



Effect of different levels of *Ipomoea batatas* flour inclusion on the ruminal pH of sheep in metabolic cages

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ABSTRACT. The accumulation of industrial by-products increases the use of sweet potato waste for ruminants, but ruminal pH characteristics are still not well known. The objective was to assess the fluctuation of ruminal pH in sheep supplemented with different levels of sweet potato flour inclusion in their diet. Four rumen-fistulated sheep were used; they were fed a diet based on ryegrass haylage (*Lolium multiflorum*) and sweet potato flour (*Ipomoea batatas*), provided according to the level of inclusion in the total diet (0, 0.5, 1.0 and 1.5%). Approximately 80 ml of ruminal fluid was collected for reading on a bench pH meter. Statistical data analysis was run on Statistical Analysis System (SAS Institute INC. Cary, NC, USA), and statistical difference was considered for $p < 0.05$. The animals that received 1.5% of sweet potato flour in their diet presented acid rumen pH; the 1.0% group presented rumen pH acidification in the first 6 hours after feeding, and the 0.5% level of inclusion did not change the rumen environment. It is concluded that the inclusion of 0.5% sweet potato flour in sheep diet proved to be an efficient energy supplementation strategy.

Keywords: carbohydrates; fermentation; ruminants; by-products.

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Introduction

Seeking to maximize the efficiency of production systems, agroindustrial waste has been used as an alternative to reduce costs, since the investment in nutrition within a property can reach 60% of the capital invested (Luz, Matos, Cardoso, & Brauner, 2019). In this context, carbohydrates are frequently used for improving ruminal fermentation, since starch is the main energy source of rumen microorganisms (Hatew et al., 2014), and corn, wheat, oats, sorghum and barley stand out among the main sources of starch (Witczak, Ziobro, Juszczak, & Korus, 2016).

Cereal grains are highly energetic, widely used in formulations and differ from each other by their degree of ruminal solubility and degradability, with oats presenting the fastest fermentation, while sorghum has one of the slowest (Demarco et al., 2019). In addition, the price fluctuation of these commodities is another factor that can interfere with the choice of which ingredient to use.

With Brazil being a major sweet potato producer in the world, and because sweet potato is preferably intended for human consumption, products that do not meet market requirements are rejected by the industry and, consequently, not commercialized, becoming a waste that is oftentimes left in the farm. This residue, when not treated, is susceptible to contamination by pests that contaminate subsequent crops, meaning losses for producers (Dias et al., 2015).

Tubers contain between 65 and 85% of starch, which is non-vitreous, making this nutrient more available for adherence and microbial fermentation in the rumen (Gómez, Posada, & Oliveira, 2016). Starch is composed of two polymers: amylopectin and amylose (Alcázar-Alay & Meireles, 2015). Amylopectin, which makes up 79% of sweet potatoes, is highly branched, therefore more susceptible to degradation by ruminal microorganisms, which makes foods with a greater amylopectin content highly fermentable (Alcázar-Alay & Meireles, 2015). Pectin, in its turn, is 100% degradable in the rumen and presents high concentrations in tubers such as beet pulp, and low proportions in grasses such as corn (Mirzaei-Aghsaghali et al., 2011).

Starch-rich diets improve protein efficiency (Silva et al., 2013) but, when used in high proportions, fast-fermenting carbohydrates cause a disproportion in the production of volatile fatty acids (VFAs), and propionic acid can reach 40% of the VFAs produced, reducing ruminal pH (Goularte et al., 2011) and providing an adequate environment for the multiplication of gram-positive bacteria that produce lactic acid, thus resulting in lower pH and compromising the viability of ruminal flora, with negative reflexes on OM digestibility (Goularte et al., 2011).

Dang, Lv, Obitsu, and Sugino (2017), through a study in which they assessed the effects of two levels of substitution (15 and 30%) in alfalfa hay (AH) by a mixture of cassava branch silage and sweet potato branch silage (CS + SPS), found that the substitution of 30% of AH by (CS + SPS) decreased the digestibility of organic matter (OM) and nitrogen in the total digestive tract, compared to the control group and 15% substitution.

Given this scenario, sweet potatoes have become a viable alternative for producers seeking to optimize costs, but their utilization requires caution, bearing in mind their possible effects on ruminal pH (Deckardt, Khol-Parisini, & Zebeli, 2013).

Studies show that the use of sweet potato by-product in livestock diet is a promising reality, with great potential for application and positive effects as to weight gain, and organic matter consumption and digestibility (Megersa, Urge, & Nurfeta, 2013; Phesatcha & Wanapat, 2013; Beckford & Bartlett, 2015; Mangwe, Rangubhet, Mlambo, Yu, & Chiang, 2016; Dang et al., 2017; Li, Ji, Wang, Qin, Hou, & Shen, 2017; Kamiya et al., 2017), but few studies have assessed the effect of using sweet potato on ruminal pH; what has been proposed is the transformation of a residue, which is not commercialized, into flour or bran, in order to compose a balanced diet. Therefore, the objective of this study is to assess the ruminal pH of sheep supplemented with different levels of sweet potato byproduct.

Material and methods

This study was conducted at the Laboratory of Bromatology and Nutrition on Ruminants (Labrumen) of the Federal University of Santa Maria. It employed four Santa Inês castrated male sheep weighing, on average, 74.4 kg, fistulated in the rumen, and housed in individual stalls. Their diet was formulated based on the National Research Council (NRC, 2001) to meet their nutritional requirements and ensure a consumption of 3% of their live weight; it consisted of ryegrass haylage (*Lolium multiflorum*) *ad libitum* and sweet potato flour (Ipomoea potatoes; Table 1).

Table 1. Dry matter (DM), crude protein (PB), neutral detergent fiber (NDF), and composition of mineral matter (MM) in the sweet potato flour and in the ryegrass haylage.

| Item (%) | Sweet potato flour (<i>Ipomoea batatas</i>) | Ryegrass haylage (<i>Lolium multiflorum</i>) |
|----------|---|--|
| DM | 83.58 | 70.03 |
| CP | 16.07 | 16.88 |
| NDF | 9.08 | 66.55 |
| MM | 2.92 | 8.79 |

The animals were divided into 4 groups - a control group with 0% sweet potato flour inclusion, and groups that received 0.5, 1.0 and 1.5% of sweet potato flour inclusion in their diet, according to methodology adapted from Tamir and Tsega (2010). Food was provided daily, twice a day, at 8:00 and 16:00. A 4 x 4 Latin square was used as experimental design; each period lasted 10 days, with the first 9 days being meant for the adaptation of the animals (Baroni et al., 2010; Ferreira, Neiva, Rodríguez, Lopes, & Lôbo, 2010) to the sweet potato flour, and the last day, for ruminal fluid collection. Ruminal fluid collection was performed over a 24 hour period, with an interval of 2h00 in between each one, starting at 8:30 in the morning, before food was supplied, and ending at 6:30 on the following day. Approximately 80 mL of ruminal fluid was collected from the intermediate area of the rumen for pH reading, with this procedure being performed immediately after collection, using a bench pH meter (Pegoraro et al., 2017).

The statistical analysis for resulting data was run on Statistical Analysis System (SAS Institute Inc. Cary, NC, USA). To do so, analysis of variance with Mixed Model was employed to compare groups, collections and their interaction (group x collection) through Tukey's HSD test ($p < 0.05$).

Results and discussion

The average ruminal pH showed statistical difference ($p < 0.01$) between groups, evidencing that higher levels of sweet potato flour inclusion in the diet result in pH acidification (Table 2).

Table 2. Comparing ruminal pH averages of fistulated sheep fed different levels of sweet potato flour inclusion in their diet.

| Parameter | Levels of sweet potato flour inclusion | | | | p value |
|------------|--|--------------|--------------|--------------|----------|
| | 0 | 0.5 | 1.0 | 1.5 | |
| Ruminal pH | 6.52 ± 0.04a | 6.38 ± 0.04a | 6.13 ± 0.04b | 5.72 ± 0.04c | p < 0.01 |

^{ab}Lower case letters differ from each other on the same row and represent statistical difference.

Figure 1 displays the behavior of ruminal pH throughout the day; it is possible to observe that the animals with 1.5% inclusion presented values below 6.1 throughout the day, reaching a minimum of 5.5 in the period preceding the afternoon meal. The 1.5% group presented a lower pH than the 0% group did during the first 4 hours after feeding (5.86 ± 0.152 and 6.78 ± 0.152 , respectively), and this decrease in pH was maintained up to 8 hours after feeding, being lower in the 1.5% group compared to the 0.5 and 0% groups (5.65 ± 0.152 , 6.53 ± 0.152 and 6.55 ± 0.152 , respectively).

pH is used for determining the H⁺ concentration of a solution; the physiological parameters of a healthy rumen for sheep stand between 6.0 and 7.0, with this variation being possibly a consequence of fermentative processes (Noro, Sepúlveda, Cárdenas, Chihuailaf, & Wittwer, 2013). Ruminal pH reduction is a consequence of the growth in the number of amylolytic bacteria, responsible for starch degradation, directly interfering with organic matter digestibility, especially fiber, for reducing the activity of cellulolytic bacteria (Silva et al., 2013). With lower digestibility, the utilization of nitrogen for microbial protein synthesis is reduced (Cantalapiedra-Hijar, Lemosquet, Rodriguez-Lopez, Messad, & Ortigues-Marty, 2014), since proteolytic and ureolytic bacteria decrease their adherence to food particles, resulting in a decreased capacity to capture the substrate in the rumen medium (Silva et al., 2013).

The group that received 1.0% of sweet potato flour inclusion in their diet had their ruminal pH dropped from 6.71 to 5.81 in the first 6 hours (p < 0.01) after feeding (Figure 1), and this drop may be related to the higher sweet potato degradation rate, just as found by Demarco et al. (2019). Brewer, Cai, and Shi (2012) report that starch composition affects enzymatic hydrolysis, with the amylopectin fraction being the easiest to access enzymes, due to their branched glucose chains. Sweet potatoes contain 79% of amylopectin in their starch fraction and, when used 100% substituting corn, present a degradation rate of 13.30% hour⁻¹ compared to corn, with 8.34% hour⁻¹ (Demarco et al., 2019), resulting in a greater accumulation of propionic acid and rendering conditions favorable for the growth of microorganisms that produce lactic acid, thus contributing to the acidification of the rumen environment (Esposito, Irons, Webb, & Chapwanya, 2014).

In a study conducted on cattle by Noro et al. (2013), during a case of subacute acidosis, with pH below 5.6, the concentration of protons in the rumen increased, causing microlesions in epithelial cells, which can affect animal metabolism, decreasing the absorption of volatile fatty acids (VFAs; Miranda Neto, Silva, Mendonça, Drummond, & Afonso, 2011), in addition to causing the death of gram-negative bacteria. This leads to an increase in lipopolysaccharides (LPS), triggering an inflammatory process (Minuti et al., 2014). According to Kim et al. (2018) and Gao and Oba (2016), pH values below 5.8 affect the fermentation of cellulolytic bacteria, whose ideal pH is between 6.2 and 6.8. The group with 1.5% sweet potato flour inclusion in their diet presented a lower average pH (p = 0.02), which may directly lead to a reduction in dietary fiber fermentation, resulting in significant production losses related to decreased acetate in the rumen (Homem Junior et al., 2010; Costa, Fernandes, Garcia, Soares, & Franzolin, 2017).

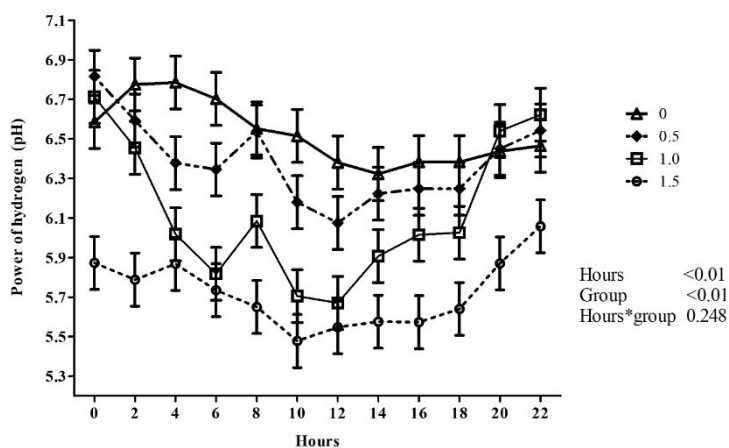


Figure 1. Behavior of ruminal pH of fistulated sheep fed different levels of sweet potato flour inclusion in their diet.

This proportion is not ideal, since the ingestion of excessive amounts of rapidly fermentable carbohydrates can cause major changes in the rumen environment, interfering with its microbial population. The first 6 hours after feeding are the most crucial, for being marked by a sharp growth of amylolytic bacteria, mainly *Streptococcus bovis*, which uses glycids to produce lactic acid, resulting in a decrease in ruminal pH, directly interfering with the motility of cellulolytic bacteria and protozoa, which can lead to the death of these microorganisms when prolonged for an excessive period, as in cases of subacute acidosis (Fernando et al., 2010).

The group with 0.5% sweet potato flour inclusion in their diet had its ruminal pH maintained within healthy physiological parameters, suggesting that this level of inclusion is a new alternative for energy supplementation without causing ruminal acidosis. Using fast-fermenting carbohydrates optimizes the protein efficiency of the diet because, when the protein reaches the rumen, it is quickly transformed into ammonia and carbon dioxide (CO₂) by the action of enzymes produced by ruminal bacteria. These fermentation products are used by ureolytic and proteolytic microorganisms for the synthesis of microbial protein. This drop in ruminal pH is maintained up to 10 hours after the morning feeding, in all groups. Ruminants have a diurnal eating behavior, which results in the variation of their ruminal fluid pH. During the day, the ruminal environment is expected to become more acid due to the high fermentation rate, which is the result of a more intense eating, while during late night and in the early morning, pH will be more alkaline due to long rumination periods (Noro et al., 2013).

Conclusion

It is concluded that the inclusion of 0.5% of sweet potato flour in sheep diet seems to be an efficient energy supplementation strategy, since it did not change ruminal pH values. However, further studies that include serum analyses to complement pH assessments are of vital importance in clarifying possible metabolic disorders.

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