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Effect of sample size on kinetic parameters of roughage and concentrated feeds by a semi-automated *in vitro* gas production system

Efeito do tamanho da amostra nos parâmetros cinéticos de alimentos volumosos e concentrados por meio de um sistema de produção de gases *in vitro* semi-automatizado

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Abstract

This study aimed to evaluate the effect of different amounts of incubated samples on the kinetic parameters of *in vitro* fermentation of roughage and concentrated food used for feeding ruminants. Samples were prepared using 200, 300, 400, and 500 mg of air-dried roughage and concentrated sample, ground to 1 mm, and placed in 120 mL glass flasks. Next, inoculum and McDougal solution were added, and the readings were obtained using a semi-automated pressure transducer up to 96 h after the beginning of the incubations. Gas production of the non-fibrous fraction increased linearly ($P < 0.05$) for sugarcane, Marandu grass silage, corn silage, dried corn distillers' grains with solubles, dried brewer's yeast, bean residue, wet brewer's grains, sunflower meal, and Jatropha meal; quadratically ($P < 0.05$) for Napier grass silage and cottonseed meal; and cubically ($P < 0.05$) for castor meal and soybean meal. The degradation rate of the non-fibrous fraction reduced linearly ($P < 0.05$) for sugarcane, Napier grass silage, and castor meal; quadratically ($P < 0.05$) for Marandu grass silage; and cubically ($P < 0.05$) for corn silage, soybean meal, dried corn distillers' grains with solubles, bean residue, and cottonseed meal. Gas production of the fibrous fraction increased linearly ($P < 0.05$) for Napier grass silage, Marandu grass silage, corn silage, dried corn distillers' grains with solubles, bean residue, wet brewer's grain, cottonseed meal, and sunflower meal; quadratically (< 0.05) for Jatropha meal; and cubically ($P < 0.05$) for sugarcane, castor meal, and soybean meal. The degradation rate of the fibrous fraction increased linearly ($P < 0.05$) for Napier grass silage, dried corn distillers' grains with solubles, dried brewer's yeast, wet brewer's grains; quadratically ($P < 0.05$) for corn silage and castor meal; and cubically ($P < 0.05$) for sugarcane, Marandu grass silage, and bean residue. The lag time reduced linearly ($P < 0.05$) for castor meal and dried corn distillers' grains with solubles; quadratically ($P < 0.05$) for Napier grass silage; and cubically ($P < 0.05$) for sugarcane, Marandu grass silage, corn silage, soybean meal, bean residue, cottonseed meal, sunflower meal, and Jatropha meal. Thus, our findings suggest that the kinetic parameters of *in vitro* fermentation were affected as a function of the amount of incubated sample.
Key words: Digestion. Headspace. Lag time. Degradation rate. Gas production.

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Resumo

Objetivou-se com este trabalho avaliar o efeito de diferentes quantidades de amostras incubadas sobre os parâmetros cinéticos de fermentação *in vitro* de alimentos concentrados e volumosos disponíveis para alimentação de ruminantes. O preparo das amostras foi realizado utilizando 200, 300, 400 e 500 mg de amostra seca ao ar, moída a 1 mm, alocadas em frascos de vidro com capacidade de 120 mL, e adicionados inóculo e solução McDougal, seguidos das leituras, por meio de um transdutor de pressão semi-automatizado, até 96 horas após o inicio das incubações. A produção de gases da fração não-fibrosa apresentou comportamento linear crescente (P < 0,05) para a cana-de-açúcar, silagem de capim marandu, silagem de milho, grão seco da destilaria do milho, resíduo de cervejaria desidratado, resíduo de feijão, resíduo úmido de cervejaria, torta de girassol e farelo de pinhão manso, quadrático (P < 0,05) para silagem de capim napier e torta de algodão, e cúbico (P < 0,05) para farelo de mamona e farelo de soja. A taxa de degradação da fração não-fibrosa apresentou redução linear (P < 0,05) para a cana-de-açúcar, silagem de capim napier e farelo de mamona, quadrático (P < 0,05) para a silagem de capim marandu, e cúbico (P < 0,05) para a silagem de milho, farelo de soja, grão seco da destilaria do milho, resíduo de feijão e torta de algodão. A produção de gases da fração fibrosa apresentou comportamento linear crescente (P < 0,05) para a silagem de capim napier, silagem de capim marandu, silagem de milho, grão seco da destilaria do milho, resíduo de feijão, resíduo úmido de cervejaria, torta de algodão e torta de girassol, quadrático (P < 0,05) para farelo de pinhão manso, e cúbico (P < 0,05) para a cana-de-açúcar, farelo de mamona, farelo de soja. A taxa de degradação da fração fibrosa apresentou comportamento linear decrescente (P < 0,05) para a silagem de capim napier, grão seco da destilaria do milho, resíduo de cervejaria desidratado, resíduo úmido de cervejaria, quadrático (P < 0,05) para a silagem de milho, farelo de mamona, e cúbico (P < 0,05) para cana-de-açúcar, silagem de capim marandu e resíduo de feijão. O lag time apresentou redução linear (P < 0,05) para o farelo de mamona e grão seco da destilaria do milho, quadrático (P < 0,05) para a silagem de capim napier, e cúbico (P < 0,05) para cana-de-açúcar, silagem de capim marandu, silagem de milho, farelo de soja, resíduo de feijão, torta de algodão, torta de girassol e farelo de pinhão manso. Os parâmetros cinéticos de fermentação *in vitro* foram afetados em função da quantidade de amostra incubada.
Palavras-chave: Digestão. Headspace. Lag time. Taxa de degradação. Produção de gases

Introduction

The nutritional value of ruminant feed is estimated using dynamic digestion models. The nutritive value of a food depends on several factors, including its chemical composition and nutrient utilization by animals, since nutrient utilization in ruminant animals depends on the symbiotic association with ruminal microbiota (Santo et al., 2017). Thus, assessing the ruminal degradation dynamics of several foods is fundamental for determining the adequacy of diets in relation to the ratios of food fractions, as well as the digestion rate and losses due to ruminal fermentation (Goes et al., 2010).

An efficient laboratory method for determining the nutritional value of food should be reproducible and have a good correlation with parameters measured *in vivo*. The *in vitro* methods are less expensive, more efficient, and allow more precise maintenance of experimental conditions than the *in vivo* assays (Getachew, Blummel, Makkar, & Becker, 1998), and hence are preferably used to measure the rate and extent of nutrient degradation in ruminants (Groot, Cone, Willians, Debersaques, & Lantinga, 1996).

Although pressure transducers a relatively simple and inexpensive technique have been used to estimate the fermentation kinetics of food or diets for ruminant animals, many factors can affect the gas production profile of foods that need to be assessed. These factors include medium agitation during fermentation; inoculum origin, conservation, and manipulation; gas production measurement system; atmospheric pressure; sample preparation and size; and buffer amount and composition (Rymer, Huntington, Williams, & Givens, 2005).

Methodologically, the amount of sample used in different studies ranges from 100 to 1000 mg (Goering & Van Soest, 1970; Menke et al., 1979; Theodorou, Williams, Dhanoa, Mcallan, & France, 1994). Small amounts of sample may increase experimental errors owing to heterogeneous weighing during sampling, as observed in the study of Menke et al. (1979), who indicated that low sample weight was a critical point in the gas production method. However, when high amounts of samples are used, the system (buffer) should be able to buffer short-chain fatty acids produced, and the accumulated pressure should not be excessively high to adversely affect the fermentation and production of gases (Rymer et al., 2005).

In general, the total amount of gases produced has been found to linearly increase with an increase in the amount of incubated sample (Theodorou et al., 1994); however, when the headspace is reduced by using flasks of equal volume, the sensitivity of the system to detect the production of small volumes of gases—usually derived from the soluble fractions of food might likely be high at the initial incubation times. This may influence the degradation rate and lag time estimated for the incubated samples. Hence, standardizing the amount of sample for *in vitro* gas measurement systems becomes necessary when semi-automated systems are used to allow the precise and accurate estimation of the kinetic parameters that can be used regardless of the food analyzed.

This study aimed to evaluate the effect of different amounts of incubated samples on the kinetic parameters of *in vitro* fermentation of roughage and concentrated food used for feeding ruminants.

Material and Methods

Characterization and chemical analysis of food

The food used for analysis was chosen owing to its high availability and frequency of use in ruminant nutrition in Mato Grosso, Brazil. The roughage foods were sugarcane *in natura*, Napier grass silage, corn silage, and Marandu grass silage; the concentrated foods were dried corn distillers' grains with solubles, soybean meal, castor meal, dried brewer's yeast, bean residue, wet brewer's grains, cottonseed meal, sunflower meal, and Jatropha meal. Food was sampled from different regions (Sinop, Cuiabá, and Rondonópolis) from Mato Grosso, Brazil. Some regional foods could be used for making only one sample, whereas three samples were obtained for less regionalized food, which was homogenized to form a composite sample. The incubations were performed at the Laboratory of Animal Nutrition of the Federal University of Mato Grosso Campus Cuiabá, MT, Brazil.

All samples were analyzed according to the standard analysis procedures of the Brazilian National Institute of Science and Technology on Animal Science (INCT-CA; Detmann et al., 2012). The samples of roughage foods were pre-dried in a convection oven at 55 ºC for 72 h to obtain dry air samples (INCT-CA G-001/1). Next, all the feeds were oven-dried at 105 ºC to determine the final dry matter (INCT-CA G-003/1). They were then milled using a 1 mm sieve to analyze crude protein (INCT-CA N-001/1), mineral matter (INCT-CA M-001/1), ether extract (INCT-CA G-004/1), neutral detergent fiber (INCT-CA F-002/1), and acid detergent fiber (INCT-CA F-004/1). Total carbohydrates (TCs) were calculated according to Sniffen, O'Connor, Van Soest, Fox and Russell (1992). The indigestible neutral detergent fiber (iNDF) of the food was obtained using *in situ* incubation for 240 h (Casali et al., 2008), followed by analysis of NDF. Potentially fermentable organic matter (pFOM) was estimated using the equation: 1000 - (MM + EE + iNDF), and the fraction of total potentially digestible carbohydrates (tpDCs) was estimated using the equation: NFC * 0.98 + NDFap - iNDF. The chemical composition of food is described in Table 1.

Table 1
Chemical composition of the concentrated and roughage food[1]

Food	DM	OM	CP	EE	pFOM	TC	NFC	NDFap	tpDC
Roughage									
Sugarcane *in natura*	300.05	965.50	23.70	23.10	733.40	919.80	522.80	397.00	700.24
Napier grass silage	180.00	891.90	65.30	42.30	601.20	784.20	200.80	583.40	531.68
Marandu grass silage	263.50	936.40	65.00	48.70	702.20	822.60	193.20	629.40	633.13
Corn silage	320.50	943.30	59.20	20.20	772.20	863.90	317.60	546.30	706.64
Concentrated									
Castor meal	919.90	905.90	493.40	33.70	585.70	378.60	14.00	364.60	91.72
Soybean meal	886.70	933.10	538.10	21.00	896.40	373.90	149.10	74.20	204.61
Dried corn distillers' grains with solubles	921.10	986.10	326.00	76.50	879.10	583.70	97.80	131.30	196.54
Dried brewer's yeast	911.20	952.50	273.90	51.80	694.40	626.70	120.10	506.60	417.89
Bean residue	899.40	958.10	207.00	24.90	919.60	726.10	536.00	190.10	701.68
Wet brewer's grains	915.80	967.60	260.30	93.00	665.40	614.20	107.90	506.30	402.74
Cottonseed meal	935.30	954.10	306.90	142.30	565.90	504.80	146.50	358.30	255.87
Sunflower meal	917.20	941.80	252.30	94.80	813.60	594.60	208.70	385.90	556.92
Jatropha meal	904.60	934.30	328.40	10.70	527.40	595.30	157.40	437.90	195.85

[1]DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; pFOM: Potentially fermentable organic matter; TC: total carbohydrates; NFC: non-fibrous carbohydrates; NDFap: neutral detergent fiber corrected for ash and protein; tpDC: total potentially digestible carbohydrates.

Gas production and kinetic parameters of ruminal fermentation

Samples for the *in vitro* incubations were prepared using the gas production technique by using 200, 300, 400, and 500 mg of air-dried sample ground to 1 mm. The samples were then placed in 120 mL amber glass flasks. To the flasks, 20 mL of 16 mL McDougal buffer solution + 4 mL inoculum, 30 mL of 24 mL McDougal buffer solution + 6 mL inoculum, 40 mL of 32 mL McDougal buffer + 8 mL inoculum, or 50 mL of 40 mL McDougal buffer solution + 10 mL inoculum was added. The McDougal buffer solution was previously reduced with CO_2 (pH 6.9–7.0), according to McDougal (1949). The inoculum was obtained from a fistulated dairy cow fed 2 kg of concentrate per day.

Immediately, the flasks were covered with rubber cap and aluminum seal and placed in a water bath at 39 °C and 45 rpm. Pressure readings (psi, pressure per square inch) were obtained using a pressure transducer (Datalogger Pressure®) at 2, 4, 6, 8, 10, 12, 24, 48, 72, and 96 h. The volume of gases from rumen liquid and buffer solution was measured by incubating two flasks without a sample (white). Thus, for each reading time, the volume of gases from the flasks with sample was subtracted from that of the flasks without sample.

Statistical analysis

The sum of the volume of gases for each reading time was used to establish the cumulative production curves of gases. Conversion from psi to mL was performed using the regression equation (Y = a $\pm$ bx), in which the coefficient "b" of the equation enabled correction and transformation of pressure (psi) into the volume of gases (mL) corrected for barometric pressure of the day. For this, a known volume of gases was injected into flasks kept under the same conditions as those of incubated samples. For each relation of the sample tested, a curve was generated according to the pressures corresponding to the injected volumes (20, 30, 40, and 50 mL), and the same measures were used to obtain the regression equation between gas pressure and volume.

The kinetics of cumulative gas production was analyzed using the logistic bi-compartmental model of Schofield, Pitt and Pell (1994): $V(t) = Vf1/(1 + \exp(2 - 4*c1*(T - L))) + Vf2/(1 + \exp(2 - 4*c2*(T - L)))$, where V(t) is the cumulative volume at time t; Vf1, the final volume of gases of the fast degradation fraction; *c1* (h^{-1}), the degradation rate of the fast degradation fraction; Vf2, the final volume of gases of the slow degradation fraction; *c2* (h^{-1}), the degradation rate of the slow degradation fraction; L, the lag time; and T, time (h).

The studied variables were analyzed using the MIXED procedure of SAS, version 9.2. Pearson's correlation coefficient analyses were performed between the chemical composition of the food and the total gas production as a function of the different amounts of incubated sample. The parameters were analyzed using *F* test in ANOVA. The LSMEANS option was used to generate the individual means for each treatment. After the quantitative factors were obtained, orthogonal contrasts were used for specifically partitioning the effects into linear, quadratic, or cubic. The effects were considered significant when $P < 0.05$.

Results and Discussion

Correlation coefficients between the chemical composition of food and total gas production as a function of the different amounts of incubated samples

The total gas production of the foods as a function of the different amounts of incubated sample showed a significant correlation ($P < 0.05$) of 0.7667, 0.6305, 0.7405, and 0.7449 with pFOM, TCs, tpDCs, and NFCs, respectively. No significant effect ($P > 0.05$) was noted for the correlation between total gas production and organic matter (OM), crude protein (CP), ether extract (EE), and neutral detergent fiber corrected for ash and protein (NDFap), which averaged 0.3914, -0.5204, -0.3638, and 0.1493, respectively (Table 2).

Table 2
Correlation coefficients between the chemical composition of the food and total gas production as a function of the different amounts of incubated samples

Variables[1]	PGT200	PGT300	PGT400	PGT500
OM	0.3887	0.3982	0.3839	0.3951
pFOM	0.7856*	0.7703*	0.7349*	0.7760*
CP	-0.4943	-0.4989	-0.5431	-0.5456
EE	-0.3869	-0.3683	-0.3924	-0.3079
BC	0.6106*	0.6122*	0.6572*	0.6421*
tpDC	0.7267*	0.7108*	0.7366*	0.7880*
NFC	0.7449*	0.7657*	0.7638*	0.7053*
NDFap	-0.1768	-0.2070	-0.1418	-0.0716

[1]DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; pFOM: Potentially fermentable organic matter; TC: total carbohydrates; NFC: non-fibrous carbohydrates; NDFap: neutral detergent corrected for ash and protein; tpDC: potentially digestible carbohydrates.

Getachew, Robinson, DePeters and Taylor (2004) evaluated the correlation between the chemical composition of different foods and the extent of gas production; their results were considerably similar to ours. They found that the protein content and available food protein were correlated negatively, whereas the NFC content was correlated positively with gas production at 6, 24, and 48 h of incubation.

The in vitro kinetic parameters

Roughage feeds

Gas production from the degradation of NFCs showed an linear increasing effect ($P < 0.05$) for *in natura* sugarcane, Marandu grass silage, and corn silage, whereas quadratic effect ($P < 0.05$) for Napier grass silage, according to the different amounts of incubated sample (Table 3). The degradation rate of NFC fraction showed a linear effect ($P < 0.05$) for *in natura* sugarcane and Napier grass silage, whereas a quadratic effect ($P < 0.05$) for Marandu grass silage and a cubic effect ($P < 0.05$) for corn silage.

Gas production from the degradation of fibrous carbohydrates showed a cubic effect ($P < 0.05$) for *in natura* sugarcane and an increasing linear effect ($P < 0.05$) for Napier grass silage, Marandu grass silage, and corn silage, according to the different amounts of incubated sample. The degradation rate of the NFC fraction showed a cubic effect ($P < 0.05$) for *in natura* sugarcane and Marandu grass silage, whereas a decreasing linear effect for Napier grass silage and a quadratic effect ($P < 0.05$) for corn silage (Table 3).

The total gas production showed a cubic effect ($P < 0.05$) for *in natura* sugarcane, Marandu grass silage, and corn silage, whereas a linear effect ($P < 0.05$) for Marandu grass silage as a function of the different amounts of incubated sample. The *lag time* was also affected by the different amounts of incubated sample, with a quadratic effect ($P < 0.05$) for Napier grass silage and a cubic effect ($P < 0.05$) for *in natura* sugarcane, Marandu grass silage, and corn silage (Table 3).

Similar to our results, Campos, Lanna, Bose and Boin (2000) varied the amount of incubated alfalfa hay between 50 and 110 mg and observed a quadratic behavior for gas production as a function of the amount of incubated sample. They also observed an alteration in the final pH, which was reduced when larger amounts of incubated samples were used after 48 h of incubation and cautioned against the use of large amounts of incubated sample as the reduction of pH may change the fermentation process once the buffer solution has no effect on the buffering of the medium.

In contrast, Theodorou et al. (1994), who used roughage amounts ranging from 200 to 2000 mg incubated with 100 mL of mixed culture in 125 mL bottles, observed a linear increase in the total gas production; however, no effect was noted on the lag time and degradation rates.

Gas production depending on available headspace showed an increasing linear effect ($P < 0.05$) for *in natura* sugarcane and Napier grass silage and a quadratic effect ($P < 0.05$) for Marandu grass silage and corn silage as a function of the amount of incubated sample. The production of gases per milligram of incubated sample showed a linear reduction ($P < 0.05$) for *in natura* sugarcane, Napier grass silage, and corn silage and a quadratic effect ($P < 0.05$) for Marandu grass silage. Gas production as a function of the amount of tpDCs and pFOM showed a quadratic effect ($P < 0.05$) for *in natura* sugarcane and an increasing linear effect for Napier grass silage, Marandu grass silage, and corn silage as a function of the amount of incubated sample (Table 4).

Table 3
Production of gases from the fraction of non-fibrous carbohydrates and fibrous carbohydrates, fraction degradation rate of non-fibrous carbohydrates and fibrous carbohydrate, total gas production and lag time as a function of the amount of incubated sample from roughage foods

Food	Amount of incubated sample (mg)				SEM[1]	*P* value*		
	200	300	400	500		Linear	Quadratic	Cubic
Gas production from degradation of the non-fibrous carbohydrate fraction (mL)								
Sugarcane *in natura*	17.36	25.64	31.76	38.62	0.36	<0.0001	0.1212	0.1497
Napier grass silage	16.56	23.93	34.51	44.53	0.47	<0.0001	0.0489	0.1507
Marandu grass silage	20.22	29.42	38.54	41.42	1.22	0.0002	0.0615	0.3248
Corn silage	27.30	37.00	37.42	40.87	1.54	0.0040	0.1136	0.1497
Degradation rate of the non-fibrous carbohydrate fraction (h^{-1})								
Sugarcane *in natura*	0.3492	0.3140	0.2109	0.2326	0.01	0.0058	0.2065	0.0845
Napier grass silage	0.0876	0.0715	0.0580	0.0398	0.00	0.0010	0.8069	0.7055
Marandu grass silage	0.0772	0.0596	0.0613	0.0588	0.00	0.0056	0.0265	0.0773
Corn silage	0.0775	0.0717	0.0595	0.1485	0.00	<0.0001	<0.0001	0.0009
Gas production from degradation of the fibrous carbohydrate fraction (mL)								
Sugarcane *in natura*	41.60	56.82	72.81	78.93	0.73	<0.0001	0.0035	0.0322
Napier grass silage	22.46	30.35	37.32	39.05	1.98	0.0031	0.1953	0.6515
Marandu grass silage	33.92	40.39	53.55	67.83	1.83	0.0002	0.1006	0.5343
Corn silage	29.69	43.22	67.72	89.48	2.33	<0.0001	0.1534	0.2604
Degradation rate of the fibrous carbohydrate fraction (h^{-1})								
Sugarcane *in natura*	0.0239	0.0236	0.0211	0.0203	0.00	0.0004	0.5154	0.0360
Napier grass silage	0.0197	0.0200	0.0174	0.0164	0.00	0.0097	0.3202	0.1626
Marandu grass silage	0.0216	0.0173	0.0187	0.0189	0.00	0.0038	0.0009	0.0036
Corn silage	0.0205	0.0183	0.0167	0.0203	0.00	0.3246	0.0021	0.0636
Total gas production (mL)								
Sugarcane *in natura*	58.96	82.46	104.58	117.56	0.91	<0.0001	0.0046	0.1312
Napier grass silage	39.02	54.28	71.83	83.58	1.74	<0.0001	0.37143	0.3583
Marandu grass silage	54.14	69.82	92.10	109.25	1.35	<0.0001	0.6154	0.1253
Corn silage	56.99	80.23	105.14	130.35	1.07	<0.0001	0.4117	0.7897
Lag time (hours)								
Sugarcane *in natura*	3.63	3.57	2.16	2.85	0.14	0.0044	0.0614	0.0059
Napier grass silage	17.01	14.56	12.52	3.39	0.93	0.0005	0.0237	0.1484
Marandu grass silage	14.31	0.1057	13.10	11.47	0.68	0.1230	0.1999	0.0272
Corn silage	14.00	12.72	5.80	9.95	0.11	<0.0001	<0.0001	<0.0001

[1]SEM: standard error of the mean; **P*-value: $P < 0.05$.

Table 4
Gas production as a function of available headspace, amount of sample (mg^{-1}), amount of potentially digestible carbohydrates (tpDC), and potentially fermentable organic matter (pFOM) incubated with roughage foods

Food	Amount of incubated sample (mg)				SEM[1]	*P* value*		
	200	300	400	500		Linear	Quadratic	Cubic
Gas production by available headspace								
Sugarcane *in natura*	0.58	0.91	1.30	1.67	0.01	<0.0001	0.1309	0.1962
Napier grass silage	0.39	0.60	0.89	1.19	0.02	<0.0001	0.1440	0.4761
Marandu grass silage	0.54	0.77	1.15	1.56	0.01	<0.0001	0.0065	0.2286
Corn silage	0.57	0.89	1.31	1.86	0.01	<0.0001	0.0008	0.6898
Gas production mg^{-1} of incubated sample								
Sugarcane *in natura*	0.29	0.27	0.26	0.23	0.00	<0.0001	0.2546	0.1441
Napier grass silage	0.19	0.18	0.17	0.16	0.00	0.0188	0.8687	0.3449
Marandu grass silage	0.27	0.23	0.23	0.21	0.00	0.0014	0.0428	0.0891
Corn silage	0.28	0.26	0.26	0.26	0.00	0.0123	0.1261	0.5871
Gas production mg^{-1} of tpDC								
Sugarcane *in natura*	0.08	0.11	0.14	0.16	0.00	<0.0001	0.0046	0.1312
Napier grass silage	0.07	0.10	0.13	0.15	0.00	<0.0001	0.3713	0.3583
Marandu grass silage	0.08	0.11	0.14	0.17	0.00	<0.0001	0.6154	0.1253
Corn silage	0.08	0.11	0.14	0.18	0.00	<0.0001	0.4117	0.7897
Gas production mg^{-1} of pFOM								
Sugarcane *in natura*	0.08	0.11	0.14	0.16	0.00	<0.0001	0.0046	0.1312
Napier grass silage	0.06	0.09	0.11	0.13	0.00	<0.0001	0.3713	0.3583
Marandu grass silage	0.07	0.09	0.13	0.15	0.00	<0.0001	0.6154	0.1253
Corn silage	0.07	0.10	0.13	0.16	0.00	<0.0001	0.4117	0.7897

[1]SEM: standard error of the mean; *P-value: $P < 0.05$.

Concentrated feeds

Gas production from the degradation of NFCF showed a linear increasing effect ($P < 0.05$) for dried corn distillers' grains with solubles, dried brewer's yeast, bean residue, wet brewer's grains, sunflower meal, and Jatropha meal; quadratic effect ($P < 0.05$) for cottonseed meal; and cubic effect ($P < 0.05$) for castor meal and soybean meal as a function of the amount of incubated sample. The degradation rate of NFC fraction showed a linear decreasing effect ($P < 0.05$) for castor meal and a cubic effect for soybean meal, dried corn distillers' grains with solubles, bean residue, and cottonseed meal, whereas no significant effect ($P > 0.05$) for dried corn distillers' grains with solubles, wet brewer's grain, sunflower meal, and Jatropha meal as a function of the amount of incubated sample (Table 5).

Gas production from the degradation of fibrous carbohydrate fraction showed a linear increasing effect ($P < 0.05$) for dried corn distillers' grains with solubles, bean residue, wet brewer's grains, cottonseed meal, and sunflower meal; quadratic effect ($P < 0.05$) for Jatropha meal; and cubic effect ($P < 0.05$) for castor meal and soybean meal as a function of the amount of incubated sample. The degradation rate of fibrous carbohydrate fraction showed a linear decreasing effect ($P < 0.05$) for dried corn distillers' grains with solubles, dried brewer's yeast, and wet brewer's grains; quadratic effect ($P < 0.05$) for castor meal; and cubic effect for bean residue. However, the amount of incubated sample had no significant effect ($P > 0.05$) on the degradation rate of fibrous carbohydrate fraction for soybean meal, cottonseed meal, sunflower meal, and Jatropha meal (Table 5).

The total gas production showed a linear increasing effect ($P < 0.05$) for almost all concentrated foods evaluated, except for Jatropha meal, which showed a cubic effect ($P < 0.05$) as a function of the different amounts of incubated sample. The *lag time* was also influenced as a function of the different amounts of incubated sample, with a linear decreasing effect for castor meal and dried corn distillers' grains with solubles and a cubic effect ($P < 0.05$) for soybean meal, bean residue, cottonseed meal, sunflower meal, and Jatropha meal.

Ramin and Huhtanen (2012) evaluated the effect of different amounts of samples (300, 600, 900, and 1200 mg) incubated with 60 mL of substrate in 260 mL flasks and observed a linear reduction in the digestibility of the fractions as the amount of incubated sample increased. Such behavior was justified because of the saturation of the medium with volatile fatty acids, which reduced the pH and then precluded digestibility. Notably, in their study, the amount of buffered rumen fluid was the same for all treatments (60 mL); in contrast, in the present study, the buffered ruminal fluid varied according to the amount of incubated sample (20, 30, 40, and 50 mL).

Cone and Van Gelder (1996) tested different concentrations of a corn by-product and found that, after 10 h of incubation, the medium was saturated when 700 and 900 mg of corn by-product was incubated with 60 mL of mixed solution of ruminal liquid and buffer solution in a 1:2 ratio, unlike when 300 and 500 mg of corn by-product was used, which led to the production of higher amounts of gases because they showed a more favorable medium for fermentation.

The production of gases depending on available headspace showed a linear increasing effect ($P < 0.05$) for castor meal, soybean meal, dried brewer's yeast, cottonseed meal, and sunflower meal; quadratic effect ($P < 0.05$) for dried corn distillers' grains with solubles, bean residue, and wet brewer's grains; and cubic effect ($P < 0.05$) for Jatropha meal as a function of the amount of incubated sample. The production of gases per milligram of incubated sample showed a linear reduction ($P < 0.05$) for castor meal, soybean meal, dried corn distillers' grains with solubles, and bean residue; quadratic effect ($P < 0.05$) for wet brewer's grains; and non-significant effect ($P > 0.05$) for dried brewer's yeast and cottonseed meal. Gas production as a function of the amount of tpDCs and pFOM showed an increasing linear effect ($P < 0.05$) for almost all concentrated foods, except for Jatropha meal, which showed a quadratic effect ($P < 0.05$; Table 6).

Table 5
Production of gases from the fraction of non-fibrous carbohydrates and fibrous carbohydrates, fraction degradation rate of non-fibrous carbohydrates and fibrous carbohydrates, total gas production, and lag time as a function of the amount of incubated sample from concentrated foods

Food	Amount of incubated sample (mg)				SEM[1]	*P* value*		
	200	300	400	500		Linear	Quadratic	Cubic
Gas production from degradation of the non-fibrous carbohydrate fraction (mL)								
Castor meal	10.43	16.35	18.09	30.64	0.73	<0.0001	0.0110	0.0105
Soybean meal	19.97	29.15	31.66	49.15	0.70	<0.0001	0.6522	0.0160
Dried corn distillers' grains with solubles	11.74	16.91	24.47	28.86	1.33	0.0006	0.7846	0.4043
Dried brewer's yeast	10.59	13.39	16.28	26.09	1.72	0.0030	0.1115	0.4245
Bean residue	41.97	54.49	68.01	78.72	0.45	<0.0001	0.1174	0.1327
Wet brewer's grain	9.09	13.45	17.26	21.76	0.71	0.0002	0.9323	0.7193
Cottonseed meal	6.99	14.85	25.17	23.45	1.62	0.0012	0.0421	0.1169
Sunflower meal	12.20	19.81	19.76	42.71	4.32	0.0091	0.1508	0.1883
Jatropha meal	10.44	12.13	17.11	28.20	2.24	0.0044	0.1039	0.7916
Degradation rate of the non-fibrous carbohydrate fraction (h^{-1})								
Castor meal	0.1506	0.1183	0.1127	0.0887	0.00	0.0073	0.6480	0.2993
Soybean meal	0.0859	0.0915	0.1140	0.1152	0.00	<0.0001	0.1821	0.0034
Dried corn distillers' grains with solubles	0.1562	0.1475	0.0667	0.0833	0.01	0.0073	0.3949	0.0465
Dried brewer's yeast	0.1033	0.1119	0.1312	0.1021	0.01	0.8118	0.2396	0.3882
Bean residue	0.0533	0.0590	0.0510	0.0516	0.00	0.0458	0.0712	0.0086
Wet brewer's grain	0.1261	0.1136	0.0973	0.1310	0.00	0.9729	0.0736	0.2786
Cottonseed meal	0.1900	0.1248	0.0740	0.1312	0.00	0.0002	0.0001	0.0062
Sunflower meal	0.1533	0.1311	0.1820	0.1236	0.02	0.7185	0.4584	0.1378
Jatropha meal	0.1325	0.1358	0.1064	0.0789	0.02	0.1381	0.5410	0.7535
Gas production from degradation of the fibrous carbohydrate fraction (mL)								
Castor meal	14.81	18.62	26.11	19.86	1.09	0.0098	0.0100	0.0236
Soybean meal	27.83	36.06	48.91	52.67	0.81	<0.0001	0.0526	0.0200
Dried corn distillers' grains with solubles	39.35	53.91	65.50	76.28	1.29	<0.0001	0.2188	0.7264
Dried brewer's yeast	30.04	38.81	55.79	73.70	12.29	0.0545	0.7288	0.9011
Bean residue	27.20	38.99	51.05	61.16	1.50	<0.0001	0.6064	0.7568
Wet brewer's grain	30.12	38.94	49.42	62.35	1.25	<0.0001	0.1769	0.8949
Cottonseed meal	16.38	21.52	21.22	27.62	1.94	0.0183	0.7619	0.2347
Sunflower meal	27.67	31.04	43.97	54.39	3.68	0.0049	0.3934	0.5046
Jatropha meal	14.24	20.03	27.48	20.58	1.99	0.0413	0.0335	0.1471

continue

continuation

Degradation rate of the fibrous carbohydrate fraction (h^{-1})								
Castor meal	0.0239	0.0218	0.0213	0.0255	0.00	0.2148	0.0092	0.3313
Soybean meal	0.0220	0.0218	0.0214	0.0232	0.00	0.2059	0.0972	0.3097
Dried corn distillers' grains with solubles	0.0250	0.0239	0.0196	0.0192	0.00	0.0016	0.6153	0.0666
Dried brewer's yeast	0.0172	0.0173	0.0176	0.0138	0.00	0.0337	0.0519	0.2573
Bean residue	0.0189	0.0227	0.0172	0.0179	0.00	0.0250	0.0495	0.0031
Wet brewer's grain	0.0180	0.0167	0.0155	0.0158	0.00	0.0120	0.1088	0.4963
Cottonseed meal	0.0240	0.0214	0.0146	0.0206	0.00	0.1619	0.1239	0.1581
Sunflower meal	0.0251	0.0254	0.0258	0.0251	0.00	0.9713	0.7892	0.8667
Jatropha meal	0.0286	0.0320	0.0220	0.0286	0.00	0.4366	0.5774	0.0635
Total gas production (mL)								
Castor meal	25.24	34.98	44.20	50.50	1.10	<0.0001	0.1955	0.6521
Soybean meal	47.81	65.21	80.57	92.83	1.32	<0.0001	0.1245	0.8650
Dried corn distillers' grains with solubles	51.10	70.83	89.97	105.15	1.22	<0.0001	0.1355	0.5688
Dried brewer's yeast	40.63	52.20	72.07	99.80	10.70	0.0146	0.4928	0.9931
Bean residue	69.18	93.48	119.06	139.89	1.23	<0.0001	0.2320	0.3357
Wet brewer's grain	39.21	52.40	66.69	84.11	0.79	<0.0001	0.0555	0.5975
Cottonseed meal	23.37	36.37	46.39	51.08	3.38	0.0035	0.2865	0.8839
Sunflower meal	39.87	50.85	63.74	97.11	7.04	0.0042	0.1871	0.5873
Jatropha meal	24.69	32.17	44.59	48.78	0.36	<0.0001	0.0106	0.0013
Lag time (hours)								
Castor meal	4.35	4.93	2.33	1.26	0.75	0.0249	0.3378	0.2372
Soybean meal	4.13	4.15	5.17	4.89	0.16	0.0110	0.4203	0.0359
Dried corn distillers' grains with solubles	10.41	10.20	5.16	5.89	1.11	0.0202	0.6962	0.1005
Dried brewer's yeast	5.78	5.86	7.10	3.63	1.17	0.3803	0.2061	0.3284
Bean residue	6.38	7.00	3.83	4.55	0.32	0.0041	0.8860	0.0063
Wet brewer's grain	7.46	6.78	4.46	6.83	0.68	0.2438	0.0909	0.1081
Cottonseed meal	4.29	3.45	0.3	4.56	0.20	0.0940	0.0017	0.0033
Sunflower meal	5.77	2.88	4.45	2.45	0.58	0.0327	0.4862	0.0372
Jatropha meal	4.80	5.41	1.49	0.16	0.21	<0.0001	0.0111	0.0019

[1]SEM: standard error of the mean; **P*-value: $P < 0.05$.

Table 6
Gas production as a function of available headspace, amount of sample (mg-1), amount of potentially digestible carbohydrates (tpDC), and potentially fermentable organic matter (pFOM) incubated with concentrated foods

Food	Amount of incubated sample (mg)				SEM[1]	*P* value*		
	200	300	400	500		Linear	Quadratic	Cubic
Gas production by available headspace								
Castor meal	0.25	0.38	0.55	0.72	0.01	<0.0001	0.3226	0.7431
Soybean meal	0.47	0.72	1.00	1.32	0.01	<0.0001	0.0878	0.9990
Dried corn distillers' grains with solubles	0.51	0.78	1.12	1.50	0.01	<0.0001	0.0263	0.7561
Dried brewer's yeast	0.40	0.58	0.90	1.42	0.15	0.0078	0.3143	0.9378
Bean residue	0.69	1.03	1.48	1.99	0.01	<0.0001	0.0061	0.5718
Wet brewer's grain	0.39	0.58	0.83	1.20	0.00	<0.0001	0.0006	0.2496
Cottonseed meal	0.23	0.40	0.58	0.72	0.04	0.0012	0.8300	0.8843
Sunflower meal	0.39	0.56	0.79	1.38	0.09	0.0020	0.9930	0.5450
Jatropha meal	0.24	0.35	0.55	0.69	0.00	<0.0001	0.0291	0.0015
Gas production mg^{-1} of incubated sample								
Castor meal	0.12	0.11	0.11	0.10	0.00	0.0027	0.9867	0.6030
Soybean meal	0.23	0.21	0.20	0.18	0.00	0.0005	0.4890	0.7630
Dried corn distillers' grains with solubles	0.25	0.23	0.22	0.21	0.00	0.0009	0.5566	0.5199
Dried brewer's yeast	0.20	0.17	0.18	0.19	0.02	0.9654	0.3258	0.8313
Bean residue	0.34	0.31	0.29	0.27	0.00	0.0001	0.0680	0.1760
Wet brewer's grain	0.19	0.17	0.16	0.16	0.00	0.0023	0.0182	0.7772
Cottonseed meal	0.11	0.12	0.11	0.10	0.00	0.2399	0.3193	0.9779
Sunflower meal	0.19	0.16	0.15	0.19	0.01	0.7287	0.1033	0.7319
Jatropha meal	0.12	0.10	0.11	0.09	0.00	0.0001	0.3594	0.0014
Gas production mg^{-1} of tpDC								
Castor meal	0.27	0.38	0.48	0.55	0.01	<0.0001	0.1955	0.6521
Soybean meal	0.23	0.31	0.39	0.45	0.06	<0.0001	0.1245	0.8650
Dried corn distillers' grains with solubles	0.26	0.36	0.45	0.53	0.00	<0.0001	0.1355	0.5688
Dried brewer's yeast	0.09	0.12	0.17	0.23	0.02	0.0146	0.4928	0.9931
Bean residue	0.09	0.13	0.16	0.19	0.00	<0.0001	0.2320	0.3357
Wet brewer's grain	0.09	0.13	0.16	0.20	0.00	<0.0001	0.0555	0.5975
Cottonseed meal	0.09	0.14	0.18	0.19	0.01	0.0035	0.2865	0.8839
Sunflower meal	0.07	0.09	0.11	0.17	0.01	0.0042	0.1871	0.5873
Jatropha meal	0.12	0.16	0.22	0.24	0.00	<0.0001	0.0106	0.0013
Gas production mg^{-1} of pFOM								
Castor meal	0.04	0.05	0.07	0.08	0.00	<0.0001	0.1955	0.6521
Soybean meal	0.05	0.07	0.08	0.10	0.00	<0.0001	0.1245	0.8650
Dried corn distillers' grains with solubles	0.05	0.08	0.10	0.11	0.00	<0.0001	0.1355	0.5688
Dried brewer's yeast	0.05	0.07	0.10	0.14	0.01	0.0146	0.4928	0.9931
Bean residue	0.07	0.10	0.12	0.15	0.00	<0.0001	0.2320	0.3357
Wet brewer's grain	0.05	0.07	0.10	0.12	0.00	<0.0001	0.0555	0.5975
Cottonseed meal	0.04	0.06	0.08	0.09	0.00	0.0035	0.2865	0.8839
Sunflower meal	0.04	0.06	0.07	0.11	0.00	0.0042	0.1871	0.5873
Jatropha meal	0.04	0.06	0.08	0.09	0.00	<0.0001	0.0106	0.0013

[1]SEM: standard error of the mean; **P*-value: $P < 0.05$.

Conclusion

Fermentation parameters of roughage and concentrated foods were influenced as a function of the amount of incubated sample. However, as the total gas production was correlated with potentially fermentable organic matter and the content of potentially digestible carbohydrates, the fermentation pattern was likely not influenced by increasing the amount of incubated sample. Another concern was that the headspace could prevent the storage of gases, affecting the fermentation of food; however, for most foods, gas production by available headspace showed linear increase, and thus was not affected by the amount of incubated sample.

References

Campos, F. P., Lanna, D. P. D., Bose, M. L. V., & Boin, C. (2000). Avaliação do Sistema de Monitoramento Computadorizado de Digestão *in vitro*. 1. Testes Preliminares. *Revista Brasileira de Zootecnia*, *29*(2), 525-530. doi: 10.1590/S1516-35982000000200028

Casali, A. O., Detmann, E., Valadares, S. C., Fº, Pereira, J. C., Henriques, L. T., Freitas, S. G., & Paulino, M. F. (2008). Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimento *in situ*. *Revista Brasileira de Zootecnia*, *37*(2), 335-342. doi: 10.1590/S1516-35982008000200021

Cone, J. W., & Van Gelder, A. H. (1996). Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. *Animal Feed Science and Technology*, *61*(1-4), 113-128. doi: 10.1016/0377-8401(96)00950-9

Detmann, E., Souza, M. A., Valadares, S. C., Fº, Queiroz, A. C., Berchielli, T. T., Saliba, E. O. E.,... Azevedo, J. A. G. (2012). *Métodos para análise de alimentos.* Viçosa, Minas Gerais: Universidade Federal de Viçosa.

Getachew, G., Blummel, M., Makkar, H. P. S., & Becker, K. (1998). *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. *Animal Feed Science and Technology*, *72*(3), 261-281. doi: 10.1016/S0377-8401(97)00189-2

Getachew, G., Robinson, P. H., DePeters, E. J., & Taylor, S. J. (2004). Relationships between chemical composition, dry matter degradation and in vitro gas production of several ruminant feeds. *Animal Feed Science and Technology*, *111*(1), 57-71. doi: 10.1016/S0377-8401(03)00217-7

Goering, H. K., & Van Soest, P. J. (1970). *Forage fiber analysis: Apparatus, reagents, procedures and some applications.* Washington: USDA.

Goes, R. H. T. E B., Souza, K. A., Patussi, R. A., Cornelio, T. C., Oliveira, E. R., & Brabes, K. C. S. (2010). Degradabilidade in situ dos grãos de crambe, girassol e soja, e de seus coprodutos em ovinos. *Acta Scientiarum*, *32*(3), 271-277. doi: 10.4025/actascianimsci.v32i3.7913

Groot, J. C. J., Cone, J. W., Willians, B. A., Debersaques, F. M. A., & Lantinga, E. A. (1996). Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. *Animal Feed Science and Technology*, *64*(1), 77-89. doi: 10.1016/S0377-8401(96)01012-7

Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D., & Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor in vitro. *The Journal of Agricultural Science*, *93*(1), 217-222. doi: 10.1017/S0021859600086305

McDougal, E. I. (1949). Studies on ruminal saliva. 1. The composition and output of sheep's saliva. *Biochemical Journal*, *43*(1), 99-109. doi: 10.1042/bj0430099

Ramin, M., & Huhtanen, P. (2012). Development of an in vitro method for determination of methane production kinetics using a fully automated in vitro gas system A modelling approach. *Animal Feed Science and Technology*, *174*(4), 190200. doi: 10.1016/j.anifeedsci.2012.03.008

Rymer, C., Huntington, J. A., Williams, B. A., & Givens, D. I. (2005). In vitro cumulative gas production techniques: History, methodological considerations and challenges. *Animal Feed Science and Technology*, *123-124*, 9-30. doi: 10.1016/j.anifeedsci.2005.04.055

Santo, A. X., Silva, L. D. F., Lançanova, J. A. C., Ribeiro, E. L. A., Mizubuti, I. Y., Fortaleza, A. P. S.,... Massaro, F. L., Jr. (2017). Fracionamento de carboidratos e proteínas, cinética de degradação ruminal in vitro pela técnica de produção de gás, de rações suplementares contendo torta de girassol.

Arquivo Brasileiro de Medicina Veterinaria e Zootecnia, *69*(1), 234-242. doi: 10.1590/1678-4162-8761

Statistical Analysis System. SAS/STAT Software Version 9.2. Cary: SAS Institute Inc., 2008.

Schofield, P., Pitt, R. E., & Pell, A. N. (1994). Kinetics of fiber digestion from in-vitro gas-production. *Journal of Animal Science*, *72*(11), 2980-2991. doi: 10.2527/1994.72112980x

Sniffen, C. J., O'Connor, D. J., Van Soest, P. J., Fox. D. G., & Russell, J. B. (1992). A net carbohydrate and protein system for evaluating cattle diets: carbohydrate and protein availability. *Journal of Animal Science*, *70*(11), 3562-3577. doi: 10.2527/1992.70113562x

Theodorou, M. K., Williams, B. A., Dhanoa, M. S., McAllan, A. B., & France, J. (1994). A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology*, *48*(3-4), 185-197. doi: 10.1016/0377-8401(94)90171-6