Research Article

Received: 5 December 2019 Revised: 31 January 2020 Accepted article published: 21 February 2020 Published online in Wiley Online Library: 11 March 2020

(wileyonlinelibrary.com) DOI 10.1002/jsfa.10342

Quantifying non-fibrous carbohydrates, acid detergent fiber and cellulose of forage through an *in vitro* gas production technique

Luis Alberto Miranda-Romero,[a†] Deli Nazmín Tirado-González,[b,d†] Gustavo Tirado-Estrada,[b†] Ricardo Améndola-Massiotti,[a] Laura Sandoval-González,[a] Rodolfo Ramírez-Valverde[a] and Abdelfattah ZM Salem[c*]

Abstract

BACKGROUND: The *in vitro* gas production (GP) technique has been useful for evaluating the potential degradability of feedstuffs in ruminal environments; GP is related to the components of feedstuff ingredients.

RESULTS: Linear models were generated and validated as alternatives of quantifying neutral detergent-soluble fiber, starch (St)/hemicellulose (Hem) and cellulose (Cel) through GP. Residuals of models obtained from the peaks of GP [0–8 h (GP-8), > 8–24 h (GP-24), > 24–48 h (GP-48) and > 24–81 h (GP-81)] of 0.02, 0.04, 0.08, 0.12 and 0.20 g of glucose (Glu), St and Cel respectively. The incubations were analyzed in mixtures of Glu, St and Cel. The best fitting models (r^2 from 0.709 to 0.935) were tested on corn stover (CS) to quantify rapid fermentation fractions (RF; equivalent to Glu), medium fermentation fractions (MF; equivalent to St) and low fermentation fractions (LF48; equivalent to Cel); in CS, RF, MF and LF models had standardized residuals < 0.09. The analysis with Leucaena (*Leucaena leucocephala* Lam. de Wit) and star grass (*Cynodon nlemfuensis* Vanderyst) consider high-protein ingredients.

CONCLUSION: The *in vitro* GP of RF, MF and LF48 fractions equivalent to Glu, St and Cel are affected by maturity and harvest time even when the chemical composition remains similar, and so RF, MF and LF48 should be considered during the design of ruminant diets. *In vitro* GP could be used to quantify the components of some forages, although further studies are necessary.

Keywords: ruminal fermentation; cell walls; forages; linear models; residuals analysis

INTRODUCTION

The quantities and degradability of nonstructural carbohydrates and neutral detergent fiber (NDF) affect the ruminal microorganisms and endogenous enzymes, the production and kinetids of gas during fermentation, and ruminal pH as well as temperature.[1–4]

In the rumen, high-quality forages, grains and concentrates increase the propionate production, and the ions of H^+ that form methane (CH_4) reduce in correlation with the proportion of nonstructural carbohydrates, improving the rate of microbial growth and microbial protein synthesis, as well as milk and meat production.[1,5] By contrast, low-quality forages increase the production of acetate, H^+ ions and CH_4 emissions[6–9]; therefore, feed utilization is related to ruminal fermentation kinetics and is a potential predictor of ruminant productivity and biogas emissions.[10–13]

Previous studies have related the kinetics of gas production (GP) and fermentation patterns to diet composition,[9,14,15] degradability[16,17] and animal productivity.[18]

Logistic and exponential models of *in vitro* systems have considered one or more poolsof degradability and GP peaks[19–22] to

* *Correspondence to: A Z M Salem, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Edo. de México, México. E-mail: salem@uaemex.mx; asalem70@yahoo.com*

† *These authors contributed equally to this work.*

a *Posgrado en Producción Animal, Departamento de Zootecnia, Universidad Autónoma Chapingo (UACh), Chapingo, México*

b *División de Estudios de Posgrado e Investigación (DEPI), Tecnológico Nacional de México/Instituto Tecnológico El Llano Aguascalientes, Aguascalientes, México*

c *Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Edo. de México, México*

d *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP)/Centro Nacional de Investigación Disciplinaria en Agricultura Familiar (CENID-AF), Ojuelos, Jalisco, México*

obtain useful parameters for the kinetics of fermentation in a comparison of different ingredients in ruminal diets.[2,23] Using *in vitro* GP to estimate the proportions of NDF, acid detergent fiber (ADF), starch (St)/hemicellulose (Hem) and cellulose (Cel) in forage ingredients would be an alternative application of the technique that could reduce the costs of analysis.

The present study aimed to model, test and validate the relationships of nonstructural carbohydrates, NDF, Hem and Cel with the *in vitro* GP as an alternative test the nutritional quality of forages.

MATERIALS AND METHODS

Substrates

The substrates used in the present study include a glucose standard (Glu) (Ricca Chemical Company), starch from maize (St) (MilliporeSigma, Burlington. MA, USA) and microcrystalline cellulose (5–40 μm) (Cel) (MilliporeSigma).

Hybrids of corn stover (CS) (subtropical germplasm) were harvested at 170 days,[24] and star grass (*Cynodon nlemfuensis* Vanderyst) and Leucaena (*Leucaena leucocephala* Lam. de Wit) were harvested at 35 and 75 days in two different seasons (rainy and dry seasons). CS, star grass and Leucaena were sampled in four places, obtaining four runs (replicates) per site.

Experiments

In vitro experiments were conducted in four phases: (i) doses of 0.02, 0.04, 0.08, 0.12 and 0.20 g of Glu, St and Cel were independently evaluated to obtain simple linear regression models to relate *in vitro* GP from 0 to 8 h, > 8 to 24 h and > 24 h (GP-8 h, GP-24 h, GP-48 h and GP-81 h, respectively) to rapid, medium and low rates of fermentation (RF, MF and LF48, respectively), with doses representing the equivalent contents of Glu, St and Cel in forage; (ii) mixtures of different combinations of 0.05, 0.1 and 0.15 g of Gl, St and Cel were fermented to test the fit of models through residuals analysis; (iii) GP of CS was also used to analyze the fit of models; and (iv) GP was measured in star grass and Leucaena to validate the models.

Chemical analysis

Glu, St and Cel, CS, star grass and Leucaena were dehydrated in an oven at 65 °C for 48 h before analysis to obtain the dry matter (DM). CS samples were dried in a forced air oven at 60 °C until they reached a constant weight (DM); then, samples were ground in a Thomas-Wiley Mill 4 (Thomas Scientific, Swedesboro, NJ, USA) with a 1-mm sieve.

The NDF, ADF and acid detergent lignin (ADL) fractions were analyzed as described by Van Soest *et al.*[25] using the reagents and filter bags of the Ankom filter bag system (Ankom Technology Corp., Macedon, NY, USA). The NDSF, Hem and Cel were calculated by subtracting the NDF from 1000 g DM, ADF from NDF, and ADL from ADF percentages, respectively.

The ash content was obtained in a muffle oven (AR-340, Felisa®, Fabricantes Feligneo S.A. de C.V., Zapopan, Jalisco, Mexico) at 520 °C for 4 h, calcining the samples. Crude protein (CP) and ether were measured according the 992.15 and 920.39 AOAC methods.[26,27]

Fermentation and *in vitro* degradability

Rumen liquid extraction and rumen inoculum preparation

Rumen liquid was extracted from two sheep on the morning and the rumen liquid was filtered with eight layers of gauze and mixed with a reduced mineral solution in a 1 to 9 dilution (v/v; ruminal liquid/mineral solution). The reduced mineral solution contained a solution I [K_2HPO_4 (6 g L^{-1} of water]; solution II [KH_2PO_4 (6 g), $(NH_4)_2SO_4$ (6 g), NaCl (12 g), $MgSO_4$ (2.45 g) and $CaCl_2{\cdot}H_2O$ (1.6 g L^{-1} of water)]; 8% sodium carbonate solution [Na_2CO_3 (8 g/100 mL of water)]; reduction solution [L-cysteine (2.5 g), NaOH 2N (15 mL L^{-1}), Na_2S (2.5 g) and 0.01% resazurin (1 drop per 100 mL of water)].[28] Once the rumen inoculum was prepared, it was placed in a 39 °C water bath with a continuous flow of CO_2.

Rumen fermentation

The gas production technique[16,29] was implemented to analyze different concentrations of individual and mixed Glu, St and Cel, as well as the CS, star grass, and Leucaena fermentations. The experiments were conducted using four runs (replicates) and repeated in two different times. Each sample was placed in a 125-mL amber flask and rumen inoculum (90 mL) was added under a continuous flow of CO_2. Flasks with rumen inoculum but without sample were used as blanks. Flasks were hermetically sealed and placed in a water bath at 39 °C.[29] Flask pressure was measured at 0, 2, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72 and 81 h of incubation with a gauge (0–1 kg cm^2; model 63100; Metron, Texcoco, México) and transformed into gas volume using the equation V = [P(kg/cm^2) + 0.0495]/0.0185.

Rapid, medium and low fermentable fractions

After initiating the *in vitro* incubation, the fermentable fractions were determined by calculating the cumulative GP from 0 to 8 h (GP-8 from RF fractions), from 9 to 24 h (GP-24 h from MF fractions) and from 25 to 48 h [GP-48 h from LF48 fractions).

Statistical analysis

Statistical analysis was performed using the SAS, version 9.2 (SAS Institute Inc., Cary, NC, USA).[30]

Linear regression models

Using the Reg procedure (Proc Reg), simple regression models of GP-8, GP-24, GP-48 from levels of Gl, Starch and Cel fermented by separately were obtained to relate the GP to RF, MF and LF48 fractions, respectively. Each experiment was run four times and the average of parameters $\beta 0$ and βi, the coefficient of determination (r^2) and r^2 adjusted were calculated.

Multiple linear regression models were obtained from the *in vitro* GP of mixtures of Glu, St and Cel [forward of stepwise method (Slentry = 0.15), r^2 adjusted and Mallow's CP are reported].

Residuals analysis

To test the fit of the generated models, they were used to calculate estimations of GP-8, GP-24, and GP-48 from fermentation of the mixtures of Gl, St and Cel, and estimations of RF, MF and LF48 fractions from GP of CS. Estimated values (Yest) were compared against the real values (Yobs) (residuals = Yobs – Yest) to obtain the residuals and calculate mean square errors (MSE), mean absolute errors (MAE), root mean square errors (RMSE), standardized residuals (SR), and correlation and determination coefficients (r and r^2), according Eqns ((1) to ((5).[31]

$$\mathrm{MSE} = \frac{\sum_{i=1}^{n} (Yest - Yobs)^2}{n} \quad (1)$$

$$\mathrm{MAE} = \sum_{i=1}^{n} \frac{|Yest - Yobs|}{n} \quad (2)$$

$$\mathrm{RMSE}=\sqrt{\frac{\sum_{i=1}^{n}(Yest-Yobs)^2}{n}} \quad (3)$$

$$\mathrm{SR}=\sum_{i=1}^{n}\frac{(Yobs-Yest)}{RMSE} \quad (4)$$

$$r(Yobs,Yest)=\frac{\sum(Yobs.Yest)-\frac{(\sum Yobs)(\sum Yest)}{n}}{\sqrt{\sum Yobs^2-\frac{(\sum Yobs)^2}{n}}-\sqrt{\sum Yest^2-\frac{(\sum Yest)^2}{n}}} \quad (5)$$

Variance analysis

The GP-8, GP-24 and GP-48 (RF, MF and LF48, respectively) and model residuals were statistically analyzed using a completely randomized design with a factorial arrangement, according to Models 1 to 4 for independently and combined substrates, and for linear regression model residuals, respectively. Analysis was performed in SAS, version 9.2[30] to obtain the variation and determination (R^2) coefficients. A general linear model procedure (Proc GLM) was implemented and significances were obtained with Proc Mixed (mixed procedure). Adjusted means (LsMeans instruction) were compared through least significant differences (LSD) calculated from the SE obtained from Proc Mixed.

$$Y=\mu+\mathrm{Rep}(T)_{ij}+S_k+D_l+TI_m+(S*D)_{kl}+(S*TI)_{km}+(D*TI)_{lm}+(S*D*TI)_{klm}+\varepsilon_{ijklm} \quad (\text{Model1})$$

where: Y = GP-8 h, GP-24 h, GP-48 h, GP-81 h; $\mathrm{Rep}(T)_{ij}$ = the random effect of the i-th run nested into the j-th time of experiment execution; S_k = the effect of the k-th substrate; D_l = the l-th effect of level of substrate; TI_m = the m-th effect of the incubation time; $(S*D)_{kl}$, $(S*TI)_{km}$, $(D*TI)_{lm}$ and $(S*D*TI)_{klm}$ = interaction between fixed effects; ε_{ijklm}, random error.

$$Y=\mu+\mathrm{Rep}(TI)_{ij}+\mathrm{Starch}_k+\mathrm{Glu}_l+\mathrm{Cel}_m+(\mathrm{Starch*Glu})_{kl}+(\mathrm{Starch*Cel})_{km}+(\mathrm{Glu*Cel})_{lm}+(\mathrm{Starch*Glu*Cel})_{klm}+\varepsilon_{ijklm} \quad (\text{Model2})$$

where: Y = GP 0 to 81 h; $\mathrm{Rep}(TI)_{ij}$ = the random effect of the i-th run nested into the j-th time of incubation time; S_k = the effect of the k-th level of starch; Glu_l = the effect of l-th level of glucose; Cel_m = the effect of the m-th level of cellulose; $(\mathrm{Starch*Glu})_{kl}$, $(\mathrm{Starch*Cel})_{km}$, $(\mathrm{Glu*Cel})_{lm}$ and $(\mathrm{Starch*Glu*Cel})_{klm}$ = interaction between fixed effects; ε_{ijklm} = random error.

$$Y=\mu+\mathrm{Rep}(T)_{ij}+Mo_k+TI_l+(Mo*TI)_{kl}+\beta 1_{(x-xi)}+\varepsilon_{ijk} \quad (\text{Model3})$$

where: Y = GP estimated, RF estimated, MF starch estimated, LF estimated, MSE, MAE, RMSE, r, r^2 and SR; $\mathrm{Rep}(T)_{ij}$ = the random effect of the i-th run nested into j-th time of experiment execution; Mo_k = the fixed effect of k-th Model; TI_l = the fixed effect of the l-th incubation period (0 to 8 h, > 8 to 24 h and > 24 h); $(Mo*TI)_{kl}$ = interaction between fixed effects; $\beta 1_{(x\text{-}xi)}$ = effect of the covariate (Starch:Glu, Cel:Glu, St:Cel ratios); ε_{ijk} = random error.

$$Y=\mu+\mathrm{Rep}(T)_{ij}+TP_k+TH_l+GD_m+(TP*TH)_{kl}+(TP*GD)_{km}+(TH*GD)_{lm}+(TP*TH*GD)_{klm}+\varepsilon_{ijklm} \quad (\text{Model4})$$

where: Y = GP-8, GP-24, GP-48, estimated equivalents of Glu, St, and Cel, ether, ashes, CP, DM, OM, NDF, NDSF, ADF, Hem, Cel and ADL; $\mathrm{Rep}(T)_{ij}$ = the random effect of the i-th run nested into j-th time of experiment execution; TP_k = the fixed effect of the k-th type of plant (SG or LL); TH_l = the fixed effect of the l-th time of harvest (rainy or dry seasons); GD_m = the fixed effect of the m-th growth days; $(TP*TH)_{kl}$, $(TP*GD)_{km}$, $(TH*GD)_{lm}$ and $(TP*TH*GD)_{klm}$ = interaction between fixed effects; ε_{ijklm} = random error.

RESULTS

In vitro gas production of different levels of Glu, St and Cel

Accumulated GP of Glu, St and Cel from 0 to 8 h, after 8 to 24 h and after 24 h

The accumulated GP-8, GP-24 and GP-48 differed among the levels of Glu, St and Cel ($P < 0.0001$) (Table 1). The highest peak of GP was between 8 and 24 h after initiating the incubation (42.34 mL). Regarding the levels of substrates, fermented Glu had higher GP from 0 to 8 h after *in vitro* incubation compared to St and Cel (average 40.80 *versus* 10.93 and 2.65 mL, respectively; $P < 0.0001$), St had the highest GP-24 (average 68.56 *versus* 47.30 from Glu and 11.16 mL from Cel; $P < 0.0001$) and Cel had the highest GP after 24 h (average 58.91 *versus* 15.79 mL from Glu and 20.00 from St; $P < 0.0001$).

Accumulated GP of combinations of different levels of Glu, St and Cel from 0 to 81 h

The GP increased with the increments of Glu and St levels from 0.05 to 0.15 g ($P < 0.0001$) (Table 2); in flasks with Cel, GP tended to be better when 0.05 g was added ($P = 0.085$); the best GP was observed in mixtures with the highest levels of Glu and the lowest of Cel ($P < 0.029$).

The r^2 adjusted and CP Mallows criteria (Table 3), from GP of a combination of substrates, multiple regression models showed that the level of Glu was primarily related to GP-8 and GP-24 (r^2 adjusted = 0.77 and 0.70), although, in both periods, St contributed with 0.13 ± 0.03 of r^2 adjusted. The Mallow's CP criteria from two different valid models obtained for GP-8 and GP-24 included the effects of starch. The multiple linear model to relate GP-48 and GP-81 with fermentable substrates had the lowest r^2 adjusted (r^2 adjusted = 0.32) and included the effects of Cel, St and Glu levels.

Simple linear regression models

Determination coefficients (r^2 and r^2 adjusted) of the models derived from *in vitro* GP-8, GP-24 and GP-48 of different levels of Glu, St and Cel varied from 0.891 to 0.995, and all were considered in further analysis (Table 4). According to simple linear models, 1 mg of Glu, St and Cel increased GP-8 by 0.369, 0.0597 and 0.0163 mL; GP-24 by 0.495, 0.703 and 0.077 mL; and GP-48 by 0.115, 0.126 and 0.252 mL, respectively.

Test and selection of best fitting models

The fit of models was tested through residual analysis of results from the flasks containing mixtures of different levels of Glu, St and Cel.

RF, MF and LF48 fractions models

Estimated values of GP accumulated from 0 to 8 h differed among the models; therefore, some of them had higher residuals (Table 5) ($P < 0.0001$). The model that considered the GP-8 relationship to the Glu level (RF Glu) without the intercept $\beta 0$

Table 1. *In vitro* gas production (GP) of different levels of glucose (Glu), starch and cellulose (Cel) among different incubation periods*

	In vitro GP (mL)† Substrate				
	0.02 g	0.04 g	0.08 g	0.12 g	0.2 g
Incubation 0 to 8 h					
Glucose	10.61	23.42	40.61	56.05	78.33
Starch	3.42	6.05	11.49	15.18	18.51
Cellulose	1.67	1.84	2.02	3.25	4.47
Incubation > 8 to 24 h					
Glucose	10.35	20.7	42.28	64.56	98.6
Starch	15.09	32.81	61.58	90.88	142.46
Cellulose	5.61	6.49	10.35	14.56	18.77
Incubation > 24 to 81 h					
Glucose	1.93	8.07	11.58	18.77	38.6
Starch	4.56	11.05	17.19	26.54	40.53
Cellulose	16.32	32.1	57.89	83.33	104.91
			P-values		
R^2	0.99	TI	< 0.0001	TI*D	< 0.0001
VC (%)	7.06	D	< 0.0001	TI*Subs	< 0.0001
SEM	1.255	Substrate	< 0.0001	D*Subs	< 0.0001
LSD (0.05)	2.088			TI*D*Subs	< 0.0001

*From statistical Model 1.
†GP, gas production; R^2, determination coefficient; VC, variation coefficient; SEM, standard error of the mean; LSD, least significant difference; TI, incubation time (0 to 8 h, > 8 to 24 h, > 24 h); D, levels of substrate (0.02, 0.04, 0.08, 0.12, 0.20 g); Subs, substrates (Glu, St, Cel).

Table 2. *In vitro* gas production (GP) of different combinations of levels of glucose (Glu), starch and cellulose (Cel) from 0 to 81 h of incubation*

	In vitro GP (mL)† Cellulose				
	0.05 g		0.1 g		0.15 g
Glucose 0.05 g					
Starch 0.05 g	35.123		25.549		27.131
Starch 0.10 g	46.872		36.363		36.956
Starch 0.15 g	56.627		47.372		44.176
Glucose 0.1 g					
Starch 0.05 g	34.629		34.731		34.679
Starch 0.10 g	43.079		44.130		39.124
Starch 0.15 g	50.051		50.472		52.426
Glucose 0.15 g					
Starch 0.05 g	42.011		41.991		43.486
Starch 0.10 g	47.994		53.798		51.702
Starch 0.15 g	60.031		51.861		58.134
			P-values		
$R^2 = 0.79$	Glu	< 0.0001		Glu*Starch	0.637
VC = 24.35%	Starch	< 0.0001		Glu*Cel	0.029
SEM = 5.062	Cel	0.085		Starch*Cel	0.813
LSD (0.05) = 8.327				Glu*Starch*Cel	0.842

*From statistical Model 2.
†GP, gas production; R^2, determination coefficient; VC, variation coefficient; SEM, standard error of the mean; LSD, least significant difference; Glu, levels of glucose (0.05, 0.10 and 0.15 g); Starch, levels of starch (0.05, 0.10 and 0.15 g), Cel, levels of cellulose (0.05, 0.10 and 0.15 g).

had the lowest MSE, MAE, RMSE and SR (residuals calculated from Yobs and Yest differences; Model 1 to Model 4) and the best r and r^2, suggesting that Glu primarily composed the RF fraction and that its fermentation explained the most of the GP-8. Similarly, considering MSE, MAE, RMSE, r and r^2 as decision of fitting criteria, the models that relate the GP-24 with St fermentation [MF St

and MF St (β0)] and the GP-48 with Cel fermentation [LF48 Cel and LF48 Cel (β0)] had the best fit ($P < 0.0001$) (Tables 6 and 7), then St, individually or combined with other carbohydrates, is primarily fermented after 8–24 h, and Cel after 24 h.

Estimated values of NSDF, Hem and Cel of CS and residuals

Table 8 shows the estimated values and residuals (MSE, MAE, RMSE and SR; (Model 1 to (Model 4) of RF, MF and LF48 fermented from 0 to 8 h, > 8 to 24 h and > 24 h CS incubation, equivalent to values for fermented Glu, St and Cel obtained from best fitting models [RF Glu, RF Glu (β0), MF St, MF St (β0), LF48 Cel and LF48 Cel (β0)].

The estimated RF were compared against their NDSF value (323.6 ± 15 *versus* 310.3 g kg^{-1} DM) with an acceptable SR (0.36), By assuming that ADF proportion of CS would be primarily produced *in vitro* gas due to Cel fermentation, LF48 Cel and LF48 Cel (β0) model estimations were compared with Cel values (399.5 *versus* 393.7 g kg^{-1} DM) and both (with and without β0) were found to have similar MSE, MAE and RMSE values ($P > 0.54$) and low SR (< 0.22). GP-24 related to St fermentation by MF models was compared with the CS Hem proportion (Hem = NDF – ADF; NDF = 1000 – NDSF) (189.3 ± 3.90 *versus* 226.3 g kg^{-1} DM) and the MF models were found to have higher values of MSE, MAE and RMSE compared to RF and LF48 models (SR > 0.82).

Table 3. Multiple linear models of *in vitro* gas production (GP) from different combination of levels of glucose (Glu), starch and cellulose (Cel) among different incubation periods

In vitro gas production (GP)		
Rapid fermentation (RF): GP from 0 to 8 h		
r^2 adjusted	Mallow's CP	Independent variables
0.86	3.17	Glu Starch
0.77	53.06	Glu
In vitro GP from 0 to 8 h = 10.105 + 0.183 Glu + 0.064 Starch		
Medium fermentation (MF): GP > 8 to 24 h		
r^2 adjusted	Mallow's CP	Independent variables
0.83	2	Glu Starch
0.7	54.92	Glu
In vitro GP > 8 to 24 h = 10.136 + 0.173 Glu + 0.384 Starch		
Low fermentation (LF48): GP > 24 h		
r^2 adjusted	Mallow's CP	Independent variables
0.32	4.00	Glu Starch Cel
0.28	6.27	Glu Cel
0.24	8.93	Cel
In vitro GP > 24 h = 16.121–0.055 Glu + 0.05 Starch + 0.122 Cel		

r^2 adjusted, adjusted determination coefficient; Glu, glucose; Cel, cellulose; GP, gas production, GP > 8 to 24 h, gas production after 8 to 24 h; GP > 24 h, gas production after 24 h (to 81 h).

Model' application in Leucaena and star grass

Leucaena has less fiber (NDF, ADF, Hem and Cel) and more CP than star grass ($P < 0.0001$) (Table 9). However, star grass harvested during the rainy season had less ADL than Leucaena harvested in the dry season. The GP-8, GP-24 and GP-48 converted in Glu, St and Cel equivalents, showed more RF and MF fractions for Leucaena and star grass harvested during the rainy season compared to those harvested in the dry season ($P < 0.0001$). Although, in this feedstuff, RF, MF and LF48 did not allow calculation of the NDSF, Hem and Cel, they were more sensitive for categorizing treatments according to the harvesting season and the time of growing than the variables NDF, ADF and Cel, which were more sensitive with respect to differentiating between type of plant.

Table 10 shows negative Pearson's correlations between NDF, ADF, Hem, Cel, ADL and ashes, and RF and MF (equivalents to *in vitro* GP as a result of Glu and St fermentation) (r^2 ranged from −0.42 to −0.64) ($P < 0.01$) RF and MF show positive correlations with CP, DM as well as Glu and St equivalents (r^2 ranged from 0.40 to 0.65) ($P < 0.0001$). However, LF48 (equivalent to *in vitro* GP as a result of Cel fermentation) positively correlated with NDF, ADF, Hem and Cel (r^2 ranged from 0.56 to 0.66) ($P < 0.01$), but negatively correlated with CP ($r^2 = -0.61$) ($P < 0.0001$).

Table 4. Simple linear models of *in vitro* gas production (GP) for different of levels of glucose (Glu), starch and cellulose (Cel) and different incubation periods

	In vitro GP			
	RF: GP from 0 to 8 h			
	r^2	r^2 adjusted	β0	βi
GP-8 h Glucose	0.977	0.97	7.846	0.369
GP-8 h Starch	0.919	0.891	2.801	0.597
GP-8 h Cellulose	0.925	0.901	1.148	0.016
	MF: GP > 8 to 24 h			
	r^2	r^2 adjusted	β0	Bi
GP-24 h Glucose	0.995	0.993	1.805	0.495
GP-24 h Starch	0.995	0.993	3.88	0.703
GP-24 h Cellulose	0.965	0.953	4.11	0.077
	LF48: GP >24 to 48 h			
	r^2	r^2 adjusted	β0	βi
GP-48 h Glucose	0.994	0.992	−1.423	0.115
GP-48 h Starch	0.987	0.982	1.101	0.125
GP-48 h Cellulose	0.986	0.981	3.684	0.253

r^2, determination coefficient; r^2 adjusted, adjusted determination coefficient; β0, intercept; β_i, increments of *in vitro* gas production by level of substrate; GP-8 h, gas production from 0 to 8 h; GP-24 h, gas production after 8 to 24 h; GP-48, gas production after 24 to 48 h.

Table 5. Residuals of models of *in vitro* gas production obtained from individual and mixtures of levels of glucose, cellulose and starch from 0 to 8 h of incubation*

	RF models: GP 0–8 h†						
	GP est	MSE	MAE	RMSE	*R*	r^2	SR
RF Glu (β0) = 7.846 + 0.369 Glu	64.304	178.1	10.95	13.064	0.973	0.948	−0.769
RF Glu = 0.369 Glu	56.443	78.838	7.017	8.612	0.973	0.948	−0.278
RF St (β0) = 2.801 + 0.0597 St	9.037	771.155	26.572	27.742	0.249	0.357	0.957
RF St = 0.0597 St	6.248	927.282	29.367	30.426	0.301	0.367	0.964
RF Cel (β0) = 1.148 + 0.0163 Cel	2.867	1141.114	32.68	33.629	0.056	0.491	0.967
RF Cel = 0.0163 Cel	1.684	1220.481	33.873	34.789	0.05	0.466	0.97
R^2	0.99	0.94	0.87	0.97	0.44	0.53	0.91
VC (%)	8.91	20.07	9.88	10.25	49.66	16.29	19.67
SEM	1.022	36.781	0.63	0.697	0.16	0.08	0.133
LSD (0.05)	1.681	60.505	1.036	1.147	0.263	0.131	0.218
P-values							
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

*From statistical Model 3.
†RF, models to relate *in vitro* gas production from 0 to 8 h with rapid fermentation proportion; RF (β0), models of RF including the intercept; Glu, glucose; Cel, cellulose; GP, gas production; MSE, mean square error; MAE, mean absolute error, RMSE, root mean square error; *r*, correlation coefficient; r^2, determination coefficient; SR, standard residual; R^2, determination coefficient; VC, variation coefficient; SEM, standard error of the mean; LSD, least significant difference.

Table 6. Residuals of models of *in vitro* gas production obtained from individual and mixtures of levels of glucose, cellulose and starch from 8 to 24 h of incubation*

	MF: GP 8–24 h†						
	GP est	MSE	MAE	RMSE	*R*	r^2	SR
MF Glu (β0) = 1.805 + 0.495 Glu	53.165	701.808	20.979	24.719	0.336	0.537	0.567
MF Glu = 0.495 Glu	51.36	758.061	21.628	25.66	0.37	0.588	0.619
MF St (β0) = 3.88 + 0.703 St	112.09	357.909	15.379	18.084	0.935	0.928	−0.48
MF St = 0.703 St	108.21	304.142	14.091	16.605	0.934	0.927	−0.294
MF Cel (β0) = 4.11 + 0.077 Cel	12.116	3509.311	55.911	58.618	0.26	0.496	0.944
MF Cel = 0.077 Cel	8.006	3985.657	60.02	62.541	0.276	0.512	0.951
R^2	0.94	0.78	0.88	0.86	0.3	0.32	0.78
VC (%)	17.44	5.22	25.4	25.27	57.3	18.5	18.76
SEM	4.824	0.38	3.044	3.232	0.157	0.094	0.128
LSD (0.05)	7.935	624.38	5.007	5.316	0.258	0.154	0.21
P-values							
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

*From statistical Model 3.
†MF, models to relate *in vitro* gas production from 8 to 24 h with medium fermentation proportion; MF (β0), models of MF including the intercept; Glu, glucose; Cel, cellulose; GP, gas production; MSE, mean square error; MAE, mean absolute error, RMSE, root mean square error; *r*, correlation coefficient; r^2, determination coefficient; SR, standard residual; R^2, determination coefficient; VC, variation coefficient; SEM, standard error of the mean; LSD, least significant difference.

DISCUSSION

It has been hypothesized previously that the proportions and degradability of NDF should be the most important predictors of animal productive behavior[32–35]; NDF and LDA impacts the partial and total degradability of feedstuffs and, as the complexity increases, the *in vivo* passage rate limits the DM intake and the potential production of milk and meat.[9,13,15]

In addition to body weight and other animal specifications, NDF and starch digestibility, other regression models had included other components of DM.[18] In the present study, starch showed the highest peak of GP between 8 and 24 h of incubation; in a complete diet, starch fermentation could enhance Hem degradability.[36–40] Then, total NDF carbohydrate equivalents to St (in the same period of fermentation) should be considered during the design of ruminant diets.

Gas production

The *in vitro* GP technique is a sensitive and reliable method for evaluating different diets simultaneously,[5,23,41] Murillo *et al.*[2]

Table 7. Residuals of models of *in vitro* gas production obtained from individual and mixtures of levels of glucose, cellulose and starch from 24 to 48 h of incubation*

	LF48: GP 24–48 h†						
	GP est	MSE	MAE	RMSE	*R*	r^2	SR
LF48 Glu (β0) = −1.423 + 0.115 Glu	10.539	460.699	17.659	19.304	0.175	0.576	0.003
LF48 Glu = 0.115 Glu	11.856	428.454	16.82	18.286	0.184	0.578	0.813
LF48 St (β0) = 1.101 + 0.125 St	14.04	290.753	14.05	15.805	0.087	0.492	0.826
LF48 St = 0.125 St	12.938	355.442	15.632	17.36	0.044	0.545	0.833
LF48 Cel (β0) = 3.684 + 0.252 Cel	42.277	107.827	8.156	9.602	0.717	0.722	−0.099
LF48 Cel = 0.252 Cel	38.593	116.052	8.259	9.781	0.709	0.707	0.248
R^2	0.95	0.37	0.51	0.47	0.23	0.11	0.77
VC (%)	15.01	47.79	41.35	41.96	51.08	31.99	22.89
SEM	0.638	0.01	0.49	0.438	5.141	9.139	8.225
LSD (0.05)	2.58	173.06	3.36	3.76	0.32	0.18	0.2
P-values							
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

*From statistical Model 3.
†LF48, models to relate *in vitro* gas production from 24 to 48 h with low fermentation proportion; LF48 (β0), models of LF48 including the intercept; Glu, glucose; Cel, cellulose; GP, gas production; MSE, mean square error; MAE, mean absolute error, RMSE, root mean square error; r, correlation coefficient; r^2, determination coefficient; SR, standard residual; R^2, determination coefficient; VC, variation coefficient; SEM, standard error of the mean; LSD, least significant difference.

Table 8. Estimated values and residuals of rapid, medium and low fermentation fractions (RF, MF and LF48) of corn stover (CS)*

	RF: GP 0–8 h†					
	NDSF g kg^{-1} DM	RF g kg^{-1} DM	MSE	MAE	RMSE	SR
RF Glu (β0) = 0.369 Glu	310.3	334.2	416.43	4.98	5.13	0.04
RF Glu = 7.846 + 0.369 Glu	310.3	313.0	362.64	4.66	4.89	0.04
R^2		0.98	0.61	0.7	0.7	0.96
LSD (0.05)		1.45	531	3.11	3.09	0.03
P-values						
Model		0.009	0.82	0.82	0.86	0.03
	MF: GP8-24 h					
	Hem g kg^{-1} DM	MF g kg^{-1} DM	MSE	MAE	RMSE	SR
MF Glu (β0) = 0.495 Glu	226.3	192.1	194.44	3.58	3.70	0.08
MF Glu = 1.805 + 0.495 Glu	226.3	186.6	224.64	4.10	4.24	0.09
R^2		0.81	0.80	0.76	0.79	0.75
LSD (0.05)		1.34	136.35	1.56	1.47	0.04
P-values						
Model		0.35	0.58	0.49	0.46	0.83
	LF48: GP 24–48 h					
	Cel g kg^{-1} DM	LF48 g kg^{-1} DM	MSE	MAE	RMSE	SR
LF48 Cel (β0) = 0.252 Cel	393.7	399.5	783.35	7.14	7.81	0.02
LF48 Cel = 3.684 + 0.252 Cel	393.7	384.9	862.07	7.21	7.94	0.04
r^2		0.76	0.71	0.75	0.75	0.99
LSD (0.05)		5.13	718.25	2.85	3.39	0.01
P-values						
Model		0.54	0.81	0.95	0.93	0.008

*From statistical Model 3.
†RF, MF and LF48, models related *in vitro* gas production from 0 to 8, > 8 to 24, and > 24 to 48 h with Glu, St and Cel estimated values; RF(β0), MF(β0) and LF48 (β0), models including the intercept; Glu, glucose; Cel, cellulose; MSE, mean square error; MAE, mean absolute error, RMSE, root mean square error; SR, standard residual; R^2, determination coefficient; LSD, least significant difference.

reported correlations from 0.95 to 0.99 for 24 and 96 h between *in vitro*, *in situ* and *in vivo* results.

GP is related to the degradation of diets as a result of enzyme and bacterial activity. The effects of production rates and types of gases produced in the rumen depend of the chemical composition of the ingredients and the interaction with the successive changes of microorganisms.[1,4] Interactions of specific cell wall structures with microorganisms and enzymes are not fully

Table 9. Rapid, medium and low fermentation fractions (RF, MF and LF48) and chemical analysis of Leucaena (*Leucaena leucocephala* Lam. de Wit) and star grass (*Cynodon nlemfuensis* Vanderyst)*

	Estimated values (g kg^{-1} DM)†			Chemical analysis (g kg^{-1} DM)									
	RF	MF	LF48	DM	Ashes	Ether	CP	NDSF	NDF	ADF	Hem	Cel	ADL
Star grass													
Rainy season													
35 days	179	358.6	666.6	944.3	118.7	6.5	111.8	365.1	634.9	382.4	252.5	267.6	53.1
70 days	119.1	236.8	464.4	947.3	128.3	5.5	96	356.3	643.7	411.2	232.5	270.3	53.4
Dry season													
35 days	83.7	218.4	479.6	952	122	13	109.3	330.1	669.9	424.5	245.4	296.1	71.8
70 days	89.4	165.8	431.5	951.7	128	10.1	96.1	298.9	701.1	449.2	251.9	298	75.4
Leucaena leucocephala													
Rainy season													
35 days	543.2	478.2	358.3	962.3	106	14.3	292.8	739.4	260.6	186.6	73.9	146.9	41.6
70 days	341.1	341.8	363.8	951	110	17.5	286.6	698.7	301.3	218.4	82.9	153.9	69.3
Dry season													
35 days	149.8	280.1	227.6	949	118.3	22.1	253.6	656.3	343.7	185.8	157.9	132.8	55.6
70 days	91. 5	220.5	248.5	946.7	114.7	32.1	234.5	656	344	214.2	129.9	160	69.4
R^2	0.9	0.8	0.8	7.8	7.2	9	9.9	9.9	9.9	9.9	9.4	9.9	7.7
VC (%)	21.3	15.8	17.8	3.1	44.6	188	53.6	27.8	29.2	33.8	106	36.1	93.5
SEM	10	10.7	17	0.69	1.2	1	2.3	8.8	3.4	2.5	4.5	1.8	1.3
LSD (0.005)	25.8	27.5	43.9	1.8	3.1	2.6	5.9	22.7	8.7	6.4	11.6	4.6	3.4
								P-values					
TP	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
TH	< 0.0001	< 0.0001	0.07	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	NS
G	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.002	< 0.0001	< 0.0001
*TP***TH*	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
*TP***G*	< 0.0001	NS	NS	< 0.0001	< 0.0001	< 0.0001	NS	NS	NS	NS	NS	< 0.0001	< 0.0001
*TH***G*	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.001	0.05	0.001	0.001	NS	NS	< 0.0001	< 0.0001
*TP***TH***G*	< 0.0001	NS	< 0.0001	< 0.0001	0.08	< 0.0001	0.01	< 0.0001	< 0.0001	NS	< 0.0001	< 0.0001	NS

*From statistical Model 4.
†RF, MF and LF48 from estimations of Glu, St and Cel, respectively; DM, dry matter; CP, crude protein; NDSF, neutral detergent soluble fiber; NDF, neutral detergent fiber; ADF, acid detergent fiber; Hem, hemicellulose; Cel, cellulose; ADL, acid detergent lignin; R^2, determination coefficient; VC, variation coefficient; SEM, standard error medium; LSD, least significant difference; TP, type of plant; TH, harvest time; G, growth days; *P*-values, probability values of *F*.

Table 10. Simple Pearson's correlations between Glu, St and Cel equivalents and chemical composition variables of Leucaena (*Leucaena leucocephala* Lam. de Wit) and star grass (*Cynodon nlemfuensis* Vanderyst)

	RF	MF	LF48	DM	OM	Ashes	Ether	CP	NDSF	NDF	ADF	Hem	Cel
MF	0.78***												
LF48	−0.09	0.13											
DM	0.62***	0.40***	−0.19										
OM	0.10	0.19	0.28***	0.29***									
Ashes	−0.64***	−0.55***	0.21**	−0.44***	−0.26								
Ether	−0.04	−0.09	−0.69***	0.07	−0.11	−0.42***							
CP	0.65***	0.54***	−0.61***	0.39***	−0.07	−0.64***	0.60***						
NDSF	0.62***	0.53***	−0.63***	0.33**	−0.14	−0.65***	0.64***	0.98***					
NDF	−0.62***	−0.53***	0.63***	−0.33**	0.14	0.65***	−0.64***	−0.98***					
ADF	−0.55***	−0.50***	0.63***	−0.24**	0.12	0.63***	−0.67***	−0.96***	−0.98***	0.98***			
Hem	−0.67***	−0.52***	0.56**	−0.43**	0.16	0.63**	−0.56***	−0.92***	−0.95***	0.95***	0.86***		
Cel	−0.53***	−0.47**	0.66**	−0.25**	0.12	0.61***	−0.65***	−0.95***	−0.97***	0.97***	0.98***	0.87***	
ADL	−0.42**	−0.44**	−0.13	−0.31	0.20	0.10	0.35**	−0.06	−0.05	0.05	0.09	0	0.10

RF, MF and LF48 from estimations of Glu, St and Cel, respectively; DM, dry matter; OM, organic matter; CP, crude protein; NDSF, neutral detergent soluble fiber; NDF, neutral detergent fiber; ADF, acid detergent fiber; Hem, hemicellulose; Cel, cellulose; ADL, acid detergent lignin; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.0001$.

understood, although pyrosequencing of ruminal microbiota have indicated that diet is one of the major factors driving the change of major ruminal phyla (*Bacteroidetes*, *Firmicutes* and *Proteobacteria*) and *archaeas*.[42–45]

Feedstuff components can be classified into two major fermentable fractions: soluble and nonstructural carbohydrates (quantified as NDSF and equivalent to Glu) and structural carbohydrates nested in cell walls (almost all NDF proportion), mainly composed of Hem and Cel. During maturity and lignification, the amount, composition and structure of lignin changes, and phenolic acids with β-*O*-4, β-β and β-5 units limit the accessibility to xylose[46–48]; after breaking simple and ether and esterified cross-linkages of ferulate and *p*-cumarate of lignin with arabinoxylan of Hem and the β-1, 4 of Cel, both Hem and Cel are sources of mostly glucose and xylose.[47–50]

The digestibility of feedstuff organic matter, DM and NDF are highly correlated with GP, volatile fatty acids (VFA), N and CH_4.[8,51] NDSF is fermented in the first 6–8 h and may be the initial energy source for bacteria populations, releasing mainly propionate, and could be a further source of N for the fibrolytic bacteria that ferment Hem after 8 h and Cel after 24 h (NDF complex) to produce primarily acetate and butyrate. During fermentation, H^+ ions can be used to produce short volatile fatty acids or methane (CH_4) in a stoichiometry where each butyrate and propionate mol captures four H ions, whereas acetate releases two H ions that are further used to form CH_4.[7,52]

Models to convert GP to Glu, St and Cel equivalents

Fermentation of almost exclusively Glu, St, Hem or Cel in the present study might lead to intervals of times with different peaks of GP and fermentation patterns related to the degradability of each component. Glu would be rapidly fermented during the first 8 h but, because of β-1, 4 linkages, Cel would hardly be available as a glucose source and would be fermented after 24 h as one of the slowest fermentable components. St, composed of chains of glucose with α linkages, would be fermented soon after Glu but before Hem, which primarily consists of xylose (48–66%) and arabinose (10.4 to 35%)[46]; however, in the rumen, both St and Hem might be fermented between 6 and 26 h after initiating the *in vitro* incubation.[53]

Some of the NDSF and St proportions are still being fermented after 8 and 24 h. Because the ADF is mostly composed of Cel, ADL and ashes, only Cel is the primary source of fermentable carbohydrates from 24 to 48 h (LF48). ADL and ashes are nonfermentable residues.

Previous studies have reported the *in vitro* GP at different times from substrates such Glu, pectin, starch and Cel. The accumulated GP at different incubation times has been defined for soluble carbohydrates such as RF,[54,55] for starch, xylose and pectin as MF [54,55] and Cel as LF fractions.[56] Sigmoidal and non-sigmoidal models described by France *et al.*,[20,57] Groot *et al.*[58] and Schofield *et al.*[22] considered the total GP from one or more pools of ingredient degradability.[13,59]

In present study, GP was divided within intervals of time with peaks at GP-8, GP-24 and GP-48 and simple linear models were obtained from exclusive Glu, St/Hem and Cel fermentations, although, overall, time series would show different peaks at different times and may or not present self-similarities. Lag, exponential increases and declines of curves of each content would be nested in the overall trend and, in the overlapped phases, residual degradability and GP of a first substrate would be related to the initial degradability of the next, but not necessarily with the

maximum GP observed in the nonoverlapped intervals. Some time series analysis methods such as fractal and multifractal detrended fluctuation focus on finding the temporospatial location of detrended data, first nesting the highest or lowest values in intervals and ignoring the general trends and shapes, where peaks are individually analyzed, and after determining short or long term correlations.[60,61] Inversely, the amount of a component might be related to the accumulated GP of peaks.

Potential of models in analysis of forage components

The maximum peak of GP of CS occurred from 8 to 24 h coinciding with the forage, grain and ruminant diet fermentation reported by García-Montes *et al.*,[62] González *et al.*[63] and Tirado-Estrada *et al.*[64] Similarly, highest degradability for DM and NDF from 0 to 24 h was reported by Trujillo *et al.*[23] who analyzed six forages (white clover, birdsfoot trefoil, ryegrass, alfalfa, barley straw), brewer's grains and citrus pulp and found that the partial percentages of degradability of DM and NDF for forages were 47.62 ± 16.76% and 22.59 ± 8.94% from 0 to 8 h after initiating *in vitro* incubation, 26.67 ± 5.54% and 41.08 ± 17.67% after 8 to 24 h, and 15.12 ± 15.11% and 22.79 ± 15.91% after 24 to 96 h [regardless of the method of analysis (*in vitro versus in situ*), all of the ingredients reached the maximum degradability at 96 h]; calculated correlations between initial values of NDF, ADL, ADF, Hem and Cel and *in vitro* GP from 8 to 24 h of ingredients were − 0.63, 0.10, −0.46, −0.53 and − 0.46, respectively, but, at 96 h, the *in vitro* GP were − 0.83, −0.73, −0.69, −0.66, −0.56.

Regarding other ingredients, the content and composition of NDF in Leucaena and star grass negatively correlated with potential GP from 0 to 24 h of incubation, although lignin content negatively impacted the GP total (from 0 to 48 h). *In vitro* NDF degradability is correlated with VFA,[41] for which the contribution of 75% of ruminants' energy requirements and individual proportions is related to the quantity and quality of production and ruminant enteric emissions.[3,9,52] In addition, although Glu, St and Cel equivalents did not predict the values of NDSF, Hem or Cel of all of the ingredients, those variables showed more sensitivity with respect to finding differences between feedstuffs as a result of conditions independent of the type of plants, such as the maturity process,[36,37] genetic selection,[32–35,38–40] supplementation of fibrolytic enzymes[65,66] and pre-fermentation processing.[67]

Limits of *in vitro* GP technique to feedstuff evaluation

Regardless of the method of analysis, at 96 h, feedstuffs had achieved the maximum degradation, although the rates of degradability change through the times,[23] and GP peaks are preceded by a lag phase.[20,56]

Forages and concentrates are complex combinations of soluble and nonstructural carbohydrate equivalents to Glu, as well as mid- or low-available sources of carbohydrates nested in cell walls (NDF complex), and the population peaks of microorganisms and the proportions of gases are interdependent, with lag phase modifying the time of fermentation of structural and nonstructural carbohydrates.

The lag phases also depend on the rumen inoculum, which might vary according to the composition and nutrient availability of diets offered to donors for which microorganisms persist despite their rapid adaptation to diets (changes of endogenous carboximetil cellulases and xylanases, microorganisms and VFA patterns reach the maximum differences 6 to 15 days after change the diet)[4]; however, characteristics of ruminal fluid like the pH and temperature can vary among the donors.[3,9]

Other sources of bias are the ruminal fluid sampling time, the inoculum preparation and the availability to sustain the anaerobic environment, the composition of buffer solutions, and the execution of *in vitro* gas production technique, which can vary between laboratories.[23]

The models derived in present study can be useful; the analyzed samples would be ≤ 0.5 g DM to measure ≤ 0.4 g of any fraction (NDSF, St and/or Hem, and Cel). However, it is necessary to correctly interpretate the GP peaks, which might be quite different for grains (the GP from 8 to 24 h would be mainly starch),[9] as well as to differentiate, from 8 to 24 h, the GP resulting from the nonstructural, the starch and the Hem fermentation.[52]

CONCLUSIONS

In vitro GP was useful for obtaining reliable approximations of Cel and NDF of CS, and considered as a forage with low CP and ether contents. In Leucaena and star grass, the estimated RF, MF and LF48 from accumulated GP-8 h, GP-24 h, and GP-48 h were significantly correlated with NDSF, Hem and Cel. They were more sensitive than chemical composition for comparing feedstuffs, suggesting that the GP equivalent to RF, MF and LF fractions would be useful for comparing the nutritional quality of ruminant diets.

ACKNOWLEDGEMENTS

All of the coauthors were closely involved in the data collection, analysis, discussion and the presentation decisions for the present manuscript. We are grateful for the financial and technical support received from the Universidad Autónoma Chapingo (UACh) and the Instituto Tecnológico El Llano Aguascalientes (ITEL)/Tecnológico Nacional de México (TecNM), as well as to INIFAP for providing the biological materials.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

REFERENCES

1 Guan LL, Nkrumah JD, Basarab JA and Moore JA, Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency. *FEMS Microbiol Lett* **288**:85–91 (2008).
2 Murillo M, Herrera E, Carrete FO, Ruiz O and Serrato JS, Chemical composition, *in vitro* gas production, ruminal fermentation and degradation patterns of diets by grazing steers in native range of North Mexico. *Asian-Australas J Anim Sci* **25**:1395–1403 (2012).
3 Lam S, Munro JC, Zhou M, Guan LL, Schenkel FS, Steel MA *et al.*, Associations of rumen parameters with feed efficiency and sampling routine in beef cattle. *Animal* **12**:1–9 (2017).
4 Xie X, Yang C, Guan LL, Wang J, Xue M and Liu JX, Persistence of cellulolytic bacteria *Fibrobacter* and *Treponema* after short-term corn stover-based dietary intervention reveals the potential to improve fibrolytic function. *Front Microbiol* **9**:1363 (2018).
5 Vallejo-Hernández L, Elghandour MMY, Greiner R, Anele UY, Rivas-Cáceres RR, Barros-Rodríguez M *et al.*, Environmental impact of yeast and exogenous xylanase on mitigating carbon dioxide and enteric methane production in ruminants. *J Clean Prod* **189**:40–46 (2018).
6 Beauchemin KA, Kreuzer M, O'Mara F and McAllister TA, Nutritional management for enteric methane abatement: a review. *Anim Prod Sci* **48**:21–27 (2008).
7 Knapp JR, Laur GL, Vadas PA, Weiss WP and Tricarico JM, Invited review: enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emmisions. *J Dairy Sci* **97**:3231–3261 (2014).
8 Zhong R, Fang Y, Sun H, Wang M and Zhou D, Rumen methane output and fermentation characteristics of gramineous forage and

leguminous forage at differing harvest dates determined using an *in vitro* gas production technique. *J Integr Agric* **15**:414–423 (2016).
9 Danielsson R, Ramin M, Bertilsson J, Lund P and Huhtanen P, Evaluation of a gas *in vitro* system for predicting methane production *in vivo*. *J Dairy Sci* **100**:8881–8894 (2017).
10 Beauchemin KA and McGinn SM, Methane emissions from feedlot cattle fed barley or corn diets. *J Anim Sci* **83**:653–661 (2005).
11 Beauchemin KA, McGinn S, Benchaar C and Holtshausen L, Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: effects on methane production, rumen fermentation, and milk production. *J Dairy Sci* **92**:2118–2127 (2009).
12 Zicarelli F, Calabro S, Cutrignelli MI, Infascelli F, Tudisco R, Bovera F *et al*., *In vitro* fermentation characteristics of diets with different forage/concentrate ratios: comparision of rumen and faecal inocula. *J Sci Food Agric* **91**:1213–1221 (2011).
13 Wang M, Wang R, Sun X, Chen L, Tang S, Zhou C *et al*., A mathematical model to describe the diurnal pattern of enteric methane emissions from non-lactating dairy cows post-feeding. *Anim Nutr* **1**:329–338 (2015).
14 Doane PH, Schofield P and Pell N, Neutral detergent fiber disappearance and gas and volatile fatty acid production during the *in vitro* fermentation of six forages. *J Anim Sci* **75**:3342–3352 (1997).
15 Warner D, Dijkistra J, Hendriks WH and Pellikaan WF, Passage kinetics of ^{13}C-labeled corn silage components through the gastrointestinal tract of dairy cows. *J Dairy Sci* **96**:5844–5858 (2013).
16 Theodorou M, Williams BA, Dhanoa MS and McAllan AB, A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim Feed Sci Technol* **48**: 185–197 (1994).
17 Getachew G, Blümmel M, Makkar HPS and Becker K, *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. *Anim Feed Sci Technol* **72**:261–281 (1998).
18 De Souza RA, Tempelman RJ, Allen MS, Weiss WP, Bernadr JK and VandeHaar MJ, Predicting nutrient digestibility in high-production dairy cows. *J Dairy Sci* **101**:1123–1135 (2017).
19 Orskov ER and McDonald I, The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J Agric Sci* **92**:99–503 (1979).
20 France J, Dhanoa MS, Theodorou MK, Lister SJ, Davies DR and Isac D, A model to interpret gas accumulation profiles associated with *in vitro* degradation of ruminant feeds. *J Theor Biol* **163**:99–111 (1993).
21 Russel J, O'Connort D, Fox DJ, Van Soest PJ and Sniffentv CJ, A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J Anim Sci* **70**:3551–3561 (1992).
22 Schofield P, Pitt RE and Pell AN, Kinetics of fiber digestion from *in vitro* gas production. *J Anim Sci* **72**:2980–2991 (1994).
23 Trujillo A, Marichal MJ and Carriquiry M, Comparison of dry matter and neutral detergent fibre degradation of fibrous feedstuffs as determined with *in situ* and *in vitro* gravimetric procedures. *Anim Feed Sci Technol* **161**:49–57 (2010).
24 Peña-Ramos A, Santana O, Briones-Reyes D, Ramírez-Díaz JL, Vidal-Martínez V and Ledesma-Miramontes A, H383: intermediate white maize hybrid for grain fodder for northern, central and western Mexico. *Rev Mex Cienc Agric* **8**:1891–1896 (2017).
25 Van Soest PJ, Robertson JB and Lewis BA, Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* **74**:3583–3592 (1991).
26 AOAC, Crude Protein in Meat and Meat Products Including Pet Foods. Method 992.15. http://www.eoma.aoac.org/methods/info.asp?ID=16519%20 (2005).
27 AOAC, Fat (Crude) or Ether Extract in Animal Feed. Method 920.39. http://www.eoma.aoac.org/methods/info.asp?ID=33043 (2005).
28 Cobos MA and Yokoyama MT, *Clostridium paraputrificum var. ruminantum*: colonization and degradation of shrimp carapaces *in vitro* observed by scanning electron microscopy, in *Rumen Ecology Research Planning, Proceedings of Workshop*, ed. by Wallas RJ and lahlou-Kassi A. *International Livestock Research Institute*, Addis Abeba, pp. 151–161 (1995).
29 Menke KH and Steingass H, Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim Res Dev* **28**:7–55 (1988).
30 Statistical Analysis System, *SAS/STAT User´s Guide. (Release 9.3)*. SAS Institute Inc., Cary (2013).
31 Canavos GC, *Probabilidad y Estadística, Aplicaciones y Métodos*. McGraw-Hill, New York, p. 652 (1988).
32 Oba M and Allen M, Effects of Brown midrib 3 mutation in corn silage on dry matter intake and productivity of high yielding dairy cows. *J Dairy Sci* **82**:135–142 (1999).
33 Oba M and Allen M, Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 1. Fiber behavior and nutrient utilization. *J Dairy Sci* **83**:1333–1341 (2000a).
34 Oba M and Allen M, Effects of Brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrates of dietary neutral detergent fiber: 2. Chewing activities. *J Dairy Sci* **83**:1342–1349 (2000b).
35 Oba M and Allen M, Effects of Brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 3. Digestibility and microbial efficiency. *J Dairy Sci* **83**:1350–1358 (2000c).
36 Khan MA, Lee HJ, Lee WS, Kim HS, Park SB, Baek KS *et al*., Starch source evaluation in calf starter: II. Ruminal parameters, rumen development, nutrient digestibilities, and nitrogen utilization in Holstein calves. *J Dairy Sci* **91**:1140–1149 (2008).
37 Khan NA, Tewoldenbrhan TA, Zom RLG and Hendriks WH, Effect of corn silage harvest maturity and concentrate type on milk fatty acid composition of dairy cows. *J Dairy Sci* **95**:1472–1483 (2012).
38 Khan NA, Yu P, Ali M, Cone JW and Hendriks WH, Nutritive value of maize silage in relation to dairy cow performance and milk quality. *J Sci Food Agric* **96**:238–252 (2015).
39 Núñez-Hernández G, Faz-Contreras R, González-Castañeda F and Peña-Ramos A, Effect of stage of maturity of maize hybrids at harvest time to improve forage production and quality. *Tec Pecu Mex* **43**:69–78 (2005).
40 Peña RA, González CF, Núñez HG, Tovar G, Preciado ORE, Terrón IA *et al*., Estabilidad del rendimiento y calidad forrajera de híbridos de maíz. *Rev Fitotec Mex* **29**:109–114 (2006).
41 Calabró S, Infascelli F, Bovera F and Moniello G, *In vitro* degradability of three forages: fermentation kinetics and gas production of NDF and neutral detergent-soluble fraction of forages. *J Sci Food Agric* **82**:222–229 (2001).
42 Hernández-Sanabria E, Goonewardene LA, Wang Z, Durunna ON, Moore SS and Guan LL, Impact of feed efficiency and diet on adaptative variations in the bacterial community in the rumen fluid cattle. *Appl Environ Microbiol* **78**:1203–1214 (2011).
43 Morgavi DP, Kelly WJ, Janssen PH and Attwood G, Rumen microbial (meta)genomics and its application to ruminant production. *Animal* **7**:1–18 (2012).
44 Saleem F, Ametaj BN, Bouatra S, Mandal R, Zebeli Q, Dunn SM *et al*., A metabolomics approach to uncover the effects of grain diets on rumen health in dairy cows. *J Dairy Sci* **95**:6606–6623 (2012).
45 Saleem F, Bouatra S, Gou A, Psychogios N, Mandal R, Dunn S *et al*., The bovine ruminal fluid metabolome. *Metabolomics* **9**:360–378 (2013).
46 Hespel RB, Microbial digestion of hemicellulose in the rumen. *Microb Sci* **5**:362–365 (1988).
47 Casler MD and Jung HG, Relationships of fibre, lignin, and phenolics to *in vitro* fibre digestibility in three perennial grasses. *Anim Feed Sci Technol* **125**:151–161 (2006).
48 Barros-Rios J, Santiago R, Malvar RA and Jung HG, Chemical composition and cell wall polysaccharide degradability of pith and rind tissues from mature maize internodes. *Anim Feed Sci Technol* **172**: 226–236 (2012).
49 Jung HG and Casler MD, Maize stem tissues: impact of development on cell wall degradability. *Crop Sci* **46**:1801–1809 (2006).
50 He Y, Mouthier TMB, Kabel MA, Dijkstra J, Hendriks WH, Struik P *et al*., Lignin composition is more important than content for maize stem cell wall degradation. *J Sci Food Agric* **98**:384–390 (2018).
51 Hatta M, Norheim H, Gustavsson A and Martinssib K, Comparison of *in vitro* degradation of temperate forages using the gas production and filter bags techniques. *J Sci Food Agric* **88**:199–206 (2007).
52 Jin D, Kang K, Wang H, Wang Z, Xue B, Wang L *et al*., Effects of dietary supplementation of active dried yeast on fecal methanogenic archaea diversity in dairy cows. *Anaerobe* **44**:78–86 (2017).
53 Yang M, Kuittinen S, Zhang J, Zhang J, Vepsalaine J, Markku K *et al*., Co-fermentation of hemicellulose and starch from barley straw and grain for efficient pentoses utilization in acetone-butanol-ethanol production. *Bioresour Technol* **179**:128–135 (2015).
54 Stefanon B, Pell AN and Schofield P, Effect of maturity on digestion kinetics of water-soluble and water-insoluble fractions of alfalfa and brome hay. *J Anim Sci* **74**:1104–1115 (1996).

55 Dul JP, Xin HS, Wan R, Shil FH and Meng QX, Fermentation kinetics of carbohydrate fractions of maize grains as determined by *in vitro* gas production curve subtraction technique. *J Anim Feed Sci* **19**: 638–650 (2010).
56 Fuente G, Belanchel A and Fondevila M, *In vitro* ruminal fermentation of carbohydrates by Spanish index. *Arch Zootec* **58**:357–362 (2009).
57 France J, López S, Kebreab E, Bannink A, Dhanoa MS and Dijkstra J, A general compartmental model for interpreting gas production profiles. *Anim Feed Sci Technol* **123-124**:473–485 (2005).
58 Groot JC, Cone JW, Williams BA, Debersaques FMA and Lantiga EA, Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feeds. *Anim Feed Sci Technol* **64**:77–89 (1996).
59 Wang M, Tang SX and Tan ZL, Modeling *in vitro* gas production kinetics: derivation of logistic-exponential (LE) equations and comparison of models. *Anim Feed Sci Technol* **165**:137–150 (2011).
60 Kantelhardt JW, Zschiegner SA, Koscielny-Bunde E, Havlin S, Bunde A and Stanley HE, Multifractal Detrended fluctuation analysis of nonstationary time series. *Phys A* **316**:87–114 (2002).
61 Ihlen EA, Introduction to multifractal Detrended fluctuation analysis in MATLAB. *Front Phys* **3**:1–18 (2012).
62 García-Montes de Oca CA, González-Ronquillo M, Salem AZM, Romero-Bernala J, Pedraza JF and Estrada JG, Chemical composition and *in vitro* gas production of some legume browse species in subtropical areas of México. *Trop Subtrop Agroecosys* **14**:589–595 (2011).
63 González GUA, González RM, Estrada FJG, Bastida GJL, Pescador SN and Salem AZM, Inclusión de heno de chícharo (*Pisum sativum* L.) y producción de gas *in vitro* en dietas para corderos en crecimiento. *Trop Subtrop Agroecosys* **14**:989–997 (2011).
64 Tirado-Estrada G, Ramos-Mijangos LM, Miranda-Romero LA, Tirado-González DN, Salem AZM, Mlambo V *et al*., Potential impacts of dietary *Lemna gibba* supplements in a simulated ruminal fermentation system and environmental biogas production. *J Clean Prod* **181**: 555–561 (2018).
65 Salem AZM, Buendía-Rodríguez G, Elghandour MM, Mariezcurrena MA, Peña FJ, Pliego AB *et al*., Effects of cellulase and xylanase enzymes mixed with increasing doses of *Salix babylonica* extract on *in vitro* rumen gas production kinetics of a mixture of corn silage with concentrate. *J Integr Agric* **14**:131–139 (2015).
66 Vallejo LH, Salem AZM, Kholif AE, Elghandour MMY, Fajardo RC, Rivero N *et al*., Influence of cellulase and xylanase on *in vitro* rumen gas production and fermentation of corn Stover. *Indian J Anim Sci* **86**:70–74 (2016).
67 Kholif AE, Elghandour MMY, Rodríguez GB, Olafadehan OA and Salem AZM, Anaerobic ensiling of raw agricultural waste with a fibrolytic enzyme cocktails as cleaner and sustainable biological product. *J Clean Prod* **142**:2649–2655 (2017).

