In vitro fermentation parameters of brachiaria straw and starchy by-products

Parâmetros de fermentação in vitro de palha de braquiária e subprodutos amiláceos

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Highlights

The starchy feedstuffs showed more intense fermentation in the first hours.
The fibrous feedstuffs fermentation occurred more slowly in the first hours.
The diets showed lower k1 values and higher k2 values when compared to corn silage.
The results confirm the possibility of using the wastes in ruminant feeding.

Abstract

Our objective was to characterize the degradation kinetics of fibrous and starchy agro-industrial wastes that can be used as feed for ruminants. Unprocessed Brachiaria straw hay (UBH), processed (briquetting) Brachiaria straw hay (PBH), a starchy by-product from potato processing (SBP), and cassava starch (CST) were evaluated. Two diets were formulated: one including UBH and CST (UBH-CST); and the other PBH and SBP (PBH-SBP). For the study of degradation kinetics, the in vitro cumulative gas production fermentation technique was used, in which the two-compartment Monomolecular-G3G1 model provided a more likely fit. The cumulative gas volumes produced were 23.2 (UBH), 29.6 (PBH), 39.1 (SBP), 36.6 (CST), 27.9 (UBH-CST), and 28.5 (PBH-SBP) mL per 0.1 g of dry matter. The starchy feedstuffs showed more intense fermentation in the first hours (k1 = 1.050 and 0.627, for CST and SBP, respectively). This agile fermentation occurs because this is a soluble and/or rapidly degradable feedstuff that contains a very small amount of fibrous matter. As for the fibrous feedstuffs, UBH and PBH, fermentation occurred more slowly in the...
first hours ($K_1=0.212$ and $0.121$, for UBH and PBH, respectively), releasing less gas. Because fiber has a slowly degradable and insoluble part, the asymptotic phase takes longer to be reached. The diets, on the other hand, exhibited fermentation intermediate to those of the starchy and fibrous feedstuffs ($K_1=0.141$ and $0.127$, for UBH-CST and PBH-SBP, respectively), what faithfully followed the gas production profile of their ingredients. Both diets showed lower $K_1$ values and higher $K_2$ values when compared to corn silage. The characterization of agro-industrial residues, using the in vitro cumulative gas production technique, confirms the real possibility of using the studied by-products in feeding ruminants.

**Key words:** Briquetting. Cassava starch. *In vitro* gas production. Potato starch. Straw processing.

**Introduction**

The harvest of tropical forage seeds generates 2.88 million tons of straw annually (consisting of remaining leaves, stems, and inflorescences) (Catuchi et al., 2017). Using this material in ruminant feeding is an obvious option; however, due to its low nutritional quality, this practice is only performed occasionally. The low density of straw is also a logistic problem in terms of transport and storage (Souza & Cardoso, 2003).
Straw can be processed, through the deconstruction of lignocellulose (Souza, 2001), to increase its digestibility and intake. To address the abovementioned issues, an industrial process denominated ‘briquetting’ can be employed, which consists of compressing raw material under high pressure, causing lignin to be liberated and bind the material into a firm briquette (Nikolaisen & Jensen, 2013). Considering that this material has the potential to be used in ruminant feeding, it is important to evaluate the kinetics of passage and ruminal degradation of this fiber.

The use of by-products in animal feeding could mitigate the issue of pollution in the agricultural industry (Pen et al., 2006), and, if not used, it presents a high cost of waste treatment (Nkosi & Meeske, 2010). Starchy by-products from the processing of cassava and potato can be an alternative in ruminant feeding, especially for the partial or complete substitution of corn, a costly diet ingredient (Marques et al., 2000; Silva et al., 2013).

World cassava production is more than 180 million tons annually. Although there are no absolute data regarding the total amount of waste produced from industrialization, it is known that around 10% of the total cassava used in the manufacture of flour is eliminated in the form of peel and around 3 to 5%, in the form of sweeping flour (Zeoula et al., 2002). Research on cassava and its by-products stands out due to the ease of its cultivation, in addition, there is the possibility of using its cultural residues (leaves and stems), and its industrial by-products (hull, flour and starch) in animal feed.

In potato processing and processing industries, approximately 35% of the potatoes produced are discarded, in addition to producing a large number of by-products. These residues can be used to feed ruminants as they have high starch content and minimize production costs. Furthermore, the possibility of using tubers unsuitable for human consumption eliminates an economic and environmental problem (Silva et al., 2013).

Knowledge of the feedstuffs used in diets is important to allow animals to express their maximum production potential (Senger et al., 2007). One of the methods to evaluate the degradability and kinetics of ruminal fermentation is the in vitro gas production approach, described by Theodorou et al. (1994). This technique has been used due to its satisfactory and rapid results, in addition to being a low-cost alternative compared with the in vivo method. Furthermore, the use of mathematical modeling based on the obtained results can generate a good estimate of the kinetic processes of degradation (Vieira et al., 2008).

The hypothesis regarding fibrous byproducts is that, in theory, briquetting processing can promote some destabilization of the material’s lignocellulosic matrix, due to the increase in pressure and temperature during the industrial process. This possible destabilization could increase the transit kinetics and ruminal degradation of the fiber. Regarding starchy by-products, due to their high starch content, they have the potential to replace corn partially or completely in rations without compromising animal performance, which configures the other hypothesis to be studied in this work. Therefore, our objective was to characterize the fermentation kinetics,
using the in vitro cumulative gas production technique, of some agro-industrial wastes that can be used as feed for ruminants.

**Material and Methods**

The present experiment was reviewed and approved by the Animal Use Ethics Committee of the State University of Norte Fluminense (Protocolo 380 da CEUA/UENF).

Four feedstuffs were evaluated to determine degradation kinetics and diet composition: (1) unprocessed *Brachiaria* straw (UBH); (2) processed (briquetting) *Brachiaria* straw (PBH); (3) a starchy by-product from potato processing (SBP); and (4) cassava starch (CST). The unprocessed *Brachiaria* straw was obtained after the harvest of tropical forage seeds. The UBH was obtained by simply baling field straw. To obtain PBH, the straw was ground and then subjected to the briquetting process. The briquetting process consists of the continuous evolution of the material in metal tubes under pressure, which increases the temperature of the material. This process considerably increases the density of the material and transforms it into a pressed material called “briquettes”, and known commercially as “briqfeno”, which in turn, considerably reduces the space used for transport and storage.

Potato starch (SPB) was obtained from the residue of a factory producing the commercial product “potato straw” provided by the company Crokes located in São João del Rei, MG. This by-product is the starch that comes off the outer part of the tubers during washing, containing a small contamination with the skin. Finally, the CST by-product used in the present study is residue from the manufacture of sweet flour, it is a waste product from a machine.

The starchy by-product, SBP, was obtained from the potato chip manufacture waste; this by-product is the starch that detaches from the external part of the tuber during the cleaning process, which is slightly contaminated with skin. Finally, the CST by-product used in the present study is residue from the manufacture of sweet flour, it is a waste product from a machine.

Two diets were formulated using the feedstuffs: one including UBH and CST (UBH-CST), containing 69.5% UBH, 28.0% CST, and 2.5% urea; and the other including PBH and SBP (PBH-SBP), containing 67.7% PBH, 29.5% SBP, and 2.8% urea. The energy and protein contents were standardized to a high enough level to meet the nutritional requirements of a cow with a body weight of 200 kg and with an average daily gain of 0.5 kg/d (Agricultural and Food Research Council [AFRC], 1993). Urea was used to balance the protein level.

Samples of the tested feedstuffs were weighed and dried in a forced-air oven at 55°C for 72 h. Then, the material was weighed again to obtain the partial dry matter (DM). Feedstuffs were ground in Wiley mills to pass through a 1-mm round-curved sieve (Theodorou et al., 1994). Total DM (method 976.03; Association of Official Analytical Chemists [AOAC], 1990), ash (method 942.05; AOAC, 1990), crude protein (method 987.04; AOAC, 1990), neutral detergent fiber (NDF; method AOAC 2002.04), acid detergent fiber (ADF), and lignin (method 973.18; AOAC 1977) in the diets and feedstuffs were determined (Table 1).
The cumulative gas production from fermentation was determined through in vitro anaerobic incubations, based on the methodology described by Malafaia et al. (1998). Rumen inoculum was made up of the combination of ruminal contents from three steers with permanent cannulas in the rumen, which were fed twice daily, at 08.00 hand at 16.00 h, for 15 days. The diet was formulated based on the metabolic weight of the cattle under maintenance (AFRC, 1993), it was composed of 35:65 (concentrate: roughage), the roughage offered was hay and the concentrate was made up of 86% ground corn; 11.5% soybean meal and 2.5% kg of urea.

The liquid and solid materials from the rumen inoculum were collected separately and stored in thermos flasks with a wide opening. The 2:1 liquid:solid ratio was used to prepare the inoculum, which was mixed in a blender for 60 hours under continuous CO2 infusion. Then, the material was filtered through four layers of gauze. The filtered inoculum was added to the culture medium at the ratio of 1:4 (Goering & Van Soest, 1970), respectively, and the mixture was kept at 39°C under continuous CO2 sparging, until the moment when the mixture was transferred to flasks containing the samples.

A partially dried 0.5-g sample was incubated in amber-colored penicillin flasks (100 mL) and culture medium and rumen inoculum were added according to the procedures reported by Hall and Mertens (2008). Three flasks without sample (blank) were used for data correction. Twenty-one flasks with sample were distributed in the digestibility machine (triplicates of each sample and three flasks without sample, or blanks). The flasks were closed with rubber stoppers, sealed with aluminum seals, and kept in a water bath at 39°C.

Pressure and volume readings were taken at initial equilibrium (zero time) and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 42, 48, 72, and 96 h after adding the rumen

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### Table 1

**Chemical composition of feedstuffs and diets**

<table>
<thead>
<tr>
<th>Item, g/kg dry matter</th>
<th>Feedstuff(^1)</th>
<th>Diet(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UBH</td>
<td>PBH</td>
</tr>
<tr>
<td>Dry matter</td>
<td>900.3</td>
<td>918.2</td>
</tr>
<tr>
<td>Crude protein</td>
<td>52.2</td>
<td>51.1</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td>760.5</td>
<td>736.6</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>453.8</td>
<td>439.4</td>
</tr>
<tr>
<td>Lignin</td>
<td>63.9</td>
<td>35.2</td>
</tr>
<tr>
<td>Mineral matter</td>
<td>44.8</td>
<td>54.5</td>
</tr>
</tbody>
</table>

\(^1\) UBH = unprocessed Brachiaria straw hay; PBH = processed (through briquetting) Brachiaria straw hay; SBP = starch by-product of potato processing; CST = cassava starch.

\(^2\) Two diets were formulated, one using UBH and CST (UBH-CST) and the other one with PBH and SBP feedstuffs (PBH-SBP).

n.a.: not available: undetectable due to very small amounts present in the feedstuff.
inoculum. The gas pressures generated from the onset of fermentation were recorded by manometric readings (0-7 psi manometer, 0.05-psi increments), as well as the volumes displaced within a graduated pipette (0-25 mL, 0.1-mL increments) by the pressurized gas diverted into the pipette using a three-way valve. The pressure and cumulative volume of rumen fermentation gases were determined by adding the corrected readings at the times performed. The volumes of gases read were standardized in mL per 0.1 g of DM of incubated sample.

Equation 1 represents the general structure attributed to the nonlinear model used to quantitatively interpret the cumulative gas production profiles:

\[ V_t = V_{f1} \times \left(1 - \exp(-k_1 t)\right) + V_{f2} \times \left(1 - \left(\delta^N \exp(-k_2 t) + \exp(-\lambda t) \sum_{i=1}^{N-1} \frac{(1-\delta^{N-i})(\lambda t)}{i!}\right)\right) + \epsilon_t \quad \text{(eq. 1)} \]

adopting \( V_t \sim \text{Normal}(\mu_{V_t}, \sigma^2_{V_t}) \), that is, we assumed that variable \( V_t \) follows a normal distribution with average function \( \mu_{V_t} \). This function (Eq. 1) represents the two-compartment model composed of the monomolecular and GNG1 models (Vieira et al., 2008), in which \( V_{f1} \) and \( V_{f2} \) (mL) are the asymptotic volumes of gases produced by the rapidly and slowly degradable fractions; \( k_1 \) is the fractional rate (1/h) of fermentation of carbohydrates of the rapidly degradable fraction; \( k_2 \) is the rate (1/h) of fermentation of carbohydrates of the slowly degradable fraction of the feedstuff DM; \( N \) is a positive integer that represents the order of time dependence; and \( \lambda \) (1/h) is the asymptotic rate of preparation of the insoluble and slowly degradable substrate for digestion.

When fitting the accumulated gas production profiles, the data were grouped using the grouped Data function of the nlme package of R software (R Foundation for Statistical Computing, Vienna, Austria), considering equations 2 and 3:

\[ V \sim \text{Time|rep / flask} \quad \text{(eq. 2)} \]
\[ V \sim \text{Time|flask} \quad \text{(eq. 3)} \]
The models were fitted to the cumulative gas production profiles using the nlme package of R (Pinheiro & Bates, 2000). In all cases, the models were fitted considering data groupings and the model combinations for interpreting the gas profiles, and the fits were compared using the Akaike information criterion (Akaike, 1974; Sugiura, 1978; Burnham & Anderson, 2004). The Akaike criterion tests which function, or functions best represent the data distribution (Akaike, 1974), that is, it chooses the model that causes the least loss of information (Burnham & Anderson, 2004). Rejection or non-rejection of the null hypothesis, used in the likelihood ratio test to test normality against another possible distribution, should be avoided. The models were fitted separately for diets, starchy and fibrous feedstuffs (Table 2). The predictive parameters were compared using the 95% confidence interval.

Table 2
Model that presented the best fit to the cumulative gas production profiles

<table>
<thead>
<tr>
<th>Feedstuffs (^1)</th>
<th>(N) (^2)</th>
<th>Variance type</th>
<th>Correlation (^3)</th>
<th>Degree of freedom</th>
<th>AICc (^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CST and SBP</td>
<td>3</td>
<td>Exponential</td>
<td>Yes</td>
<td>12</td>
<td>119.8</td>
</tr>
<tr>
<td>UBH and PBH</td>
<td>3</td>
<td>Homogeneous</td>
<td>Yes</td>
<td>13</td>
<td>-92.1</td>
</tr>
<tr>
<td>PBH-SBP and UBH-CST</td>
<td>3</td>
<td>Homogeneous</td>
<td>Yes</td>
<td>13</td>
<td>134.0</td>
</tr>
</tbody>
</table>

\(^1\) UBH = unprocessed Brachiaria straw hay; PBH = processed (through briquetting) Brachiaria straw hay; SBP = starch by-product of potato processing; CST = cassava starch; UBH-CST= diet were formulated using UBH and CST; PBH-SBP = diet were formulated using PBH and SBP

\(^2\) \(N\) is a positive integer that represents the order of time dependence

\(^3\) Correlation auto regressive (AR1 in nlme package)

\(^4\) Akaike information criterion.

Results and Discussion

The models fitted for the diets and starchy and fibrous feedstuffs was the Monomolecular-G3G1 model, in which increments in the value of \(N\) up to 3 provided a more likely fit of the model to the cumulative gas production data. The inclusion of the correlation function and the variance function provided considerable improvement in the fitting of the model to the profiles. The parameters estimated using the Monomolecular-G3G1 model are analogous to what the graphs illustrate (Figures 1 and 2).
In vitro degradability by gas production follows a sigmoid curve and can be divided into three phases, according to Cheng et al. (1980): 1) the slow phase, without gas production (initial phase); 2) the fast gas production phase (exponential phase); and 3) the phase in which the gas production rate decreases, reaching zero (asymptotic phase). The gas released from feedstuffs inoculated with rumen fluids reflects microbial activity and can provide information on degradability and fermentation kinetics. The curves generated by the two diets followed the same pattern, as did the respective curves for the feedstuffs. The difference in exponential and asymptotic phases between the curves of starchy and fibrous feedstuffs was noteworthy (Figure 1).

![Figure 1. Sigmoid curves of the accumulation of gas volume produced during the 96 hours of incubation.](image)

The curves of the starchy and fibrous feedstuffs were different during the exponential phase because of the more intense fermentation in starchy feedstuffs in the first hours. The asymptotic phase of the starchy feedstuffs was reached quickly, demonstrating the accelerated fermentation rate of the starch from the feedstuffs evaluated here. This agile fermentation occurs because this is a soluble and/or rapidly degradable feedstuff that contains a very small amount of fibrous matter, as confirmed by chemical analysis (Table 1).

The sigmoid curves of the fibrous feedstuffs, UBH and PBH, show that in the first hours, fermentation occurred more slowly, releasing less gas, which is due to the greater amounts of NDF, ADF, and lignin present in this material (Table 1). Because fiber has a slowly degradable and insoluble part, the asymptotic phase takes longer to
be reached, when compared with starchy ingredients. The curves generated by the two diets followed the same pattern as the food curves and were intermediate between the curves of starchy and fibrous foods, that is, their gas production values were lower than those of starchy foods and higher than those of fibrous foods.

Cumulative gas production data allow us to compare the different diets regarding their final gas production and the degradation rates of the soluble and fibrous fractions. The accumulated gas production values were 39.1, 36.6, 29.6, 23.2, 27.9 and 28.5 for SBP, CST, UBH, PBH, UBH-CST and PBH-SBP, respectively. The volume of cumulative gas produced from SBP was greater than that produced from CST. Initially, CST produced a greater amount of gas, that is, greater initial fermentation. Gas production by SBP, from the twelfth reading (24 h after incubation), exceeded that of CST and remained greater until the last reading (Figure 2), producing a cumulative gas volume of 39.1 mL/0.1g DM, which is greater than the 36.6 mL/0.1g DM produced by CST.

Figure 2. Gas production (mL per 0.1 g dry matter per hour) during the 96 hours of incubation of fibrous (UBH and PBH) and starch (SBP and CST) feedstuffs, and diets (UBH-CST and PBH-SBP)\. UBH = unprocessed Brachiaria straw hay; PBH = processed (through briquetting) Brachiaria straw hay; SBP = starch by-product of potato processing; CST = cassava starch (CST). Two diets were formulated, one using UBH and CST (UBH-CST) and the other one with PBH and SBP feedstuffs (PBH-SBP).
The gas production profiles of UBH and PBH were similar; however, in the last readings, the lines representing gas production by PBH were above those of UBH (Figure 2), resulting in cumulative gas volumes of 29.6 and 23.2 mL/0.1 g DM, respectively. The cumulative volume of gas produced by the PBH-SBP diet (28.5) was greater than that produced by the UBH-CST diet (27.9 mL/0.1g DM), similarly to what happened with the respective starchy and fiber feedstuffs in each diet (Fig. 2).

Cabral et al. (2002) used the two-compartmental logistic model to estimate the kinetics of cumulative gas production and found final gas volumes of 20.03, 23.35, 25.77, 26.95 and 28.47 for corn silages with 0, 15, 30, 45 and 60% grains. According to bromatological analyses, the diets in studies present a chemical composition (crude protein, neutral detergent fiber, acid detergent fiber, lignin and mineral matter) between silage with inclusion of 0 to 30% of grains, but the gas production of diets was similar to silage with inclusion of 60% grains, which had the best nutritional value according to the estimated parameters. This was probably due to the greater effective degradability of starch from tubers compared to starch from cereals, especially corn. The greater degradability is due to the lack of pericarp, horny and peripheral endosperm, protein matrix and possibly due to a lower proportion of amylose and lipids in the starch granules, which reduces the amount of hydrogen bonds in the starch molecule and increases the capacity expansion of tuber starch in an aqueous medium (Zeoula & Caldas, 2001; Rangel et al., 2008).

According to the monomolecular-G3G1 model (Table 2), a greater rate of degradation of the soluble fraction (k1) in any diet or feedstuff means greater degradation of the soluble or rapidly degradable fraction. The second rate (k2) allows differentiating the diets in terms of the degradation of the slowly degradable fraction, proving to be an important measure in the selection of the diets with the highest-quality fibrous fraction. Table 3 presents the estimated values for \( Vf_1 \), \( Vf_2 \), \( K_1 \), \( K_2 \), e \( \lambda \) starchy and fibrous foods and their respective diets. Based to the degradation rates, k1 and k2, and their respective standard errors, the diets showed similar fermentation (k1=0.127 and k2=0.040 for PBH-SBP and k1=0.141 and k2=0.037 for UBH-CST). As for the fibrous feedstuffs, UBH exhibited a greater k1 than PBH, whereas k2 was greater in PBH (k1=0.212 and k2=0.031 for UBH and k1=0.121 and k2=0.034 for PBH). Between the starchy feedstuffs, CST showed greater values than SBP for both rates (k1=1.050 and k2=0.157 for CST and k1=0.627 and k2=0.091 for SBP).
The lower fermentation of fibrous foods in the first hours of incubation is demonstrated by the lower fermentation rates of the rapidly degradable fraction ($K_1$), with values of 0.212 and 0.121 for UBH and PBH, respectively. Because fiber has a slowly degradable and insoluble part, the asymptotic phase takes longer to be reached, when compared with starchy ingredients, where $K_1$ were 1.050 and 0.627 for CST and SBP, respectively (Table 3). Although UBH exhibited greater $k_1$, in the last readings, gas production from PBH was greater than that of UBH. Further research is needed on the processing of Brachiaria straw by briquetting; however, the results suggest that processing had a positive effect, since it increased the cumulative volume of gas produced, indicating greater fermentation of this straw compared with unprocessed straw.

Malafaia et al. (1999), estimating kinetic parameters of dry matter degradation of some tropical forages using the gas production technique, found higher values for the volume of gas produced, as well as higher degradation rates for brachiaria grass (Brachiaria decumbens), both for slowly degradable and rapidly degradable fractions. The lower degradation rates of the brachiaria
straw studied here are probably since it is a residue coming from a more mature material (90% DM) (Table 1) when compared to the brachiaria grass used in the study above (28.9 % DM).

Regarding starchy foods, a possible explanation for the lower degradation rate of the slowly degradable fraction (k2) of SBP is the larger amount of fiber present in this feedstuff when compared with CST. The greater amount of fiber present in SBP compared with CST is a consequence of the greater quantity of peels present in this by-product resulting from the industrial process. The by-product used to re-treat cassava starch (CST) is a residue from the manufacture of sweet tapioca flour, a machine waste product. Because it is a purely starchy product, the NDF of CST was not detected in the chemical analyses (Table 1).

The gas production profile of the diets faithfully followed the gas production profile of their ingredients (Figure 2). Both diets evaluated in the present study exhibited similar degradation rates. When compared to corn silage, they showed lower k1 values. Conversely, the k2 values of the tested diets (0.040 and 0.037) were greater than those of corn silage (Processi et al., 2016). Bendia et al. (2021) evaluated the nutritional potential of corn hybrids using the in vitro gas production technique and the values they estimated are comparable to those found in this study for diets. The k1 values, according to the confidence interval presented in the study, were similar between corn (G1= 0.140; G2=0.141 and G3=0.160) and the diets studied here (UBH-CST=0.141 and PBH-SBP = 0.127). The k2 values of the diets were higher (UBH-CST=0.037 and PBH-SBP = 0.040) than those of corn (G1= 0.024; G2=0.021 and G3=0.026).

**Conclusion**

The characterization of agro-industrial residues, using the in vitro cumulative gas production technique, confirms the real possibility of using the studied by-products in feeding ruminants.

**References**


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