Communication

[*Comunicação*]

Use of F57 bags by the semi-automatic *in vitro* technique of gas production

[*Uso de sacos F57 pela técnica* in vitro *semi-automática de produção de gases*]

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In vitro techniques based on the production of gases during ruminal fermentation simulate the ruminal environment and ruminants digestive processes (Theodorou *et al.*, 1994). These techniques allow the description of ruminal fermentation kinetics and also, indirectly, to estimate animal consumption (Blümmel, Ørskov, 1993). Therefore, these techniques have contributed for the studies of forages (Getachew *et al.*, 1998).

The semi-automatic *in vitro* gas production technique (Maurício *et al.*, 1999) enables the evaluation of a large number of substrate, presents high repeatability and offers the possibility to describe the rumen fermentation kinetic, estimating the rate and the extension of degradation for different foods and diets. Moreover, it is a low cost technique if compared to others *in vivo* techniques (Guimarães Júnior *et al.*, 2008).

This technique was developed at the University of Reading (England). It is based on the measurement of the pressure of gases produced by the anaerobic degradation of substrate incubated in fermentation flasks containing ruminal liquid and a buffering culture medium. Afterwards, the pressure data were used to calculate the volume of gases by means of a regression equation (P x V) specific for each laboratory where the technique was implemented. The relationship between P and V

is dependent on the altitude and, therefore, varies according to the geographic position of each research center (Mauricio *et al.*, 2003). Maurício *et al.* (1999) have modified the technique described by Theodorou *et al.* (1994) eliminating the need for manual measurements of gas volume and considering the use of the equation derived from the relation between P and V. This way, the degradation kinetics, more specifically the initial period of lag phase fermentation, could be described with greater accuracy due to the larger number of measurements and therefore were able to contribute to studies linked to feed evaluation for ruminants.

The technique described by Maurício *et al.* (1999) uses glass crucibles with porosity 1 and borosilicate plaques for filtering the material from the *in vitro* fermentations. However, when the analyzed sample is a substrate with high starch concentration (ex. concentrated), this process becomes inefficient due the blockage of the pores by sample residues. Alternatively, the use of the F57 (Ankom®) bag, in lieu of the filter crucibles, could avoid the filtering process since the previously weighed bag would be removed from the flask, dried and weighed for quantification of the sample without interference of the filtration. However, it is necessary to reduce the amount of sample incubated; since, according to recommendation from Ankom® (New York City, New York State, United States), wthese bags were designed

to store samples up to 0.8 gram. Due to the reduction of the amount of incubated sample, it also becomes necessary to reduce the volume of culture medium and inoculum, in order to preserve the original relationship described for Maurício *et al.* (1999).

Therefore, the objective of this study was to develop an equation that can be used to calculate the volume of gases produced using the pressure data obtained from the *in vitro* fermentation of substrate incubated in F57 bags (Ankom®). The Federal University of Minas Gerais (UFMG) ethical committee (CETEA n. 66/2011) approved

all the procedures of this research. The experiment was carried out at the Laboratory of *in vitro* Gas Production, of Veterinary School at the Universidade Federal de Minas Gerais (UFMG), Brazil, located at an altitude of 836m. Sixty-four samples of sorghum (BRS 506) were used. The samples were pre-dried at 55°C and grounded (1mm) including individual parts of the plant (stalk, leaf and panicle) as well as the entire plant. The values of dry matter, crude protein, neutral detergent fiber, acid detergent fiber and lignin fiber, following methods of AOAC (1990), are described in Table 1.

Table 1. Chemical composition of forage samples from the whole plant and plant fractions of the sweet sorghum variety

Sample	DM (%)		Chemical composition (% DM)							
			CP		NDF		ADF		Lignin	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Entire plant	24,8	0,6	6,3	0,5	52,4	0,8	31,9	0,6	7,3	0,2
Stalk	21,5	0,4	0,6	0,1	46,6	0,6	29,3	0,5	4,6	0,1
Leaves	34,9	1,4	11,7	0,3	75,2	6,9	41,5	0,9	13,6	1,2
Panicle	45,3	2,4	9,8	0,1	47,1	2,7	23,6	1,4	9,5	0,3

DM = dry matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, SEM = standard error of the mean.

The samples (0.5 gram) were placed in F57 (Ankom®) bags, previously weighed, and hot sealed. The bags were incubated in glass flasks (160mL) previously gasified with CO_2 aiming at maintaining anaerobiosis within the bags. Flasks containing only ruminal liquid, culture medium (blanks) and empty F57 (Ankom®) bags were included as control. In each flask, 45mL of culture medium were manually added (Theodorou *et al.*, 1994) and later sealed with a silicone stopper and kept at 4ºC during the night previous to the inoculation. Five hours before inoculation, the flasks were placed in an incubator for stabilization of the temperature in accordance with the ruminal environment (39°C). The ruminal inoculum was removed from an adult bovine fistulated in the rumen which was kept in a plot of *Paspalum notatum* and fed two kilograms of a corn and soybean meal concentrate (220g kg/DM of protein) for eight days prior to the collection of the inoculum. The ruminal liquid was removed and stored in previously warmed thermal bottles. In the laboratory, the ruminal liquid was filtered in two layers of cotton gauze, subject to a continuous

CO_2 injection and kept in a warm thermostatic bath at 39°C. The inoculation was done by means of an injection of 5mL of inoculum per flask, using a plastic syringe. A Luer Lock valve with three exits was connected to a 60mL syringe and to a pressure transducer (T443A, Bailey & Mackey, England). A needle (for perforation of the silicone cork) was used for measuring the volume (syringe) and pressure (transducer) of the gases produced in the interior of the fermentation flasks. After the insertion of the needle in the flask, through the stopper, internal pressure was recorded. Afterwards, while rotating the valve (Luer Lock), the accumulated gases pushed the syringe's piston up to the point when the pressure recorded by the transducer reached zero, which was the indication that all gases had been removed from the flask. This was the moment when the volume of gases produced was measured according to the reading in the graduated syringe (Fig. 1). Simultaneous P and V data were obtained, with measurements at 0, 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 hours after inoculation.
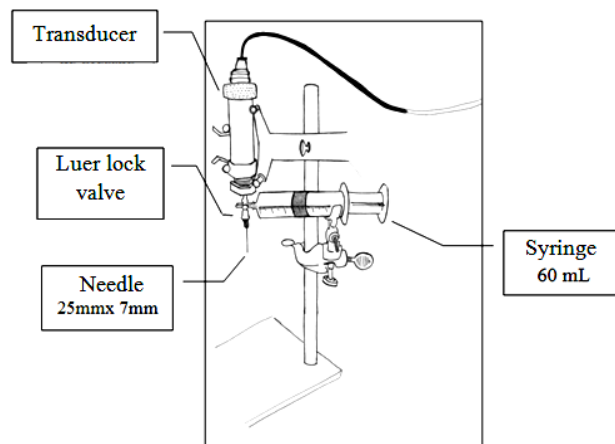
Figure 1. Schematical drawing of the equipment used for measuring pressure and volume of the gases during *in vitro* fermentation.

The polynomial regression procedure was applied for data analysis using SAEG (Statistical and Genetics Analyses System), according to Ribeiro Júnior (2001), in which the volume of gases was considered a dependent variable and pressure an independent variable.

Pressure ranged from 0 the 4.8 psi and volume from 0 to 36.7mL, considering data obtained from 474 observations (Figure 2). According to Theodorou *et al*. (2004), the maximum pressure recommended for the *in vitro* system of gas production is 7 psi, since for values above this limit the linearity of the P x V relationship tends to be reduced due to the dispersion or instability of the data. Pressure data in this study was within this limit. The equation found for the data in this study was: V (mL) = 0.747 (s.e. 0.0733) + 7.23 P (s.e. 0.1165) + 0.101 P^2 (s.e. 0.0372), (R^2 = 0.99). This equation differed from the previous one obtained by Mauricio *et al*. (2003), which used filter crucibles; [V (mL) = - 0.004 (s. e. 0.06) + 4.43 P (s.e. 0.043) + 0.051 P^2 (s.e. 0.007), (R^2= 0.99)]. These differences occurred due to changes in the volumes of culture medium (90mL versus 45mL) and ruminal liquid (10mL versus 5mL), the amount of fermented substrate (1g versus 0.5g), the apparatus used for residue collection (filter crucible versus F57 bag) and, consequently, the volume (60mL versus 110mL) to be occupied by the gases in each flask (head space) that were used according to the technique described by Mauricio *et al*. (2003) as opposed to the ones proposed in this study, respectively. In the equation originated in this study, the linear (7.23 versus 4.43) and angular (0.202 *versus*

0.102) coefficients linked to the variation of the volume of gases in relation to the pressure generated using F57 bags, were superior to the values of the equation proposed by Mauricio *et al*. (2003), respectively.

Note that in the equation described by Maurício *et al*. (2003), for each 1.0 psi of pressure registered by the transducer, estimate a production of 4.38mL of gases. Using the equation obtained in this study, each 1.0 psi of pressure corresponded to a volume of 8.11mL of gases. Although the values are different, results calculated from the volume of gases obtained employing both equations is coherent. Much more so, since the strategy used in the present study for segregation of the incubation residues required the use of only 50% of the amounts of sample, culture medium and inoculum compared to the original protocol described by Maurício *et al*. (1999). Therefore, there was approximately double the volume available (head space) for accumulation of gases in the incubation flask.

Thus, the experimental planning using substrate including different parts of the sorghum plant fermented in F57 (Ankom®) bags, generated a high variability in the data and, therefore, the development of an equation that allowed the estimate of volume by means of the pressure in agreement with the incubation methodology proposed for application in the Laboratory of Gas Production owned by the College of Veterinary Medicine at the Universidade Federal de Minas Gerais (UFMG).
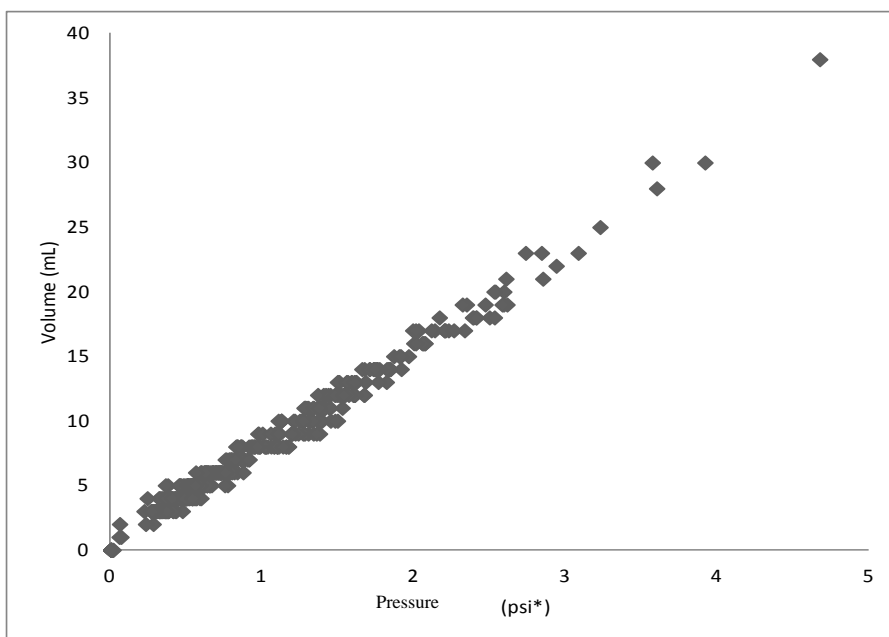
Figure 2. Pressure and volume data obtained through the semi-automatic *in vitro* technique of gas production, gases which were obtained through the fermentation of parts of the plant (stalk, leaf and panicle) and of the entire sorghum plant BRS506 (*pressure by square inch)

The development of the mathematical equation estimating the volume of gases by means of the pressure using F57 (Ankom®) bags as a vehicle for substrate fermentation increased the accuracy of the volume determinations and residue deriving from the fermentations using the *in vitro* semi-automatic technique of gas production installed in the Laboratory of Gas Production of Veterinary School at the Universidade Federal de Minas Gerais.

Keywords: *in vitro*, forage evaluation, F57 bags, gas production

RESUMO

Os cadinhos filtrantes (porosidade 1) originalmente usados na técnica in vitro *semi-automática de produção de gases estabelecida no Laboratório de Produção de Gases da Escola de Veterinária da UFMG podem ocasionar interferências nos estudos de quantificação da degradação de alimentos, principalmente os ricos em amido, devido à obstrução dos poros da placa filtrante pelo material residual após fermentação. Este estudo objetivou avaliar a substituição dos cadinhos filtrantes por sacos F57 (Ankom®) visando a realização de experimentos* in vitro *para descrição da cinética de fermentação e degradação ruminal. Esta substituição causou a redução do peso/volume da amostra incubada e desta forma, interferiu na relação pressão (P) / volume (V) e consequentemente inviabilizou a utilização de equação previamente estabelecida por Mauricio et al. (2003). Desta forma, objetivou-se neste estudo estabelecer equação para estimar o volume de gases produzidos a partir de dados de P e V obtidos pela fermentação de sorgo forrageiro, incluindo partes da planta e planta inteira, incubados em sacos F57 (Ankom®). Foram quantificados 474 dados simultâneos de pressão e volume os quais variaram de 0 a 4.670 psi e os de volume entre 0 e 38mL. A equação relacionando P e V obtida foi: V (mL) = 0.747 (s.e. 0.0733) + 7.23 P (s.e. 0.1165) + 0.101 P^2 (s.e. 0.0372), (R^2 = 0.99). O desenvolvimento desta equação viabilizou a utilização dos sacos F57 (Ankom®) pela na técnica* in vitro *semi-automática de produção de gases e, portanto permitindo a quantificação dos resíduos da fermentação favorecendo a acurácia dos resultados.*

Palavras chaves: in vitr*o, avaliação de forrageiras, sacos F57, produção de gases*

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