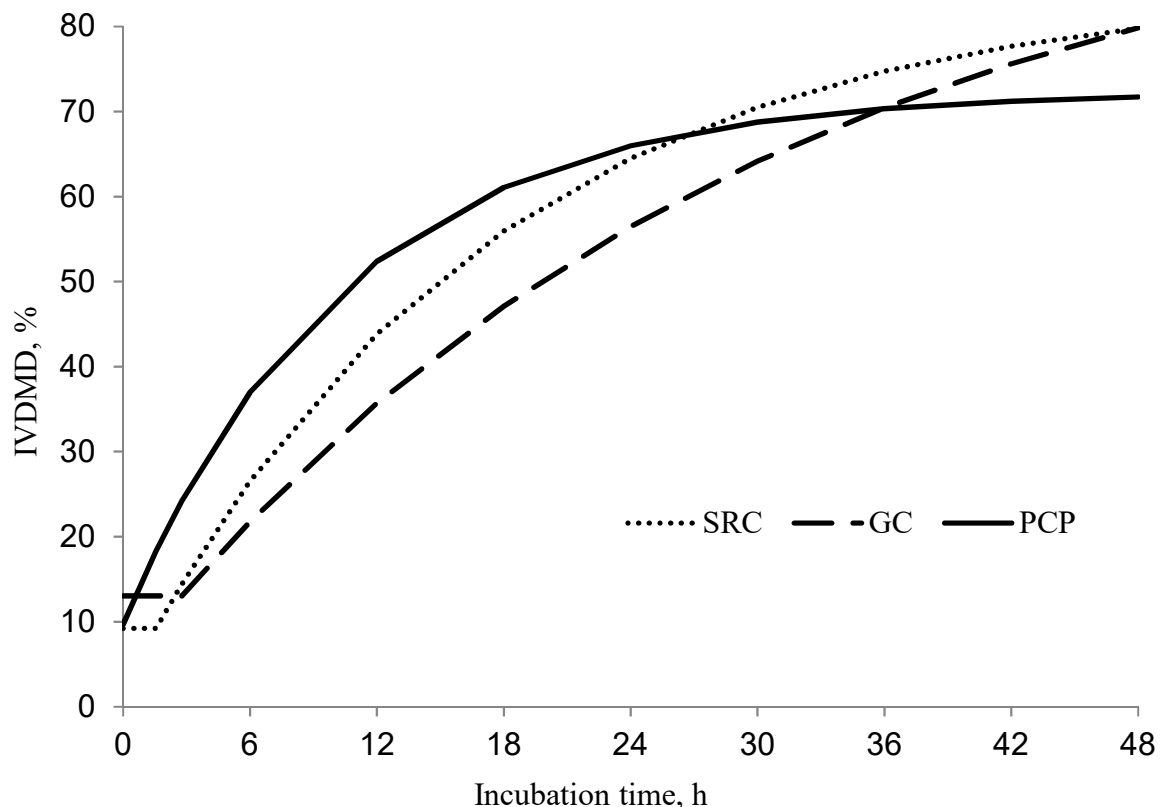


Figure 1 – Potential degradation curve of DM for steam-rolled corn (SRC), ground corn (GC) and pelleted citrus pulp (PCP) at 0, 6, 12, 18, 24, 30, 36, 42 and 48 h after feeding with the Lag time

Table 3 – Feed fractions of the concentrate feedstuff, dry matter degradation rate and Lag time, Pirassununga – SP, Brazil – Feb. 2018

Item ¹	Treatment			SEM ²
	GC	SRC	PCP	
	----- Mean (95% CI) -----			
A (%)	13 (5-21)	9 (1-17)	10 (2-18)	4.3
B (%)	86 ^a (78-93)	76 ^a (69-83)	63 ^b (55-70)	3.8
k _d (%.h ⁻¹)	3.3 ^c (1.9-4.7)	5.9 ^b (4.5-7.3)	9.5 ^a (8.1-10.9)	0.7
Lag (h)	2.77 ^a (1.34-4.20)	1.55 ^a (0.12-2.98)	0.00 ^b (-1.43-1.43)	0.728

¹ A = Soluble fraction; B = Potentially degradable insoluble fraction; k_d = rate of degradation of the B fraction; Lag = Lag-time; GC = ground corn; SRC = steam-rolled corn; PCP = pelleted citrus pulp. ² Standard error of means

The rumen fluid from animals fed with SRC decreased both DM (P = 0.03) and NDF (P = 0.02) digestibility of the roughage sources, when measured after 48 h of incubation (Table 3). There was no effect of treatments on either DM or NDF in vitro digestibility when measured after 30 h of incubation

(Table 3).

The degradation curve of the three NFC sources is presented in (Figure 2). Analyzing the curve parameters, PCP had shorter Lag Time, smaller B fraction and greater k_d compared to both corn sources (Table 4). Also, SRC had greater k_d than GC (Table 4).

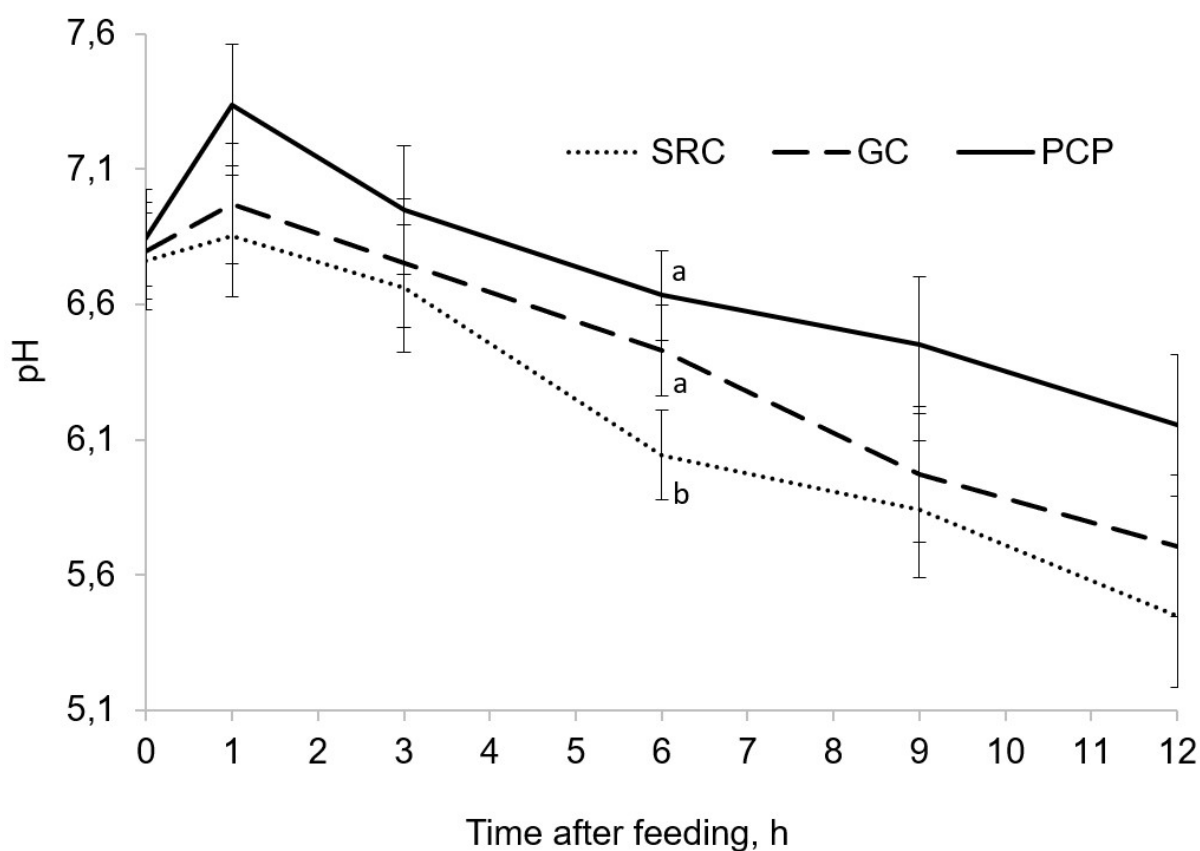


Figure 2 – Rumen pH from animals fed diets with Steam rolled corn (SRC), Ground Corn (GC), Pelleted *citrus* pulp (PCP). ^{a,b} Means within the same time period with different lowercase letters differ (P < 0.05)

Discussion

In vitro digestibility and rumen pH

Citrus pulp is composed of approximately 25% pectin, which is a carbohydrate with rapid fermentation (CAMPBELL et al., 2002) but lower potential for rumen acidification. Pectin digestion produces greater concentrations of acetic acid, a weak acid, and less lactic acid in the rumen compared to starch (STROBEL; RUSSELL, 1986). Marino et al. (2011) evaluated diets containing approximately 70% concentrate and observed that animals fed citrus pulp presented pH means above 6.0 throughout the 12 h period after feeding, similar to the present study. However, in that study, when animals were fed finely-ground corn or high-moisture corn silage, authors reported pH means around 5.6 from 2 to 6 h after feeding. In the current study, despite the ground corn being a processed starchy feedstuff, the effect on rumen pH was similar to PCP.

The only observed difference in the present study was a lower rumen pH after 6 h of feeding when GC was partially replaced with SRC. Starch digestion can be improved by processing the grain, which includes decreasing particle size by grinding or breaking the kernel, crushing using rolls, adding or not moisture or heat (OWENS; BASALAN, 2016). The steam-rolling method breaks the protein matrix associated with starch granules, causes starch gelatinization, increases the surface area of the kernel, and facilitates the digestion of starch granules by amylolytic enzymes (NOCEK; TAMMINGA, 1991). In this regard, Zinn et al. (1995) observed lower rumen pH 4 h after feeding for steam-processed corn compared to dry-rolled corn, and they associated this effect to increased SCFA concentration in the rumen. In our trial, the only difference between the NFC sources was 6 h after feeding because there was a sharper drop for rumen pH at that time point for SRC compared to the other diets.

The cellulolytic activity of the rumen fluid of animals fed with SRC might have been lower than the other two NFC sources, when measured after 48 h of incubation. When rumen pH values fall below 6.0, the growth of the cellulolytic organisms can be reduced, allowing for an increase in the amylolytic flora which

are propionate-producing microbes (JOUANY, 2006), therefore impairing fiber digestion. The results of the present study agree with those of Owens and Soderlund (2006) who found that rumen and total tract digestibility of fiber decreased 42.4 and 12.7%, respectively, when animals were fed steam-processed corn compared to animals fed dry-rolled corn. Similarly, Manríquez et al. (2016) observed a 25.1% decrease in the rumen fiber digestibility when animals were fed SRC compared to dry-rolled corn.

Comparing the three roughage sources analyzed in the current experiment, the *in vitro* DM digestibility was associated with the fiber content of the feedstuffs. Corn silage presented greater NFC content than sugarcane silage and Bermuda-grass hay, and had greater *in vitro* DM digestibility. Oliveira et al. (2011) reported DM digestibility of corn silage to range from 72.2 to 74.8% and NDF digestibility from 44.8 to 46.3% after 48 h of incubation, means slightly greater than the present study (66.4 and 43.1% for DM and NDF digestibility, respectively). Reis et al. (2003) reported DM digestibility of 43.7% for Bermuda-grass hay, and Oliveira et al. (2007) evaluated sugarcane silage and reported *in vitro* digestibility of 60.8 and 40.1% for DM and NDF, respectively. The lower fiber digestibility of sugarcane was again demonstrated in this experiment. Sugarcane had a higher proportion of lignin in the cell-wall, which likely explains the lower *in vitro* NDF digestibility (YOU et al., 2017).

Degradation curve

To better characterize the three NFC sources used in this study, the feedstuffs were incubated at several time points, and the parameters of the degradation curve were generated. Pelleted citrus pulp had lower lag-time, lower potentially degradable fraction and faster rate of degradation than the other two corn sources. The lower lag-time and faster rate of digestion of PCP is likely related to the accessibility of particles for microbe degradation. When the pulp reaches the rumen and gets some moisture, the pellet expands and facilitates the microbe access. In addition, pectin has

high cation exchange capacity that can cause the plant to attract and bind hydrogen ions (MCBURNEY et al., 1983). When these ions are bound to the feed rather than free in the liquid phase, less acid is in the rumen, and pectin also increases the rate at which the rumen microbes digest the feed (MCBURNEY et al., 1983). The pectin in PCP, although quickly and extensively degraded in the rumen, does not generate lactic acid in the rumen (KIM et al., 2007), explaining the absence of differences in rumen pH regarding citrus pulp. The total degradation of PCP was lower than corn sources, likely reflecting the greater total NDF and ADL content in the feedstuff.

According to McAllister et al. (1994), reduction of particle size increases starch digestion, due to increases in surface area for microbial attachment,

colonization and enzymatic attack. Although some starch granules remain embedded within a protein matrix even after grinding, the rate of starch digestion is still increased by reducing particle size (MCALLISTER et al., 1993). However, the steam-rolling processing probably improves starch accessibility to rumen microbes, accelerating the rate of DM degradation compared to ground corn (NOCEK; TAMMINGA, 1991). The effect of steam-rolling corn was demonstrated in the degradation curve, with faster degradation rate for SRC than GC.

Conclusion

In conclusion, the SRC diet decreased rumen pH at 6 h after feeding and decreased fiber digestibility of the tropical forage sources evaluated. Although PCP has a faster rate of degradation of the B fraction, lower lag time, and lower degradable insoluble fraction than ground corn, it did not negatively affect rumen pH or fiber digestibility of forage.

References

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTRY (AOAC). **Official methods of analysis**. 17th ed. Washington, DC: AOAC, 2000. 2200 p.

ARTHINGTON, J. D.; KUNKLE, W. E.; MARTIN, A. M. Citrus pulp for cattle. **Veterinary Clinics North America: Food Animal Practice**, v. 18, n. 2, p. 317-326, 2002. doi: 10.1016/S0749-0720(02)00023-3.

BAMPIDIS, V. A.; ROBINSON, P. H. Citrus by-products as ruminant feeds: a review. **Animal Feed Science and Technology**, v. 128, n. 3-4, p. 175-217, 2006. doi: 10.1016/j.anifeedsci.2005.12.002.

BENGOCHEA, W. L.; LARDY, G. P.; BAUER, M. L.; SOTO-NAVARRO, S. A. Effect of grain processing degree on intake, digestion, ruminal fermentation, and performance characteristics of steers fed medium-concentrate growing diets. **Journal of Animal Science**, v. 83, n. 12, p. 2815-2825, 2005. doi: 10.2527/2005.83122815x.

CAMPBELL, J. L.; WILLIAMS, C. V.; EISEMANN, J. H. Fecal inoculum can be used to determine the rate and extent

of in vitro fermentation of dietary fiber sources across three lemur species that differ in dietary profile: *Varecia variegata*, *Eulemur fulvus* and *Haplemur griseus*. **The Journal of Nutrition**, v. 132, n. 10, p. 3073-3080, 2002. doi: 10.1093/jn/131.10.3073.

FEDERATION OF ANIMAL SCIENCE SOCIETIES (FASS). **Guide for the care and use of agricultural animals in agricultural research and teaching**. Savoy: FASS, 1999. 169 p.

GOUVÊA, V. N.; BATISTEL, F.; SOUZA, J.; CHAGAS, L. J.; SITTA, C.; CAMPANILI, P. R. B.; GALVANI, D. B.; PIRES, A. V.; OWENS, F. N.; SANTOS, F. A. P. Flint corn grain processing and citrus pulp level in finishing diets for feedlot cattle. **Journal Animal Science**, v. 94, p. 665-677, 2016. doi: 10.2527/jas.2015-9459.

HALL, M. B.; EASTRIDGE, M. L. Invited Review: Carbohydrate and fat: Considerations for energy and more. **The Professional Animal Scientist**, v. 30, n. 2, p. 140-149, 2014. doi: 10.15232/S1080-7446(15)30101-7.

- HENDRIX, D. L. Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. **Crop Science**, v. 33, n. 6, p. 1306-1311, 1993. doi: 10.2135/cropsci1993.0011183X003300060037x.
- HOLDEN, L. A. Comparison of methods of in vitro dry matter digestibility for ten feeds. **Journal of Dairy Science**, v. 82, n. 8, p. 1791-1794, 1999. doi: 10.3168/jds.S0022-0302(99)75409-3.
- HUNTINGTON, G. B.; HARMON, D. L.; RICHARDS, C. J. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. **Journal of Animal Science**, v. 84, p. e14-e24, 2006. Supplement 13. doi: 10.2527/2006.8413_supplE14x.
- JOUANY, J. P. Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. **Animal Reproduction Science**, v. 96, n. 3-4, p. 250-264, 2006. doi: 10.1016/j.anireprosci.2006.08.005.
- KIM, S. C.; ADESOGAN, A. T.; ARTHINGTON, J. D. Optimizing nitrogen utilization in growing steers fed forage diets supplemented with dried citrus pulp. **Journal of Animal Science**, v. 85, n. 10, p. 2548-2555, 2007. doi: 10.2527/jas.2007-0059.
- LEI, Y. G.; LI, X. Y.; WANG, Y. Y.; LI, Z. Z.; CHEN, Y. L.; YANG, Y. X. Determination of ruminal dry matter and crude protein degradability and degradation kinetics of several concentrate feed ingredients in cashmere goat. **Journal of Applied Animal Research**, v. 46, n. 1, p. 134-140, 2018. doi: 10.1080/09712119.2016.1276916.
- MANRÍQUEZ, O. M.; MONTANO, M. F.; CALDERON, J. F.; VALDEZ, J. A.; CHIRINO, J. O.; GONZALEZ, V. M.; SALINAS-CHAVIRA, J.; MENDOZA, G. D.; SOTO, S.; ZINN, R. A. Influence of wheat straw pelletizing and inclusion rate in dry rolled or steam-flaked corn-based finishing diets on characteristics of digestion for feedlot cattle. **Asian-Australasian Journal of Animal Sciences**, v. 29, n. 6, p. 823-829, 2016. doi: 10.5713/ajas.15.0062.
- MARINO, C. T.; OTERO, W. G.; RODRIGUES, P. H. M.; DICOSTANZO, A.; MILLEN, D. D.; PACHECO, R. L. D.; DILORENZO, N.; MARTINS, C. L.; ARRIGONI, M. D. B. Effects of adding polyclonal antibody preparations on ruminal fermentation patterns and digestibility of cows fed different energy sources. **Journal of Animal Science**, v. 89, n. 10, p. 3228-3235, 2011. doi: 10.2527/jas.2010-3062.
- MCALLISTER, T. A.; PHILLIPPE, R. C.; RODE, L. M.; CHENG, K. J. Effect of the protein matrix on the digestion of cereal grains by ruminal microorganisms. **Journal of Animal Science**, v.71, n. 1, p. 205-212, 1993. doi: 10.2527/1993.711205x.
- MCALLISTER, T. A.; BAE, H. D.; JONES, G. A.; CHENG, K. J. Microbial attachment and feed digestion in the rumen. **Journal of Animal Science**. v.72, n. 11, p. 3004-3018, 1994. doi: 10.2527/1994.72113004x.
- MCBURNEY, M. I.; VAN SOEST, P. J.; CHASE, L. E. Cation exchange capacity and buffering capacity of neutral-detergent fibres. **Journal of the Science of Food and Agriculture**, v. 34, n. 9, p. 910-916, 1983. doi: 10.1002/jsfa.2740340903.
- MCDONALD, I. A revised model for the estimation of protein degradability in the rumen. **Journal of Agricultural Science**, v. 96, n. 1, p. 251-252, 1981. doi: 10.1017/S0021859600032081.
- MILLEN, D. D.; PACHECO, R. D. L.; ARRIGONI, M. D. B.; GALYEAN, M. L.; VASCONCELOS, J. T. A snapshot of management practices and nutritional recommendations used by feedlot nutritionists in Brazil. **Journal of Animal Science**, v. 87, n. 10, p. 3427-3439, 2009. doi: 10.2527/jas.2009-1880.
- NATIONAL RESEARCH COUNCIL (NRC). **Nutrient requirements of beef cattle**. 7th ed. Washington: National Academy Press, 2000. 408 p.
- NATIONAL RESEARCH COUNCIL (NRC). **Nutrient requirements of beef cattle**. 8th ed. Washington: National Academy Press, 2016. 475 p.
- NOCEK, J. E.; TAMMINGA, S. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. **Journal of Dairy Science**, v. 74, n. 10, p. 3598-3629, 1991. doi: 10.3168/jds.S0022-0302(91) 78552-4.
- OLIVEIRA, F. C. L.; JOBIM, C. C.; SILVA, M. S.; CALIXTO JUNIOR, M.; BUMBIERIS JUNIOR, V. H.; ROMAN, J. Produtividade e valor nutricional da silagem de híbridos de milho em diferentes alturas de colheita. **Revista Brasileira de Zootecnia**, v. 40, n. 4, p. 720-727, 2011. doi: 10.1590/S1516-35982011000400004.
- OLIVEIRA, M. D. S.; ANDRADE, A. T.; BARBOSA, J. C.; SILVA, T. M.; FERNANDES, A. R. M.; CALDEIRÃO, E.; CARABOLANTE, A. Digestibilidade da cana-de-açúcar hidrolisada in natura e ensilada para bovinos. **Ciência Animal Brasileira**, v. 8, n. 1, p. 41-50, 2007.
- OLIVEIRA, C. A.; MILLEN, D. D. Survey of the nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil. **Animal Feed Science**

and Technology, v. 197, p. 64-75, 2014. doi: 10.1016/j.anifeeds.2014.08.010.

OWENS, F. N.; BASALAN, M. Ruminal fermentation. In: MILLEN, D. D.; ARRIGONI M. D. B.; PACHECO R. D. L. **Rumenology**. Cham: Springer, 2016. p. 63-102.

OWENS, F. N.; SODERLUND, S. Ruminal and post ruminal starch digestion by cattle In: Cattle Grain Processing Symposium. 2006, Stillwater. **Proceedings...** Stillwater: Oklahoma State University, 2006. p. 116-128. Available from: <<https://goo.gl/bUWjgo>>. Viewed: 25 May 2017.

REIS, R. A.; BERCHIELLI, T. T.; ANDRADE, P.; MOREIRA, A. L.; SILVA, E. A. Valor nutritivo do feno de capim coast-cross (*Cynodon dactylon* L. Pers.) submetido à amonização. **ARS Veterinaria**, v. 19, n. 2, p. 143-149, 2003.

STROBEL, H. J.; RUSSELL, J. B. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. **Journal of Dairy Science**, v. 69, n. 11, p. 2941-2947, 1986. doi: 10.3168/jds.S0022-0302(86)80750-0.

THEURER, C. B.; LOZANO, O.; ALIO, A.; DELGADO-ELORDUY, A.; SADIK, M.; HUBER, J. T.; ZINN, R. A. Steam-processed corn and sorghum grain flaked at different densities alter ruminal, small intestinal, and total tract

digestibility of starch by steers. **Journal of Animal Science**, v. 77, n. 10, p. 2824-2831, 1999. doi: 10.2527/1999.77102824x.

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

VAN SOEST, P. J.; ROBERTSON, J. B.; LEWIS, B. A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v. 74, n. 10, p. 3583-3597, 1991. doi: 10.3168/jds.S0022-0302(91)78551-2.

VAN SOEST, P. J.; ROBERTSON, J. B. **Analysis of forages and fibrous foods. a laboratory manual for animal science**. Ithaca: Cornell University Press, 1985. 613 p.

YOU, Y.; LI, P.; LEI, F.; XING, Y.; JIANG, J. Enhancement of ethanol production from green liquor-ethanol-pretreated sugarcane bagasse by glucose-xylose cofermentation at high solid loadings with mixed *Saccharomyces cerevisiae* strains. **Biotechnology for Biofuels**, v. 10, n. 92, p. 1-11, 2017. doi: 10.1186/s13068-017-0771-7.

ZINN, R. A.; ADAMS, C. F.; TAMAYO, M. S. Interaction of feed intake level on comparative ruminal and total tract digestion of dry-rolled and steam-flaked corn. **Journal of Animal Science**, v. 73, n. 5, p. 1239-1245, 1995. doi: 10.2527/1995.7351239x.