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Ruminal fermentation kinetics of by-products using the semi-automatic technique of in-vitro gas production

Cinética de fermentação ruminal de coprodutos utilizando a técnica semiautomática de produção de gases in vitro

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ABSTRACT

The objective of this study was to develop a specific equation for the conversion of pressure values (psi) to volume (ml) for the Laboratory of Bromatology of the Federal University of Western Pará. To this end, the ruminal fermentation kinetics of regional feedstuffs were evaluated using the semi-automatic technique of in-vitro gas production. To set up the targeted equation, samples of ground corn, soybean meal, rice bran, Mombasa grass, cupuassu pie, cassava residues, and banana leaves were incubated and the pressure and volumes of the gases produced during the fermentation process were measured at predetermined times and related. These data on the volume of produced gases were used to determine, by applying the bi-compartmental logistic model, the ruminal fermentation kinetics parameters. The equation found for the laboratory was $V = 0.3757P^2 + 1.5972P + 0.2189$. Ground corn and cassava residue showed a higher degradation rate of non-fibrous carbohydrates (0.120 and 0.163 %/h respectively) and higher final gas volume (228.91 and 273.17 ml/g of DM, respectively). As for the degradation rate of fibrous carbohydrates, ground corn (0.023 %/h), rice bran (0.023 %/h), and cassava

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residue (0.021 %/h) presented the highest degradation rate. Thus, a specific equation to be used at the Laboratory of Bromatology of the Federal University of Western Pará was identified, according to the method applied and the altitude of the premises. Ruminal fermentation kinetics of cassava residue and rice bran showed the same parameters as corn, which may suggest the possibility of replacing corn in the diet of ruminant animals.
Keywords: Alternative feeding, feed analysis, in-vitro incubation

RESUMO

O objetivo deste estudo foi desenvolver uma equação específica para a conversão de valores de pressão (psi) em volume (ml) para o Laboratório de Bromatologia da Universidade Federal do Oeste do Pará. Para tanto, avaliou-se a cinética fermentativa ruminal de rações regionais por meio da técnica semiautomática de produção de gases in vitro. Para configurar a equação alvo, amostras de milho moído, farelo de soja, farelo de arroz, capim Mombaça, torta de cupuaçu, resíduos de mandioca e folhas de bananeira foram incubadas e a pressão e os volumes dos gases produzidos durante o processo de fermentação foram medidos em tempos predeterminados e relacionado. Esses dados de volume de gases produzidos foram utilizados para determinar, por meio da aplicação do modelo logístico bicompartimental, os parâmetros cinéticos da fermentação ruminal. A equação encontrada para o laboratório foi $V = 0,3757P^2 + 1,5972P + 0,2189$. Milho moído e resíduo de mandioca apresentaram maior taxa de degradação de carboidratos não fibrosos (0,120 e 0,163% / h respectivamente) e maior volume final de gás (228,91 e 273,17 ml / g de MS, respectivamente). Quanto à taxa de degradação dos carboidratos fibrosos, milho moído (0,023% / h), farelo de arroz (0,023% / h) e resíduo de mandioca (0,021% / h) apresentaram as maiores taxas de degradação. Assim, foi identificada uma equação específica para ser utilizada no Laboratório de Bromatologia da Universidade Federal do Oeste do Pará, de acordo com o método aplicado e a altitude das instalações.
Palavras-chave: Alimentação alternativa, análise de ração, incubação in vitro

INTRODUCTION

Significant advances in agribusiness performance have led to increased input intake and residue generation. At the same time, these residues have generated some opportunities for the ruminant production system, and the possibility of using by-products has attracted the interest of many producers. However, the use of these by-products requires knowing their bromatological composition and degradation profile in the rumen.

The in-vivo technique is the most accurate resource for evaluating animal nutrition. However, its applicability is limited by the high cost, since it requires the use of animals, feedstuffs, labor, and a great deal of time (MAURÍCIO, 2003). Therefore, there is a need for alternatives that are easy to carry out and that deliver accurate results.

In-vitro evaluation techniques simulate the ruminal environment and identify the behavior of the samples in this artificial environment (THEODOROU et al., 1994). The in-vitro gas production technique, used to determine ruminal fermentation kinetics, provides information about the rate and extent of degradation of the feedstuffs tested (GETACHEW et al., 1998; MAURÍCIO et al., 2003). The technique is based on

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the assumption that the production of gas in a growth medium inoculated with rumen microorganisms and under conditions similar to those of reticulum-rumen (temperature, pH, and anaerobiosis) is proportional to the amount of fermented substrate (LÓPEZ et al., 2007).

The method quantifies the pressure produced by the gases inside compartments of known volume. The relation between pressure and volume, however, varies with the altitude of the laboratory where the test is conducted, making it necessary to determine a specific equation for each laboratory (RYMER et al., 2005).

Thus, this study sought to determine the equation for the transformation of pressure values into volume, to be used at the Laboratory of Bromatology of the Federal University of Western Pará, and to identify the in-vitro fermentation kinetics parameters of some feedstuffs and regional by-products.

MATERIAL AND METHODS

The experiment was carried out at the Laboratory of Bromatology of the Federal University of Western Pará, in the city of Santarém-PA, at an altitude of 51 m.

Standard ruminant feedstuffs (ground corn, soybean meal, and Mombasa grass) and regional by-products that could be introduced into the animal diet (rice bran, cassava residue, cupuassu pie, and banana leaves) were evaluated, totaling 7 feedstuffs.

The feedstuff was dried and ground to 2 mm using a Willey mill. It was then analyzed to determine the dry matter (DM) (ID 934.01), crude protein (CP) (ID 984.13), organic matter (OM) (ID 942.05), and ether extract (EE) (ID 920.39), according to the AOAC (1990) methods. For analyses of neutral detergent fiber (NDF), the samples were treated with thermostable alpha-amylase, without the use of sodium sulfite (MERTENS, 1992). The non-fibrous carbohydrate (NFC) contents were calculated as proposed by Hall (2000): NFC = 100 - (%CP + %NDF + %EE + %ashes). Table 1 shows the bromatological composition of the feedstuffs.

Table 1. Chemical-bromatological composition of tested feedstuffs.

Feedstuffs	DM (%)	OM (%)	NDF (%)	CP (%)	EE (%)	NFC (%)
Soybean meal	88.61	92.95	11.21	45.74	1.90	34.1
Ground corn	87.94	97.60	13.97	9.04	4.04	70.55
Cassava residue	19.24	98.55	22.27	2.05	0.65	73.58
Mombaça grass	26.60	93.60	68.86	3.50	2.76	18.48
Banana leaf	12.63	90.06	55.36	6.24	3.56	24.90
Rice bran	88.95	89.40	23.15	13.42	16.38	36.45
Cupuassu pie	90.41	94.88	52.22	20.30	2.44	19.93

The samples were incubated in glass vials (50 ml) previously injected with CO2. In each vial, 300 mg of feedstuff sample were weighed. Five vials were used per tested feedstuff, in addition to two vials containing only ruminal fluid and culture medium, as a control, totaling 47 vials. With the aid of a pipette, 27 ml of culture medium were added to each vial, according to

Theodorou et al. (1994), as well as 3 ml of ruminal fluid (inoculum). Due to the unavailability of ruminal cannula, the inoculum was obtained in a slaughterhouse, stored in a preheated thermos and immediately taken to the laboratory. In the laboratory, the ruminal fluid was filtered through a double layer of cotton gauze with continuous CO2 injection. The vials were sealed with rubber stoppers (14 mm) and placed in a forced ventilation oven at 39°C.

The pressure of the gases accumulated in the upper part of the bottles (headspace) was measured, in psi (pressure per square inch), through a pressure transducer (Press DATA 800) connected to a three-outlet valve. The first outlet was connected to a needle (0.6 mm), the second to the pressure transducer, and the third to a plastic syringe used for volume measurement. Pressure and volume measurements were taken more frequently during the initial period of fermentation and subsequently reduced (1, 2, 3, 4, 6, 8, 10, 12, 14, 17, 20, 24, 28, 36, 48, 72, 96, 120, 144 and 168 hours), according to the methodology proposed by Maurício et al. (1999) adapted for 50 ml bottles. The accumulated gases were removed using the syringe, until the pressure recorded in the transducer reached zero, confirming the total removal of the gases.

The data on pressure and volume obtained during fermentation were used to determine the regression equation, with the aid of the SAS (2001) statistical software.

To determine the in-vitro ruminal fermentation kinetics parameters, a bi-compartmental logistic model proposed by Schofield et al. (1994), adjusted by non-linear regression using the Gauss-Newton method, was applied on the gas volume values produced.

RESULTS AND DISCUSSION

The pressure and volume data obtained from a total of 740 measurements allowed determining a pressure/volume ratio equation for the local conditions. Table 2 presents the descriptive statistics for the data set used to generate the equation.

Table 2. Descriptive statistics of the data set used to predict the volume of gases from the pressure values.

Variables	Maximum	Minimum	Mean	Standard deviation	CV(%)
Pressure (psi)	4.92	0.06	0.974	0.911	93.59
Volume (ml)	16.00	1.00	2.46	2.747	112.32

CV = coefficient of variation

Maximum values of 4.92 psi and 16 ml were observed for pressure and volume, respectively. According to Theodorou et al. (1994), pressure readings above 7 psi cause instability in the correlation between variables. To avoid this effect, measurements were taken at shorter intervals in the beginning of the fermentation process, as the highest gas production occurs in this stage.

The equation found, i.e. $V\ (ml) = 0.2189 + 1.5972\ P + 0.3757\ P^2$, ($R^2 = 0.98$), differs from equations found by other studies around the world (Mauricio et al.

1999; Mauricio et al. 2003; Posada et al. 2006) for two main reasons. First, the different capacity of the bottles used results in different pressure/volume ratios, such that larger bottles need greater gas volume for their internal pressure to increase, while internal pressure of smaller bottles increases with lower gas volume (ROTBART et al., 2018). The second reason is that the pressure/volume ratio varies according to the altitude of the study site, such that the volume of gas required per pressure unit is greater in laboratories located at higher altitudes (RYMER et al., 2005).

Mauricio et al. (1999) used 125 ml bottles at an altitude of 66 m in Reading, England; Mauricio et al. (2003) used 160 ml bottles at an altitude of 836 m in Belo Horizonte, Brazil; and Posada et al. (2006) used 100 ml bottles at an altitude of 1,583 m in Medellín, Colombia. Since the equation found by the present study was formulated using 50 ml bottles at an altitude of 51 m, the difference with the equations found by these authors was expected. The specific equation determined for the Laboratory of Bromatology of the Federal University of Western Pará - UFOPA will enable greater precision and accuracy in determining the feedstuff fermentation profile from data on gas volume obtained by in-vitro gas production.

Table 3 shows the mean values of final gas volume (FV), degradation rates of fibrous and non-fibrous carbohydrates (KdFC and KdNFC), and the lag time of the tested feedstuffs.

Table 3. In-vitro degradation kinetics parameters of tested feedstuffs.

Feedstuff	Parameters of in vitro degradation kinetics			
	FV (ml/g DM)	Lag Time (h)	KdNFC %/h)	KdFC (%/h)
Soybean meal	142.80	10.39	0.063	0.012
Ground corn	228.91	9.88	0.120	0.023
Cassava waste	273.17	15.24	0.163	0.021
Mombaça grass	221.28	19.09	0.042	0.012
Banana leaf	163.57	15.32	0.028	0.010
Rice Bran	112.23	7.26	0.075	0.023
Cupuassu pie	63.80	3.40	0.070	0.013

FV - Final volume of gases produced in 168 hours; Lag Time - Time of microbial colonization; KdNFC - Degradation rate of non-fibrous carbohydrates; KdFC - Degradation rate of fibrous carbohydrates.

The volume of gas produced during fermentation depends on the composition of the incubated sample; higher fiber contents result in slower gas production, while for higher non-fiber carbohydrate contents there is a lower gas production at a higher rate (NOGUEIRA et al., 2006).

Cassava residue had the highest final gas volume and degradation rates of fibrous and non-fibrous carbohydrates, similar to ground corn. The high degradation rates may be associated with the high amount of readily fermentable substrates, such as soluble carbohydrates (SILVA et al. 2014). Cabral et al. (2019) incubated a sample of cassava scrapings and observed degradation rates of non-fibrous and fibrous carbohydrates (0.176 and 0.037 %/h respectively) slightly higher than those found in the present study. It is worth noting that cassava

scrapings have higher NFC content than cassava residue, and this greater availability of readily available carbohydrates can explain the gap between rates.

The fermentation profile of cassava residue, a by-product of the extraction of cassava starch that maintains a high starch content, makes it a possible substitute for corn as feedstuff for ruminants, with great reduction in feedstuff costs. However, such substitution should be tested in-vivo, and intake parameters, digestibility, and animal performance should be evaluated.

In the present study, the observed values for ground corn incubation were 0.12%/h for KdNFC and 9.88 h for lag time, while Olivo et al. (2017) observed a KdNFC of 0.13%/h and lag time of 3.31 h for the same product. The different lag times observed in the two studies can be explained by the type of inoculum used. While the referred authors used inoculum collected with a vacuum pump from animals on a controlled diet in their study, the inoculum used in the present study was collected from animals on an unknown diet, 12 hours after fasting. This can result in the depletion of the microbiota, requiring a longer incubation time to reestablish them.

The cupuassu pie had the lowest FV among the tested feeds. This by-product, resulting from the extraction of oil from cupuassu almonds, has been used as a protein concentrate; however, it has a high fiber content, as can be seen in Table 2. Feedstuff protein fraction results in small gas production, while lipids produce no gas (Nogueira et al., 2006) or even reduce the production of gases from bulky diets (Morais et al., 2015). In addition, the high lignin content of this product (Rogério et al., 2016) may make fibrous carbohydrates unavailable for fermentation, thus resulting in lower gas production. Silva et al. (2015) evaluated licuri pie, which has CP and NDF contents similar to those in cupuassu pie, and observed a volume (51.01 ml/g) and degradation rate (0.065 %/h) similar to those observed for the cupuassu pie.

The highest degradation rates of FC were observed for ground corn, rice bran and cassava residue. The low NDF content reduces the amount of substrate for cellulolytic microorganisms, thus potentiating its action on the substrate resulting in a higher degradation rate. This higher degradation rate suggests that ground corn, rice bran and cassava residue cause less of a ruminal repletion effect when compared to other evaluated feedstuffs. On the other hand, banana leaf had the lowest degradation rate of FC, probably due to the high amount of NDF and low amount of soluble carbohydrates. This combination may cause an energy deficit for cellulolytic microorganisms, which in turn results in low degradation rate of fibrous carbohydrates. This lower degradation rate may indicate a large ruminal repletion effect when consumed by the animal, thus limiting dry matter intake and damaging animal production.

From the above, it can be concluded that the specific equation for the conversion of pressure values to volume, to be used in the UFOPA Laboratory of Bromatology, is $V\ (ml) = 0.2189 + 1.5972\ P + 0.3757\ P^2$. This equation allows for the production of more precise data from the application of a semi-automatic technique of in-vitro gas production in this laboratory.

Rice bran and potato starch residue showed in-vitro ruminal degradation kinetics parameters similar to ground

corn and should be investigated as potential substitutes. However, such substitution should be tested in-vivo, and intake parameters, digestibility, and animal performance should be evaluated.

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