









In vitro fermentation of diets containing sweet potato flour as a substitute for corn in diets for ruminants

Claudia Faccio Demarco¹  Fabian Manuel Guerrero Paredes¹ Claudio Antonio Pozo² 
Marilisa Mibach¹ Gilberto Vilmar Kozloski²  Lisandre de Oliveira³ Eduardo Schmitt¹ 
Viviane Rohrig Rabassa¹  Francisco Augusto Burkert Del Pino¹
Marcio Nunes Corrêa¹ Cassio Cassal Brauner^{1*} 

¹Núcleo de Pesquisa, Ensino e Extensão em Pecuária (NUPEEC), Universidade Federal de Pelotas (UFPEL), 96160-000, Pelotas, RS, Brasil. E-mail: cassiocb@gmail.com. *Corresponding author.

²Programa de Pós-graduação em Zootecnia, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.

³Instituto Federal Sul Rio-grandense (IFSul), Pelotas, RS, Brasil.

ABSTRACT: *With the intensification of production systems, dairy cow feeding has undergone changes creating the need to increase substitute feed options, focusing on more efficient, modern, and sustainable alternatives. Few researches were carried out evaluating the inclusion of sweet potato flour as an energy source in substitution of maize for ruminants. The aim of this study was to evaluate the in vitro gas production of ground corn replacement by sweet potato flour at different levels. For in vitro gas production, four treatments were performed, consisting of corn replacement by sweet potato flour at the levels of 0, 33, 66, and 100%, in a diet consisting of corn silage, soybean meal, and ground corn. In vitro incubations were conducted in sealed bottles containing 50 ml of the inoculum prepared using ruminal fluid and 0.5 g of each treatment. Gas production was determined in 96 consecutive hours. The cumulative gas production was greater when the corn was 100% replaced by SPF (224 ± 1.45 and 231.9 ± 1.45 ml/g DMi for 0 and 100% of replacement, $P = 0.01$). Degradation rates were 7.10, 7.59, 8.08, and $8.59 \pm 0.06\%$ per hour for the 0, 33, 66, and 100% replacement rates, respectively ($P < 0.001$). There was also a difference ($P = 0.002$) in the lag time, in which diets with the highest SPF inclusion had a lower colonization time. In conclusion, sweet potato flour produced more gas and was more rapidly degraded than corn.*

Key words: fermentation, starch, ruminant nutrition, tubers.

Fermentação *in vitro* de dietas contendo farinha de batata-doce como substituto do milho em dietas para ruminantes

RESUMO: *Com a intensificação dos sistemas de produção e o aumento das exigências alimentares das vacas leiteiras criou-se a necessidade de diversificação nas opções de alimentos, focando em alternativas mais eficientes, modernas e sustentáveis. Poucas pesquisas foram realizadas avaliando a inclusão da farinha de batata-doce como fonte de energia em substituição ao milho para ruminantes. O objetivo deste trabalho foi avaliar a produção de gás in vitro da farinha de batata-doce (SPF) em substituição ao milho moído em diferentes níveis. Para a produção de gás in vitro, foram realizados quatro tratamentos, com substituição de milho por farinha de batata-doce a 0, 33, 66 e 100%, em uma dieta com silagem de milho, farelo de soja e milho moído. As incubações foram conduzidas em frascos selados contendo 50 ml do inóculo preparado utilizando o fluido ruminal, solução tampão e 0,5 g de cada tratamento. A produção de gás acumulada foi maior na substituição do milho pela SPF em 100% (224 ± 1.45 e $231,9 \pm 1.45$ ml/g MSi para as substituições 0 e 100%, $P = 0,01$). A taxa de degradação foi 7,10, 7,59, 8,08 e $8,59 \pm 0,06\%$ por hora nas substituições 0, 33, 66 e 100%, respectivamente ($P < 0,001$). Houve também diferença ($P = 0,002$) no lag time, em que as dietas com maior inclusão de SPF tiveram tempo de colonização bacteriana menor. Em conclusão, a farinha de batata-doce produziu mais gás e foi degradada mais rapidamente que o milho.*

Palavras-chave: fermentação, amido, nutrição de ruminantes, tubérculos.

In high-producing ruminants, meeting the energy requirements is important in order to maintain the health status of the animal and to support high milk yields and rapid weight gains. Cereals represent the primary source of energy in ruminant diets

(GOZHO & MUTSVANGWA, 2008). Starch is a major energy source for both ruminant animals and ruminal microorganisms (MOHARRERY et al., 2014), and cereal grains contain high concentrations of this component. Among the cereals used are corn,

sorghum, barley, wheat, and oats and differences exist for ruminal starch degradability among these grains.

In addition to these differences in degradability that will result in different ruminant performance, other factors such as availability and price determine which cereal to use. The high cost of some feeds encourages the use of alternative local sources of energetic feed, notably the starchy tubers in temperate and tropical countries (WHEATLEY et al., 1995). Among the starch-rich tuber feeds studied in substitution of cereals for ruminants are cassava (*Manihot esculenta Crantz*) (WANAPAT & KANG, 2015), beet and by-products (EVANS & MESSERSCHMIDT, 2017), potato (*Solanum Tuberosum*) (BABAEINASAB et al., 2015) and sweet potato (*Ipomoea batatas*) (MATHER et al., 1948).

Sweet potato is an annual crop grown widely in the tropical and subtropical countries. Sweet potato roots have high levels of energy (85% of soluble carbohydrate) and low levels of crude protein (3.87%) (ROSTAGNO, 2005). Using sweet potato flour as animal feed to supply energy is not a new concept. MATHER et al. (1948), tested dehydrated sweet potatoes in comparison to corn and did not find an impact on milk production, but an increase in vitamin A.

Bromatological similarities between the two carbohydrate rich substrates, corn and sweet potato, is not sufficient to consider the sweet potato eligible for replacing corn in ruminant diets. Differences in the ruminal starch disappearance rates among cereal grains and tubers makes the results of previous studies inconsistent. For instance, starch degradability in the rumen is greater for potato and maize than for barley and pea (CHAI et al., 2004). Techniques that characterize the ruminal metabolism and degradability of these ingredients, such as technical *in vitro* gas production, are indispensable for describing the kinetics of the activity of microorganisms in response to a potential new substrate (SILVA et al., 2015). Thus, the aim of this study was to evaluate the *in vitro* gas fermentation parameters of sweet potato flour at different levels of substitution for ground corn. The hypothesis of this study is that the substitution of corn by sweet potato alters the *in vitro* fermentation parameters.

The *in vitro* gas production assays and laboratory analyzes were carried out in the facilities of the Laboratory of Bromatology and Nutrition of Ruminants of the Department of Animal Science, Federal University of Santa Maria, from March to July of 2017. Sweet potato roots were obtained from family farms of Mariana Pimentel, geographic location 30 °

21 '10 "S, 51 ° 34' 58" W, in the South-Central region of the State of Rio Grande do Sul, Brazil.

Samples of sweet potato flour (SPF) were from four varieties: Beauregard, Cabeluda, Catarina and Rubisol. The SPF was obtained from the artisanal processing of the root. After harvesting, the tubers were washed and crushed in a knife grinder, resulting in pieces of 2 to 3 cm, which were then dried in a static grain dryer. Forced hot air was used, not exceeding the temperature of 40 °C, avoiding loss of nutrients and altering the chemical composition of sweet potato (ARAÚJO et al., 2015). After 6 hours, the dried sweet potato passed through a hammer crusher with medium sieve and was stored hermetically. After this process, the SPF samples were dried in an oven at 55 °C, ground to 1 mm and stored for later bromatological analysis and *in vitro* gas incubation. A preliminary *in vitro* gas production test with one run was performed to describe the *in vitro* gas parameters of SPF varieties (Beauregard, Cabeluda, Catarina, and Rubisol) and ground corn.

The experimental treatments tested in the *in vitro* gas assays were diets composed by either of four SPF varieties included in the diets at a level of 33, 66 or 100% in substitution of ground corn. A control diet with ground corn and without SPF (0%) was also included. The diets (Table 1) were formulated to meet the requirements of a dairy cow (650 kg of body weight (BW), 75 days in milk) producing 24 kg/d of milk composed of 3.4% fat and 3.2% protein (NRC, 2001). Three *in vitro* runs were performed for each diet and, in each run, the diet samples were incubated in triplicates. Additional blank bottles were also incubated. Thus, 42 bottles were incubated in each run.

Samples were weighed (0.5 g) into 125 mL glass bottles. Subsequently, 40 mL of buffer solution (MOULD et al., 2005) was added and then kept refrigerated at 4 °C for 12 hours to allow hydration of the substrate. After that, the bottles were put in a water bath with agitation at 39 °C and 10 mL of ruminal inoculum was added. The inoculum was collected from the rumen of a fistulated bovine kept on pasture and supplemented with 2 kg/day of a concentrate with 18% of CP, composed of 75% of ground corn and 25% of soybean meal. All procedures were performed under continuous CO₂ injection.

Gas production was manually recorded after 3, 6, 9, 12, 18, 24, 36, 48, 72, and 96 hours of incubation using a three-outlet valve. The first outlet was connected to a needle (0.6 mm), which was inserted in the rubber cap. The second outlet was connected to the pressure gauge, which has a graduate column filled with distilled water and

Table 1 - Ingredients (mg/500mg of DM) for each of the treatment diets replacing ground corn by sweet potato flour (SPF) in 0, 33, 66, and 100%.

Item, mg/500mg of DM	-----Replacement of ground corn by SPF (%)-----			
	0	33	66	100
Corn silage	208	208	208	208
Soybean meal	92	92	92	92
Ground corn	200	134	66	0
Sweet potato	0	66	134	200

the third remained free being used to remove gases from inside the bottles at each reading, down to zero pressure. The volume of gas generated by each bottle at each measurement was recorded and the volume of gas produced by blanks was subtracted in order to estimate the evolution of the fermentation. The volume of produced gas was expressed in ml of gas produced per gram of incubated dry matter (DMi).

The unicompartamental model proposed by SCHOFIELD et al. (1994) was used to describe the kinetics of the fermentation process through the cumulative production of gases as follows:

$$V = Vf * (1 + \exp(2 - 4 * S * (t - L)))^{-1},$$

where: Vf = final volume of gas (ml) at time t; S = rate of degradation (/ h); L = colonization time of the bacteria on the substrate (in hours).

Total DM content was determined by drying at 105 °C until a constant weight was reached. Ash was determined by combustion at 600°C for 3 h and OM by mass difference. Total N was determined by the Kjeldahl method (Method 984.13; AOAC, 1997). The neutral (NDF) and acid (ADF) detergent fiber analyses included ash and were based on the procedures described by MERTENS (2002) and AOAC (1997); respectively, except that samples were weighed in polyester filter bags (porosity of 16 µm) and treated with neutral or acid detergent in an autoclave at 110 °C for 40 min. For the NDF analysis also was added α-amylase (SENGER et al., 2008). Ether extract (EE) was determined using a fat extractor (Ankom XT15; Ankom Technology, Macedon, NY) and petroleum ether as solvent. The total digestible nutrients (TDN) was calculated according NRC (2001) and starch was analyzed using acid hydrolysis followed by glucose determination using the spectrophotometric method of Glucose-Oxidase (TRINDER, 1969), with changes in the hydrolysis time and the temperature of the digestion based on the study of KOZLOSKI et al. (1999). Amylose was analyzed according

to MCGRANCE et al. (1998). The values of the bromatological analysis of the diet and sweet potato varieties are presented in table 2.

For analysis, data of triplicates in each run were averaged and each run was considered a replicate. After verifying normality of residuals and homogeneity of variance, data of *in vitro* gas parameters of diets were analyzed using GLM procedure (NCSS 7.0, Statistical System, Utah, USA, 2005), according to the following model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ij}$$

where: i = 1, 2, 3, 4 (4 SPF varieties); j = 1, 2, 3, 4 (4 substitution levels); μ: overall mean; α_i: effect of level i of SPF β_j: effect of level j of substitution; αβ_{ij}: effect of the interaction of levels i and j of these two experimental factors, and e_{ij}: experimental error. The calculation was done on the supposition that the terms μ, α_i, β_j, and αβ_{ij} are fixed effects, and e_{ij} uncorrelated random effects.

Tukey Kramer multiple range test was used to compare averages. Orthogonal polynomial contrasts were used to test statistical significance of the linear, quadratic, or cubic components of treatments. Statistical differences were declared significant at P≤0.05.

Results from the preliminary *in vitro* gas production test are demonstrated in figure 1 and T

table 3. It is noticeable that the maize degradation curve separates from the set of SPF and SPF varieties showed higher gas production in the first hours of incubation. The fermentation parameters obtained for the four varieties of sweet potato and ground corn are presented in the table below (Table 3).

The fermentation parameters obtained for the four levels of substitution on the second *in vitro* gas production test are presented in the table and figure below (Table 4 and Figure 2). The only significant effect was observed on the replacement levels on fermentation parameters (PG Total, Kd, and Lag time, P=0.01; <0.001; 0.002, respectively).

Table 2 - Chemical Composition of ingredients including 4 varieties of SPF, the control diet (i.e. only ground corn) and for diets where sweet potatoes flour (SPF) was included at either rate 33, 66 or 100% in substitution of ground corn.

-----as % of DM-----										
Item	DM ¹	Ash	OM ²	CP ³	NDF ⁴	ADF ⁵	EE ⁶	TDN ⁷	Amylose	Starch
Corn silage	25.56	5.61	94.39	8.75	46.47	-	-	-	-	-
Soybean meal	88.45	7.54	92.46	47.05	20.23	11.11	3.87	-	-	-
Ground corn	85.69	1.75	98.25	8.51	11.12	4.33	3.83	-	-	-
-----Sweet potato-----										
Beauregard	90.57	2.73	97.27	5.89	6.83	1.61	2.20	96.19	-	-
Catarina	88.43	2.20	97.80	3.64	5.49	1.39	2.19	95.76	-	-
Rubisol	86.79	2.39	97.61	5.65	4.54	2.53	1.74	95.72	-	-
Cabeluda	85.01	2.93	97.07	3.26	13.41	5.92	2.11	93.77	21.06	75.7
-----Total mixed diet-----										
Control	61.18	4.42	95.58	15.70	27.50	3.78	2.24	-	-	-
33	61.45	4.53	95.47	15.19	27.03	3.58	2.01	-	-	-
66	61.72	4.64	95.36	14.66	26.55	3.38	1.77	-	-	-
100	61.99	4.75	95.25	14.14	26.08	3.19	1.54	-	-	-

1: Dry Matter; 2: Organic Matter; 3: Crude Protein; 4: Neutral Detergent Fiber; 5: Acid Detergent Fiber; 6: Ethereal Extract; 7: Total Digestible Nutrients.

There was no significant difference between varieties (PG Total, Kd, and Lag time, $P=0.99$; $P=0.39$; $P=0.54$, respectively).and there was no interaction between SPF varieties and replacement levels for the total GP, Kd and Lag time variables (PG Total, Kd, and Lag time, $P=0.94$; $P=0.98$; $P=0.97$, respectively). There was a

significant difference between Control and 100% of SPF replacement for the PG ($P = 0.01$), Kd ($P<0.001$) and lag time ($P=0.002$) parameters (Table 2). The interaction varieties vs replacements are demonstrated in figure 2.

Including SPF in the mixtures lead to an increase in the volume of gas produced in the flasks

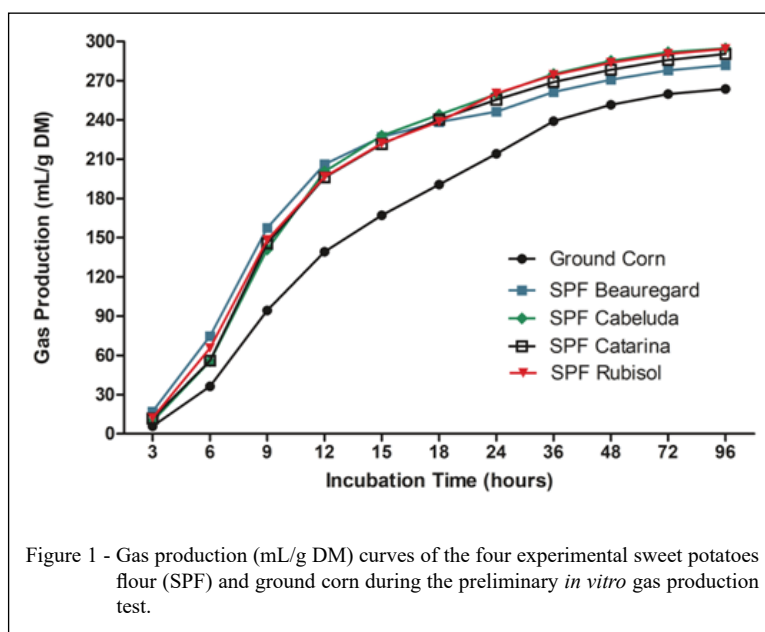


Figure 1 - Gas production (mL/g DM) curves of the four experimental sweet potatoes flour (SPF) and ground corn during the preliminary *in vitro* gas production test.

Table 3 - Descriptive gas production parameters of the four experimental sweet potatoes flour and of ground corn during the preliminary *in vitro* gas production test.

Parameters	Corn	Beauregard	Catarina	Rubisol	Cabeluda
Total gas production (mL/g of DMi)	257.10	271.70	277.50	286.00	286.50
Gas production rate (%/h)	8.34	13.30	11.66	11.57	11.62
Lag time (h)	3.22	2.75	3.00	2.90	3.09

and therefore during the fermentation, the production of gas was linearly higher as the level of substitution increased ($P=0.005$). In addition, the inclusion of SPF at higher levels caused a linear increase in the fermentation rate ($P<0.001$) and the fermentation began earlier ($P=0.001$). The greater the level of replacement of ground corn by SPF, the faster the substrate was degraded with larger gas production.

In vitro gas production has been developed as a predictive tool of nutrient content (BLÜMMEL & BECKER, 1997). One of the advantages of the gas production technique makes it possible to conduct frequent simultaneous measurements on a large number of samples of small size. It has been widely used (CHAI et al., 2004; HATEW et al., 2015; LUTAKOME et al., 2017) to assess the nutritional quality of feeds due to its high correlation with *in vivo* digestibility, which results from its ability to simulate the process of digestion in ruminant animals (HATEW et al., 2015).

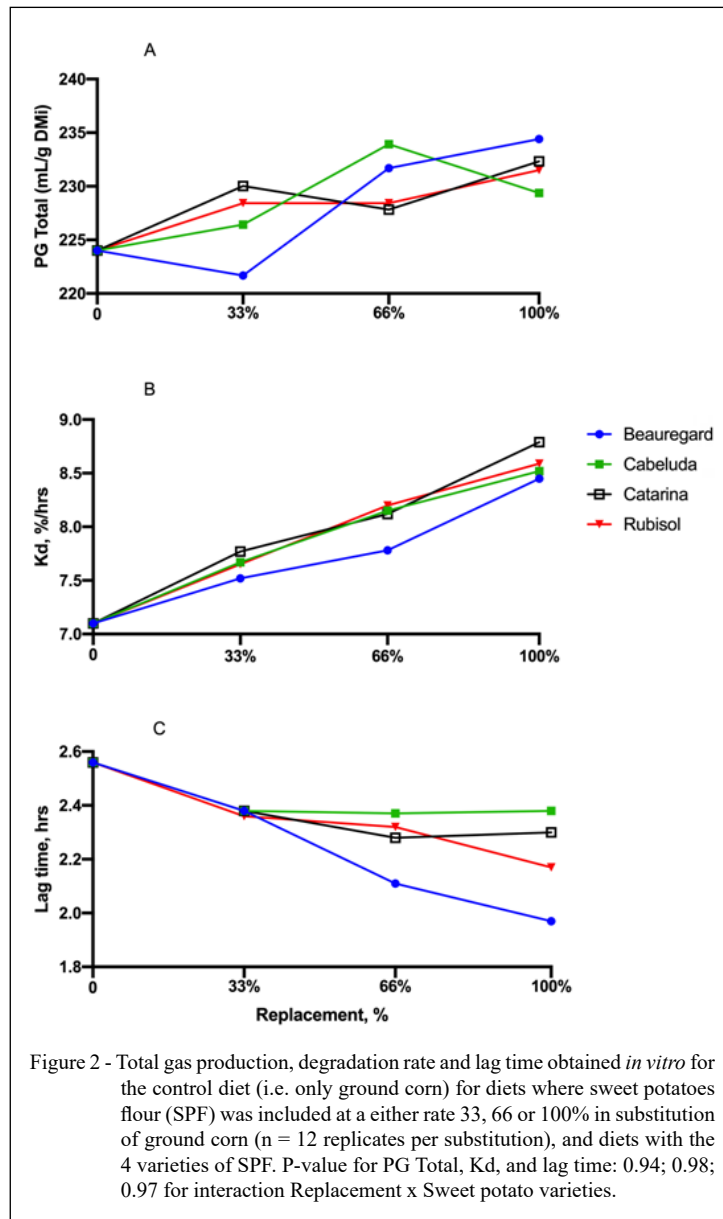
The increased in total gas production (mL/g DMi) may be related to the level and source of starch in each diet. This linear increase of the gas production observed with the increase of sweet potato inclusion probably occurs due to the increase in ruminal fermentation as shown in Table 4. In this study, the value of starch was reported to be 75.7% for sweet potato, while ROSTAGNO (2005) reported 62.9%. However, these values are higher than the levels found by Brazilian researchers, who determined the starch values of sweet potato to range from 13.4% to 29.2% (OLIVEIRA et al., 2005). The wide range of sweet potatoes produced in Brazil could explain this difference. In addition, phosphorus present in the soil may influence the accumulation of starch in sweet potatoes. Another influential factor may be the source of organic fertilization (OLIVEIRA et al., 2013).

The higher and more rapid gas production explained by the content of starch found in sweet

Table 4 - Means \pm standard error of means (S.E.M) of total gas production, degradation rate and lag time obtained *in vitro* for the control diet (i.e. only ground corn) for diets where sweet potatoes flour (SPF) was included at a either rate 33, 66 or 100% in substitution of ground corn ($n = 12$ replicates per substitution), and diets with the 4 varieties of SPF. P-value for PG Total, Kd, and lag time: 0.01; <0.001; 0.002 for replacement levels. P-value for PG Total, Kd, and lag time: 0.99; 0.39; 0.54 for varieties. P-value for PG Total, Kd, and lag time: 0.94; 0.98; 0.97 for interaction Replacement x Sweet potato varieties.

	PG Total (mL/g DMi)	Kd (%/hr)	Lag time (hours)
-----Replacement (%)-----			
Control (0%)	224.00 \pm 1.45 ^A	7.10 \pm 0.06 ^A	2.56 \pm 0.04 ^A
33%	226.64 \pm 1.45 ^{AB}	7.59 \pm 0.06 ^B	2.37 \pm 0.04 ^{AB}
66%	230.50 \pm 1.45 ^B	8.08 \pm 0.06 ^B	2.27 \pm 0.04 ^B
100%	231.90 \pm 1.45 ^B	8.59 \pm 0.06 ^B	2.21 \pm 0.04 ^B
-----Sweet potato-----			
Beauregard	227.94 \pm 2.41	7.66 \pm 0.01	2.25 \pm 0.08
Cabeluda	228.43 \pm 2.41	7.86 \pm 0.01	2.42 \pm 0.08
Catarina	228.55 \pm 2.41	7.94 \pm 0.01	2.38 \pm 0.08
Rubisol	228.11 \pm 2.41	7.88 \pm 0.01	2.35 \pm 0.08

^{A,B} Values followed by the same capital letter on the same line do not differ significantly.



potato as well as others tubers like cassava (LUNSIN et al., 2010) is due to the readily available non-fiber carbohydrates (NFC). These provide energy for microbial growth and for the host (STEVNEBØ et al., 2009) through the production of organic acids, CH₄ and CO₂.

The extent of starch degradation is dependent on its nature and structure. These include the chemical composition of the feed, starch granule size and shape, amylose and amylopectin ratio, presence of a protein matrix, and adaptation of rumen bacteria to different starch sources (GIUBERTI et

al., 2014). These factors should be considered as sources of variation for fermentable parameters. In agreement, MCALLISTER et al. (1993) suggested that differences in ruminal starch digestibility between cereals are related to the structural components within the endosperm rather than structural components within the starch granules. As there have been few *in vitro* studies evaluating tubers, we will discuss both the components within the starch granules and the structural components.

Understanding of the relationships between amylose and amylopectin ratio and starch

digestion in the rumen is still limited and inconsistent (STEVNEBØ et al., 2009). *In vitro* studies revealed a negative relationship between amylose content and starch digestion rate in cereals (LI et al., 2001). The decreased starch digestion potential of high amylose starch structures is hypothesized to occur because hydrogen bonding in the glucose chains of amylose is more extensive and; therefore, making high amylose starch less accessible for enzymatic hydrolysis as compared to amylopectin, which has many branched chains of glucose and a larger surface area per molecule (BREWER et al., 2012).

Data from literature shows amylose contents varying between 19.1 to 28% for sweet potatoes (HOOVER, 2001); in this study, the value was 21.06% for the Cabeluda variety, whereas for ground corn these values quite varied (2.7 to 70%), depending on the botanical variety and differences in the geographical origin (ALCÁZAR-ALAY & MEIRELES, 2015). CONE & WOLTERS (1990) pointed out that the rapid ruminal *in vitro* digestion obtained with tapioca and rice starch was due to the lower amylose content in this starch sources. Contrastingly, in others starch rich cereals with cultivars of varying amylose content (e.g. barley) it was observed that the starch amylose proportion and granule particle size had only a minor influence on the *in vitro* ruminal rate of starch digestion (STEVNEBØ et al., 2009).

According to CALDAS NETO et al. (2000), cassava starch has a higher concentration of amylopectin than corn (83.0 and 76.0%, respectively). These values of amylopectin in cassava and the non-association of starch grains with the protein matrix that surrounds these granules interfere with the grain digestibility, favoring greater use by the ruminal microorganisms, decreasing the lag time. Replacing corn for sweet potato, the time of bacterial colonization decreases from 2.56 h on Control to 2.20 h on 100% of sweet potato flour replacement ($P = 0.002$). Under these conditions, the starch found in energy sources other than cereals, such as cassava and sweet potato, do not present a protein matrix and this may explain the shorter colonization time (RICACHESKI et al., 2017).

PATTON et al. (2012), estimated the *in vitro* kd of various ruminant feeds and others starch rich sources such as corn co-products (kd = 7.0%/h) and wheat germ meal (kd = 6.8%/h). There are differences in the rate of corn degradation reported by several studies (CORREA et al., 2002; PEREIRA et al., 2018) and this can be explained by the corn hybrid used and the maturity of the plant, as the

degradability of corn starch is strongly influenced by these factors (PEREIRA et al., 2004). Tubers such as cassava and sweet potato have a higher degradability than cereals due to their lack of pericarp, protein matrix and, peripheral endosperm (GOMÉZ et al., 2016).

The adaptation of the rumen inoculum to different sources and amounts of starch can affect the *in vitro* gas parameters and was discussed by HATEW et al. (2015) and CONE & VAN GELDER (2006). In these studies, when comparing *in vitro* and *in vivo* starch degradation from different sources, a discrepancy was found between the *in vivo* starch degradation and the degradation rate estimated based on an *in vitro* gas production experiment, in which the donor animals were not adapted to the diets containing the used starch source. Taking this into account and knowing the complexity of rumen fermentation it can be considered a weakness of this study that the donor animals of the rumen inoculum was not fed with the different sources of starch used in this trial and this may be could generate inconsistent results.

It is important to understand the structural characteristics of starch, its ruminal and post-ruminal digestion and the factors affecting its digestibility, in order to improve the performance and profit of livestock systems. The use of sweet potato as a source of starch provides faster fermentation of the starch when compared to corn, allowing better synchronization with rapidly degraded nitrogen sources, such as urea. (QIAO et al., 2018)

The replacement of ground corn with sweet potato flour resulted in a difference between the fermentation parameters of diets with 0 and 100% sweet potato flour content, with an increasing linear response to the substitution level of sweet potato flour. The sweet potato may be a viable source of starch in diets for ruminants. Nevertheless, a larger scale study would be required to clarify the effects of sweet potato on ruminant performance and metabolism.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the design and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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