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Fermentation kinetics of stems of sorghum and millet genotypes

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Abstract

Gas production profiles were obtained from in vitro fermentation of stems of six genotypes of sorghum and millet grown at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India. The ranking of sorghum and millet genotypes by cumulative gas production was consistent throughout the 96-hour fermentation period. However, differences were proportionally greater during the initial 3 and 6 h of fermentation.

The multiphase model described by Groot et al. [Groot, J.C.J., Cone, J.W., Williams, B.A., Debersaques, F.M.A., Lantinga, E.A., 1996. Anim. Feed Sci. Technol. 64:77–89] was used to fit the in vitro fermentation gas observations of these substrates, and fermentation kinetics parameters were calculated using the fitted model. The final estimates of the model parameters (A, B, C), tested by varying the initial estimates obtained with the monophasic model by $\pm 50\%$, were stable, showing no dependence on the starting values of the model parameters. However, in millet stems, the C parameter has shown a tendency to converge near unity. The stability of the final values of the parameters of the model in this study suggests the potential applicability of the multiphase model when only nine gas observations over a period of 96 h were available. However, the indeterminacy in the parameters of phase 1 for some millet stems indicates the need of an intermediate gas value between 0 and 3 h.

In both, sorghum and millet the asymptotic gas of the first phase (A_1) was negatively correlated with NDF ($r = -0.82$, $p < 0.05$; $r = -0.80$, $p < 0.05$, respectively) and lignin ($r = -0.86$, $p < 0.05$; $r = -0.95$, $p < 0.01$, respectively). The estimated maximum fractional rate of substrate digestion in the second phase (R_{m2}) showed a strong inverse relationship with lignin ($r = -0.93$, $p < 0.01$) in millet but not in sorghum. On the other hand, the time at which the rate of fermentation reached its maximum in phase 1 (t_{max1}) was negatively correlated with ADF and lignin ($r = -0.88$, $p < 0.05$

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and $r = -0.87$, $p < 0.05$, respectively) in sorghum, whereas in millet only t_{max2} (phase 2) was negatively correlated with lignin ($r = -0.88$, $p < 0.05$). Estimated differences in the contribution from the different phases to similar cumulative gas at 48 h in stems of different sorghum genotypes indicate the need to complement measurements of total gas production with selected kinetics parameters. The degree of variability between genotypes observed for kinetics parameters related to both, phases 1 and 2 suggest that these parameters have potential for discriminating feed quality differences between crop residues or plant parts. These results point out the difficulty in finding a single feed quality parameter to be used to rank different crop residues of different genotypes. Additional studies are needed to associate these parameters with voluntary intake and in vivo rumen outflow rates with which these phases could be associated.

Keywords: Gas production; Genotypes; Millet; Sorghum; Stems; Fermentation models

1. Introduction

Genetic enhancement of the feed quality of non-grain plant parts of cereal and legume crops is one way to increase their use by ruminant livestock, especially in developing countries. In order to differentiate genotypic differences, heritable and variable traits related to use by ruminants must be identified. Gas production alone, or associated with other tests or chemical analyses, has been used to estimate in vivo digestibility (Getachew et al., 1998; Khazaal et al., 1993) and voluntary intake (Blummel and Becker, 1997; Blummel and Bullerdieck, 1997; Blummel and Orskov, 1993) of roughages. The degradation kinetics of roughage and concentrate feeds determined from the gas produced during fermentation has been described in the literature, and a number of models describing degradation kinetics have also been reported (Cone et al., 1996; Groot et al., 1996; Schofield et al., 1994; Theodorou et al., 1994; Beuvink and Kogut, 1993; France et al., 1993).

The multiphase model proposed by Groot et al. (1996) estimates a number of parameters describing the fermentation process. It requires estimates of the number of phases in a fermentation profile and the initial values of the corresponding model parameters from the observed gas data. Groot et al. (1996) applied this model to fermentation profiles with frequent gas observations obtained with sophisticated instruments; however, such frequency of measurements as well as instruments may not be available in many developing countries.

The objective of this study was to investigate fermentation kinetics of in vitro gas production from incubations of sorghum and millet residues using multiphasic analysis of nine cumulative gas measurements obtained using a modification of the Menke method (Menke et al., 1979; Osuji et al., 1993).

2. Materials and Methods

2.1. Feeds and experimental procedure

Stems of sorghum and millet of six different genotypes grown at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India, during the main

Table 1
Mean chemical composition of stems of six genotypes of sorghum and millet (rep = 2)

Crop	Genotype	N (g/kg DM)	ASH (g/kg DM)	LIGNIN (g/kg DM)	ADF (g/kg DM)	NDF (g/kg DM)
Sorghum	CSV-15	3.18	62.3	54.2	464.5	695.3
	ICSV-700	3.24	39.0	39.6	322.5	532.2
	ICSV-735	2.18	40.4	47.1	355.2	574.3
	ICSV-89057	2.47	58.5	48.8	399.0	617.1
	LOCALFSR	4.38	49.5	52.0	418.6	669.3
	M35-1	3.10	38.8	46.4	380.0	535.3
	SE[a]	0.50	3.4	2.6	16.8	15.2
Millet	BK560	3.82	65.0	94.9	510.4	808.0
	CZ-IC41	2.80	53.1	91.8	480.6	772.2
	ICMP9282	2.30	50.7	90.5	495.7	786.5
	NokhaLocal	2.94	56.9	85.2	474.6	778.9
	RCB-IC911	2.67	50.8	71.5	463.4	698.1
	RCB-IC912	2.61	52.9	77.9	464.1	755.5
	SE[a]	0.22	4.5	4.9	29.4	20.5

[a] Standard error of the least-squares means.

rainy season in 1995 were used. Stems were obtained from plants of two field replicates of each sorghum and millet genotypes. Stems of sorghum and millet were ground in a Wiley mill to 1-mm particle size. Nitrogen content (N) was determined with a Technicon Auto Analyser and acid and neutral detergent fibres (ADF, NDF) were determined by the method of Van Soest and Robertson (1985). Dry matter and ash were determined according to AOAC (1980). Chemical composition of these feeds is presented in Table 1. Gas produced from incubated feeds was estimated according to the method of Menke et al. (1979) as modified by Osuji et al. (1993). Two hundred milligrams of each sample, standards (groundnut hay, pearl millet, foxtail millet, sorghum and starch) and blanks were incubated in triplicate with 30 ml of rumen fluid mixture as described in Osuji et al. (1993). Rumen fluid was obtained from four bullocks (average live-weight = 387.5 kg, SD = 40.3) adapted to a diet of sorghum residues. The buffer solution was made up with sodium hydrogen carbonate. Cumulative fermentation gas was measured at 3, 6, 12, 18, 24, 36, 48, 72, and 96 h after incubation.

2.2. Fitting of multiphase model

The number of phases in the fermentation gas profile and corresponding initial parameters of the multiphase model were based on a fitted monophasic model using the approach described by Groot et al. (1996). The starting values of A and B parameters for fitting a monophasic model were taken as the gas value at 96 and 48 h, respectively. The starting value of any C parameter of any phase was kept at 2. Starting at the origin, the first phase was identified by a change in the sign of the residuals from positive to negative. The observation with the lower absolute value of the residual at this shift of sign was taken as an estimate of $(2B_1, A_1)$. The subsequent change in the sign of the residuals from negative to positive identified the second phase. The observation with the lower

Table 2
Mean cumulative fermentation gas (ml/ 200 mg DM) at 3, 6, 12, 18, 24, 36, 48, 72 and 96 h of incubation for stems of six genotypes of sorghum and millet (rep = 2)

Crop	Genotype	Hours of incubation[b]								
		3	6	12	18	24	36	48	72	96
Sorghum	CSV-15	1.36b	3.47c	5.55e	7.45d	9.56d	14.78d	20.01d	30.2d	36.29d
	ICSV-700	1.19cb	4.31bc	9.78ab	13.82a	17.05a	24.23a	31.03a	40.78a	45.74ab
	ICSV-735	1.16cb	4.62b	8.5cb	11.97b	14.55b	21.09b	27.07b	36.59b	41.32cb
	ICSV-89057	0.59c	4.45bc	7.32cd	10.07c	12.55c	17.93c	23.78c	33.92c	39.7cd
	LOCALFSR	1.62ab	4.49bc	6.92d	9.36c	11.99c	18.5c	24.39c	35.06bc	40.18cd
	M35-1	2.18a	6.36a	10.94a	15.02a	18.29a	26.09a	32.29a	41.86a	46.6a
	SE[a]	0.20	0.3	0.36	0.47	0.5	0.54	0.45	0.7	1.24
Millet	BK560	0.81c	1.66d	3.78d	6.2c	8.54d	13.89c	18.09d	23.02d	25.48e
	CZ-IC41	1.89b	3.42cb	5.76c	8.38b	10.96cb	15.81cb	20.95bc	26.3bc	29.19cd
	ICMP9282	2.04b	3.38cb	5.79c	8.84b	11.23ab	16.12cb	21.55b	27.95b	31.29cb
	NokhaLocal	1.16c	2.74cd	4.4d	6.84c	9.15cd	14.03c	18.69dc	24.5dc	27.11ed
	RCB-IC911	2.93a	4.85a	7.95a	10.7a	13.24a	19.09a	24.99a	32.72a	35.52a
	RCB-IC912	2.16b	4.02ab	6.72b	9.31b	12.05ab	17.91ab	23.89a	30.98a	33.91ab
	SE[a]	0.15	0.3	0.24	0.32	0.54	0.63	0.63	0.79	0.74

[a] Standard error of the least-squares means.
[b] Means within a column without common letters differ ($p < 0.05$).

absolute value of the residual at this shift of sign was taken as an estimate of (B_2, $A_1 + 0.5A_2$). Proc NLIN of SAS (Statistical Analysis Systems Institute, 1989), was used to fit the multiphase model to the observed data (Appendix A).

3. Results

3.1. Cumulative gas production

Cumulative volumes of gas produced from stems of different genotypes of sorghum and millet are reported in Table 2. At 12, 18, 24, 36, 48 and 72 h of incubation, differences in total gas production between sorghum genotypes were consistent. At 3 h of fermentation, the major difference observed was between ICSV-89057 (lowest) and M35-1 genotypes, which consistently showed the highest gas production over the 96 h fermentation time. At 6 h of incubation, differences in total gas production were significant only between the CSV-15 (lowest) and M35-1 (highest) genotypes. The ranking of millet genotypes by cumulative gas production was consistent throughout the 96-h fermentation period. However, differences were proportionally greater during the initial phase at 3 and 6 h of fermentation.

Correlations between gas production and fibre components, ash and N content of sorghum and millet stems are given in Table 3. In both, sorghum and millet NDF was strongly correlated with cumulative gas at 6, 12, 24 and 48 h. Other fibre fractions, such as ADF and lignin were strongly correlated with gas at 24 and 48 h in sorghum while they

Table 3
Pearson's correlation coefficients of fermentation gas at 6, 12, 24, 48 h of incubation with nutritive value indices ASH, NDF, ADF, LIGNIN and N ($n = 6$)

Crop	gas6	gas12	gas24	gas48
Sorghum				
ASH	−0.64	−0.88[a]	−0.90[a]	−0.92[b]
NDF	−0.65	−0.95[b]	−0.96[b]	−0.94[b]
ADF	−0.37	−0.79	−0.82[a]	−0.82[a]
LIGNIN	−0.33	−0.80	−0.83[a]	−0.83[a]
N	−0.05	−0.18	−0.17	−0.08
Millet				
ASH	−0.88[a]	−0.82[a]	−0.85[a]	−0.78
NDF	−0.91[a]	−0.90[a]	−0.86[a]	−0.86[a]
ADF	−0.82[a]	−0.74	−0.70	−0.72
LIGNIN	−0.84[a]	−0.82[a]	−0.77	−0.82[a]
N	−0.76	−0.68	−0.73	−0.67

[a] Significant at $p < 0.05$.
[b] Significant at $p < 0.01$.

were more correlated with gas at 6 and 12 h in millet. The apparent negative correlation of N with gas in millet was not significant.

3.2. *Estimates of model parameters*

The stability of the final estimates of the model parameters (A, B, C) was tested by varying the initial estimates obtained with the monophasic model by ±50%.The final estimates of the A, B and C parameters obtained for sorghum and millet stems were stable, showing no dependence on the starting values of the model parameters (Table 4). However, in millet stems, the C parameter has shown a tendency to converge near unity. This could be due to a lack of observations on the increasing rate of gas production during the first phase of the fermentation profile. On the other hand, in the profile of sorghum stems, the 3-h reading was still part of the increasing rate of gas production, providing information to fix the point of inflection of phase 1. Fig. 1 shows the monophasic and diaphasic model fitted to the gas observations of one each of the sorghum and millet stem genotypes. In the millet stem, the 3-h observation falls above the fitted monophasic model curve and shows that the 3-h gas observation was past the initial increasing rate of gas production. On the other hand, for the sorghum stem the 3-h observation is below the monophasic curve, indicating that it is part of the initial increasing rate of gas production in the first phase.

3.3. *Goodness of fit*

The significance of the final parameters of the model and of the phases was not tested because only three degrees of freedom remained for the error term. Autocorrelation

Table 4
Variation of final estimates of model parameters (A_1, B_1, A_2, B_2) due to imposed ±50% variation of their initial estimates (est) and the corresponding Durbin–Watson coefficient (DW) and R^2 for one replicate of stems of six genotypes of sorghum and millet

Crop	Genotype		Initial estimates				Final estimates						DW	R^2	CSS[a]	SSE1[b]	SSE2[c]	F[d]	F2[e]	P[f]	P2[g]
			A_1	B_1	A_2	B_2	A_1	B_1	C_1	A2	B2	C2									
Sorghum	CSV-15	0.5*est	3.43	4.50	12.41	24.00	4.34	4.83	2.41	49.14	70.74	2.08	3.16	0.9999	1212.4	2.80	0.11	6612.5	23.57	<0.00001	0.014
		est	6.86	9.00	24.82	48.00	4.34	4.83	2.41	49.14	70.74	2.08	3.16	0.9999							
		1.5*est	10.29	13.50	37.23	72.00	4.34	4.83	2.41	49.14	70.74	2.08	3.16	0.9999							
	ICSV-700	0.5*est	7.035	4.50	17.23	24.00	14.34	9.14	2.13	39.69	52.24	2.57	3.71	0.9999	2087.3	3.79	0.29	4318.0	12.12	<0.00001	0.035
		est	14.07	9.00	34.46	48.00	14.34	9.14	2.13	39.69	52.24	2.57	3.71	0.9999							
		1.5*est	21.105	13.50	51.69	72.00	14.34	9.14	2.13	39.69	52.24	2.57	3.71	0.9999							
	ICSV-735	0.5*est	5.82	4.50	15.05	24.00	16.16	12.19	1.63	31.00	55.15	2.96	3.10	0.9997	1610.5	3.47	0.56	1724.9	5.16	<0.00001	0.105
		est	11.64	9.00	30.10	48.00	16.16	12.19	1.63	31.00	55.15	2.96	3.10	0.9997							
		1.5*est	17.46	13.50	45.15	72.00	16.16	12.19	1.63	31.00	55.15	2.96	3.10	0.9997							
	ICSV-89057	0.5*est	4.43	4.50	14.32	24.00	6.46	5.76	3.59	38.81	52.67	2.54	2.92	0.9995	1408.6	7.13	0.75	1126.3	8.56	<0.00001	0.056
		est	8.86	9.00	28.64	48.00	6.46	5.76	3.59	38.81	52.67	2.54	2.92	0.9995							
		1.5*est	13.29	13.50	42.96	72.00	6.46	5.76	3.59	38.81	52.67	2.54	2.92	0.9995							
	LOCALFSR	0.5*est	3.135	3.00	17.17	24.00	4.81	3.80	3.22	51.90	62.36	2.01	3.37	0.9994	1551.7	5.95	0.94	989.8	5.36	<0.00001	0.101
		est	6.27	6.00	34.34	48.00	4.81	3.80	3.22	51.90	62.36	2.01	3.37	0.9994							
		1.5*est	9.405	9.00	51.51	72.00	4.81	3.80	3.22	51.90	62.36	2.01	3.37	0.9994							
	M35-1	0.5*est	7.275	4.50	11.06	18.00	12.07	6.63	2.26	37.72	44.90	2.55	3.74	0.9999	1893.8	5.45	0.25	4544.5	20.44	<0.00001	0.017
		est	14.55	9.00	22.12	36.00	12.07	6.63	2.26	37.72	44.90	2.55	3.74	0.9999							
		1.5*est	21.825	13.50	33.18	54.00	12.07	6.63	2.26	37.72	44.90	2.55	3.74	0.9999							
Millet	BK560	0.5*est	1.93	3.00	9.84	18.00	8.42	21.91	1.10	20.08	40.84	2.31	3.09	0.9998	631.6	0.87	0.12	3157.4	6.19	<0.00001	0.084
		est	3.85	6.00	19.68	36.00	8.42	21.91	1.10	20.08	40.84	2.31	3.09	0.9998							
		1.5*est	5.78	9.00	29.52	54.00	8.42	21.91	1.10	20.08	40.84	2.31	3.09	0.9998							
	CZ-IC41	0.5*est	2.88	3.00	10.78	18.00	3.53	3.00	2.37	29.66	39.58	2.02	3.48	0.9996	793.7	3.17	0.34	1400.0	8.37	<0.00001	0.057
		est	5.75	6.00	21.56	36.00	3.53	3.00	2.37	29.66	39.58	2.02	3.48	0.9996							
		1.5*est	8.63	9.00	32.34	54.00	3.53	3.00	2.37	29.66	39.58	2.02	3.48	0.9996							
	ICMP9282	0.5*est	2.81	3.00	11.79	18.00	5.48	5.74	1.00	31.08	41.98	2.11	2.93	0.9997	964.1	3.34	0.33	1752.3	9.22	<0.00001	0.050
		est	5.62	6.00	23.58	36.00	5.48	5.74	1.00	31.08	41.98	2.11	2.93	0.9997							
		1.5*est	8.43	9.00	35.37	54.00	5.48	5.74	1.00	31.08	41.98	2.11	2.93	0.9997							

NokhaLocal	0.5*est	2.02	3.00	9.79	18.00	2.74	3.71	2.86	27.49	42.25	2.23	3.11	0.9997	707.2	2.47	0.19	2232.7	11.92	<0.00001	0.036
	est	4.03	6.00	19.58	36.00	2.74	3.71	2.86	27.49	42.25	2.23	3.11	0.9997							
	1.5*est	6.05	9.00	29.37	54.00	2.74	3.71	2.86	27.49	42.25	2.23	3.11	0.9997							
RCB-IC911	0.5*est	4.09	3.00	12.01	18.00	20.66	18.00	1.00	21.37	46.55	3.26	2.42	0.9996	1217.1	7.33	0.48	1520.8	14.21	<0.00001	0.028
	est	8.18	6.00	24.02	36.00	20.66	18.00	1.00	21.37	46.55	3.26	2.42	0.9996							
	1.5*est	12.27	9.00	36.03	54.00	20.66	18.00	1.00	21.37	46.55	3.26	2.42	0.9996							
RCB-IC912	0.5*est	3.18	3.00	12.38	18.00	34.57	51.65	1.00	11.50	39.71	5.09	2.01	0.9999	1137.4	7.04	0.09	7582.1	80.97	<0.00001	0.002
	est	6.36	6.00	24.76	36.00	34.57	51.65	1.00	11.50	39.71	5.09	2.01	0.9999							
	1.5*est	9.54	9.00	37.14	54.00	34.57	51.65	1.00	11.50	39.71	5.09	2.01	0.9999							

[a] Corrected sum of squares of gas values.
[b] Residual sum of squares of the fitted monophasic model.
[c] Residual sum of squares of the fitted diaphasic model.
[d] 3*(CSS-SSE2)/(5*SSE2) (F test statistics for significance of the diaphasic model).
[e] 3*(SSE1-SSE2)/(3*SSE2) (F test statistics for significance of the second phase).
[f] Probability > F.
[g] Probability > F2.

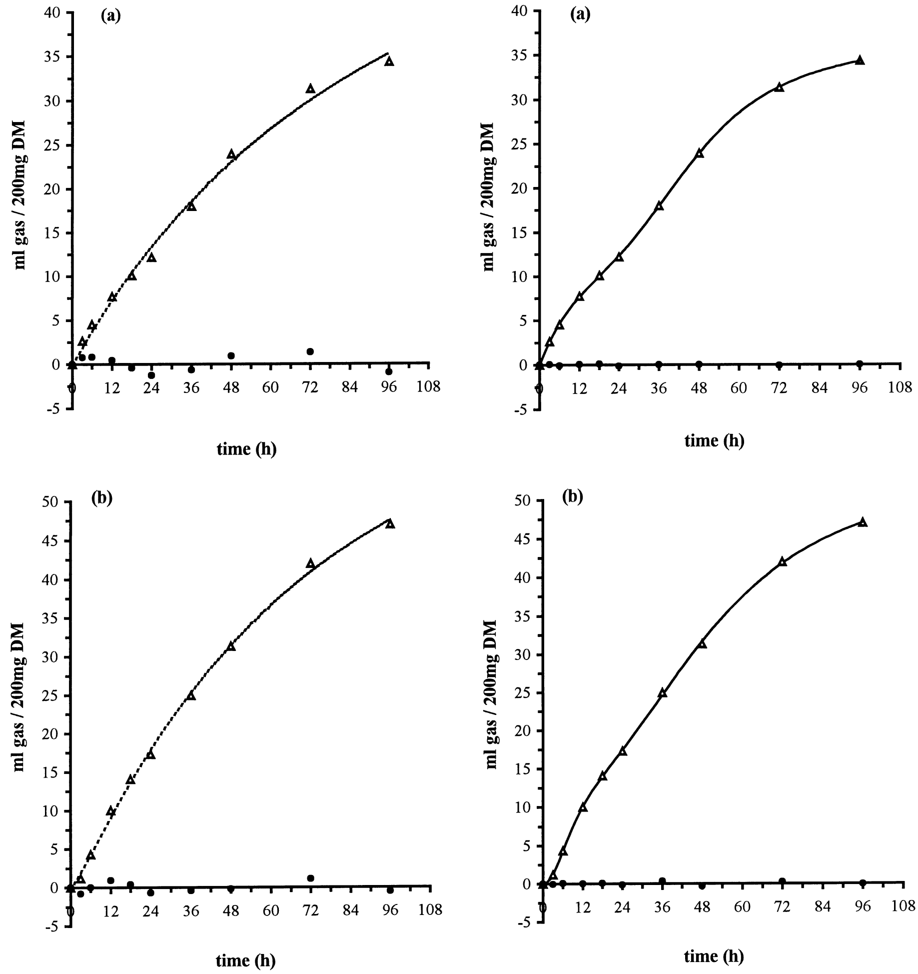

Fig. 1. Observed gas values (△), fitted monophasic model (- -), fitted diaphasic model (———), residuals (●). (a) millet genotype RCB-IC911; (b) sorghum genotype ICSV-700.

was tested using the Durbin–Watson (DW) coefficient (Theil, 1971) calculated as follows:

$$DW = \frac{\int_{i=2}^{n}(e_i - e_{i-1})^2}{\int_{i=1}^{n} e_i^2}$$

where $e_i = g_i - \text{ghat}_i$, $\text{ghat}_i$is the predicted gas; and $g_i$the gas at t_i.

As the Durbin–Watson (DW) table does not provide lower and upper bounds for the Durbin–Watson test statistics at the 5% level for number of observations <15, the critical values were extrapolated linearly using the values at $n = 15$ and $n = 16$, $k = 6$ and $k = 3$ (Theil, 1971). The lower critical value at 5% for $n = 9$ and $k = 6$ was 0.265. The Durbin–

Watson test statistics showed no significant autocorrelation for the fermentation profiles of sorghum and millet stems (DW greater than the lower critical value of 0.265 at the 5% significance level in all cases) (Table 4). The DW obtained was also greater than the lower critical value at $n = 15$ (1.08) at a significance level of 0.05. Therefore, potential errors in the linear extrapolation of the critical value of DW could be overcome.

3.4. Kinetics parameters

A set of parameters calculated from the fermentation curve is presented in Table 5:

$R_{m1}$and R_{m2} are the maximum fractional rates of substrate digestion (%/h) in the 1st and 2nd phases, respectively (Groot et al., 1996)
t_{m1} and t_{m2} the times (h) of occurrence of R_{m1} and R_{m2} (Groot et al., 1996)
t_{max1} and t_{max2} the times (h) of occurrence of maximum rates of gas production in the 1st and 2nd phases (Groot et al., 1996)
dg/dt_1 and dg/dt_2 the maximum rates of gas production (ml gas/200 mg DM/h) in the 1st and 2nd phases $(A_i * C_i * B_i^{C_i} * t_{max_i}^{(-C_i-1)}/(1+(B_i/t_{max_i})^{C_i})^2)$
When we varied the estimates of the initial parameters of the multiphase model, the parameters related to phase 2 of the model (R_{m2},t_{m2}, t_{max2} and dg/dt_2) varied relatively less in comparison to the parameters related to phase 1 (Table 4).

Correlations between fermentation kinetics parameters and fibre components, ash and N content in sorghum and millet stems, are presented in Table 6.

3.5. Robustness of fermentation kinetics parameters

Fermentation kinetics parameters A_1, A_2, t_{m1}, t_{m2}, R_{m1}, R_{m2}, t_{max1}, t_{max2}, dg/dt_1 and dg/dt_2 have shown stability with respect to the initial estimates of the model parameters. However, measurement errors can be a significant source of variation. The syringes used for the in vitro fermentation have a least count of 0.5 ml. With 200 mg of low-quality feed such as sorghum and millet residues; measurement errors at early hours of fermentation can produce large variations in the fermentation kinetics parameters. We can assume a 50% chance of a $\pm$0.25-ml deviation in gas observations. A simulation study was carried out to investigate the stability of fermentation-kinetics parameters with respect to measurement errors. The reading errors were generated from a probability distribution taking values of -0.25, 0.25 and 0 with a probability of 0.25, 0.25 and 0.5, respectively. The RANTBL function call of SAS (Statistical Analysis Systems Institute, 1989) was used to generate random samples of measurement error from the above-mentioned probability distribution at 3, 6, 12, 18, 24, 36, 48, 72, and 96 h. The generated reading errors were introduced in gas observations at each hour to produce an additional set of observations for each genotype of sorghum and millet stems. The multiphase model was then fitted to each of the 11 sets of observations for stems of each genotype of sorghum and millet with starting values of the model parameters reported above (Table 4). The iterations converged in all the cases. ANOVA (Statistical Analysis Systems Institute, 1989) was carried out on the simulation results. Table 7 shows the least square means and

Table 5
Least-squares means of fermentation kinetics parameters for stems of six genotypes of sorghum and millet (rep = 2)

Crop	Genotype	A_1[c,d]	A_2[c,e]	t_{m1} (h)[c,h]	R_{m1} (h)[c,f]	t_{m2} (h)[c,i]	R_{m2} (h^{-1})[c,g]	t_{max1} (h)[c,j]	t_{max2} (h)[c,k]	dg/dt_1[c,l]	dg/dt_2[c,m]
		(ml/200 mg DM)								(ml/200 mg DM/h)	
Sorghum	CSV-15	5.85b	42.93a	5.31c	0.199a	71.50a	0.019a	3.09c	42.7a	0.699b	0.470b
	ICSV-700	14.13a	37.97a	9.62a	0.115a	61.39b	0.026a	5.62a	37.5a	1.025ab	0.559ab
	ICSV-735	12.67ab	35.13a	8.24ab	0.146a	63.26ab	0.026a	4.77ab	38.8a	0.980ab	0.506b
	ICSV-890	5.91b	54.23a	6.95cb	0.513a	59.76b	0.017a	4.72ab	34.4ab	1.285a	0.506b
	LOCALFSR	6.56ab	44.01a	5.50c	0.323a	61.45b	0.022a	3.36c	36.7ab	1.052ab	0.557ab
	M35-1	9.71ab	48.31a	6.18cb	0.258a	47.45c	0.022a	3.73cb	27.7b	1.330a	0.628a
	SE[a]	2.08	9.35	0.64	0.101	2.3	0.006	0.29	2.4	0.100	0.028
Millet	BK650	5.83a	23.17a	4.71a	0.083a	47.1a	0.029ab	2.60a	28.1a	0.287a	0.395a
	Cz-IC41	4.13a	28.89a	3.42a	0.399a	39.9a	0.026b	2.05a	23.1a	0.836a	0.489a
	ICMP9282	6.08a	30.31a	0.00a	ne[b]	44.1a	0.025b	0.00a	25.8a	ne[b]	0.494a
	NokhaLocal	8.76a	22.49a	4.18a	0.219a	53.9a	0.030ab	2.40a	33.1a	0.533a	0.377a
	RCB-IC911	19.04a	20.98a	0.56a	0.055a	60.7a	0.040a	0.28a	39.3a	0.914a	0.417a
	RCB-IC912	18.87a	20.62a	0.74a	0.044a	64.8a	0.037ab	0.37a	42.0a	0.828a	0.370a
	SE[a]	4.72	6.2	1.80	0.250	5.6	0.003	0.85	4.2	0.067	0.090

[a] Standard error of the least-squares means.
[b] Not estimable.
[c] Means within a column without common letters differ ($p < 0.05$).
[d] Asymptotic gas in the first pahse
[e] Asymptotic gas in the second phase.
[f] Estiamted maximum fractional rate of substrate digestion in the first phase.
[g] Estiamted maximum fractional rate of substrate digestion in the second phase.
[h] Time of occurrence of R_{m1}.
[i] Time of occurrence of R_{m2}.
[j] Time of occurrence of maximum rate of gas production in the first phase.
[k] Time of occurrence of maximum rate of gas production in the second phase.
[l] Maximum rate of gas production in the first phase.
[m] Maximum rate of gas production in the second phase.

Table 6
Pearson's correlation coefficients of nutritive value indices ASH, NDF, ADF, LIGNIN and N with fermentation kinetics parameters ($n = 6$)

Crop	ASH	NDF	ADF	LIGNIN	N
Sorghum[c]					
A_1	−0.86[a]	−0.82[a]	−0.91[a]	−0.86[a]	−0.29
t_{m1}	−0.60	−0.73	−0.91[a]	−0.90[a]	−0.43
t_{m2}	0.60	0.68	0.42	0.42	0.00
R_{m2}	−0.84[a]	−0.55	−0.72	−0.61	0.06
t_{max1}	−0.48	−0.70	−0.88[a]	−0.87[a]	−0.49
t_{max2}	0.46	0.59	0.31	0.32	−0.00
dg/dt_1	−0.37	−0.56	−0.40	−0.37	−0.13
Millet[c]					
A_1	−0.40	−0.80[a]	−0.77	−0.95[b]	−0.31
t_{m1}	−0.82[a]	0.56	0.43	0.58	−0.82[a]
t_{m2}	−0.20	−0.62	−0.71	−0.87[a]	−0.17
R_{m2}	−0.25	−0.81[a]	−0.72	−0.93[b]	−0.13
t_{max1}	0.79	0.56	0.41	0.59	0.79
t_{max2}	−0.22	−0.65	−0.72	−0.88[a]	−0.18
dg/dt_1	−0.98[b]	−0.81	−0.86	−0.70	−0.93[a]

[a] Significant at $p < 0.05$.
[b] Significant at $p < 0.01$.
[c] A = asymptotic gas in the first phase; R_{m2} = estimated maximum fractional rate of substrate digestion in the second phase; t_{m1} = time of occurrence of Rm_1; t_{m2} = time of occurrence of Rm_2; t_{max1} = time of occurrence of maximum rate of gas production in the first phase; t_{max2} = time of occurrence of maximum rate of gas production in the second phase; dg/dt_1 = maximum rate of gas production in the first phase.

standard errors for the calculated parameters. These values should be compared with those in Table 4.

As expected, the variation in the calculated parameters of the first phase was relatively large. However, the parameters of the second phase have shown stability. The above simulation result indicates that in situations where measurement errors can be significant, a simulation study can be carried out to assess the applicability of feed quality parameters calculated from the multiphase model.

4. Discussion

This study has shown the applicability of the multiphase model with only nine gas observations over a period of 96 h. However, in some instances, lack of observations during the first increasing rate of gas production produced indeterminacy in the parameters of phase 1. Therefore, an intermediate value between 0 and 3 h should be chosen.

The kinetics of the gas profile provided a set of parameters that differentiated the probable substrates in sorghum and millet stems. Stems and leaves of sorghum and millet are not a uniform substrate, but a mixture of components, which can vary from genotype to genotype, and describing their digestion with a mathematical model is difficult

Table 7
Least square means of fermentation kinetics parameters for stems of six genotypes of sorghum and millet for one replicate after application of reading errors

Crop	Genotype	A_1[c]	A_2[d]	t_{m1}(h)[g]	R_{m1} (h^{-1})[e]	t_{m2} (h)[h]	R_{m2} (h^{-1})[f]	t_{max1} (h)[i]	t_{max2} (h)[j]	dg/dt_1[k]	dg/dt_2[l]
		(ml/200 mg DM)								(ml/200 mg DM/h)	
Sorghum	CSV-15	4.91	49.71	5.28	0.30	74.78	0.015	3.20	43.62	0.674	0.453
	ICSV-700	13.89	40.83	9.64	0.13	61.89	0.024	5.68	37.52	1.056	0.572
	ICSV-735	15.19	33.55	8.85	0.11	67.22	0.026	4.99	41.71	0.845	0.473
	ICSV-890	6.77	38.67	7.65	0.35	63.19	0.025	4.97	38.54	1.084	0.546
	LOCALFSR	6.92	49.57	4.04	0.50	65.08	0.017	2.61	38.08	1.164	0.525
	M35-1	11.83	38.23	7.34	0.18	53.06	0.029	4.38	32.24	1.277	0.630
	SE[a]	0.96	1.49	0.31	0.05	1.26	0.001	0.19	1.01	0.050	0.010
Millet	BK560	17.51	22.98	0.75	0.20	38.75	0.028	0.41	22.55	0.342	0.380
	CZ-IC41	3.74	31.72	0.74	0.26	39.97	0.022	0.42	22.82	0.884	0.466
	ICMP9282	3.58	35.61	0.04	0.19	41.58	0.021	0.02	23.73	0.774	0.507
	NokhaLocal	2.37	30.56	1.66	0.57	45.70	0.022	1.06	26.42	0.649	0.431
	RCB-IC911	12.94	31.23	0.00	ne[b]	55.39	0.026	0.00	33.33	ne[b]	0.460
	RCB-IC912	17.37	24.54	0.65	0.12	51.23	0.051	0.35	32.60	0.684	0.484
	SE[a]	3.84	1.66	0.37	0.25	0.75	0.006	0.22	0.60	0.220	0.014

[a] Standard error of the least-squares means.
[b] Not estimable.
[c] Asymptotic gas in the first phase.
[d] asymptotic gas in the second phase.
[e] Estimated maximum fractional rate of substrate digestion in the first phase.
[f] Estimated maximum fractional rate of substrate digestion in the second phase.
[g] Time of occurrence of R_{m1}.
[h] Time of occurrence of R_{m2}.
[i] Time of occurrence of maximum rate of gas production in the first phase.
[j] Time of occurrence of maximum rate of gas production in the second phase.
[k] Maximum rate of gas production in the first phase.
[l] Maximum rate of gas production in the second phase.

(Beuvink and Kogut, 1993). However, mathematical modeling could provide insight into changes in the digestion kinetics of the carbohydrate components and the contribution of carbohydrates to total output of rumen fermentation (Doane et al., 1997).

4.1. Feed quality indicators from the kinetic curves

The juxtaposition of sorghum stem-fermentation curves with the multiphasic model shows the production of gas at two distinct rates corresponding to two phases, possibly from the fermentation of two distinct substrates. The fit of a single phase for the millet stems, with a relatively uniform fermentation rate, suggests the predominance of one substrate type of slowly degradable components with little contribution derived from soluble components.As the neutral detergent fibre (NDF) and lignin content of sorghum and millet stems increased, the asymptotic curve for gas in the first phase, representing the highly degradable fraction of the residue, decreased (NDF: $r = -0.82$, $p < 0.05$; $r = -0.80$, $p < 0.05$, respectively. Lignin: $r = -0.86$, $p < 0.05$; $r = -0.95$, $p < 0.01$, respectively). This is in agreement with results reported by Doane et al. (1997) for forage samples. However, the same authors cautioned against the establishment of associations between the identified highly degradable substrate pool through mathematical analysis of the gas profile and the chemical or physical analysis of unfractionated forage.

At 48 h, genotypes ICSV 700 and M35-1 had similar volumes of cumulative gas. However, whereas in ICSV 700 the contribution of gas from phase 2 was similar to the contribution of phase 1, in M35-1 the contribution of phase 2 was almost double that of phase 1 (Fig. 2). Similar comparisons can be made at different hours with other genotypes. These estimated differences in the contribution from the different phases — possibly representing the highly and slowly degradable fractions — to similar cumulative gases at 48 h in sorghum stems indicate the need to complement measurements of total gas production with selected kinetics parameters. The objective of in vivo experimentation currently being conducted at our Institute is to identify and validate appropriate parameter(s) that will characterize more adequately feed quality of sorghum and millet residues. This information will allow crop breeders to incorporate the validated traits into crop improvement programmes.

R_{m1}, which represents an estimate of the maximum fractional rate of digestion of the highly digestible substrate stem component, was not significantly different between genotypes. However, there was a high degree of variation and the magnitude of the differences observed could affect the total utilization of the sorghum and millet residues. In sorghum, t_{m1} was significantly different between genotypes and was negatively correlated with ADF and lignin ($r = -0.91$, $p < 0.05$; $r = -0.80$, $p < 0.05$, respectively). These results show that the type of fibre present in the roughage affects the time at which the maximum fractional rate of highly degradable substrate digestion occurs. R_{m2} of stems of millet genotypes were significantly different, while R_{m2} of sorghum were similar. In sorghum stems, R_{m2} ranged from 1.7 to a maximum of 2.6%/h and was negatively correlated with ash content ($r = -0.84$, $p < 0.05$), ADF and lignin. In millet, R_{m2} ranged from 2.5 to 4%/h and was negatively correlated only with lignin ($r = -0.93$, $p < 0.01$). R_{m2}values obtained in this study relate well with in vivo estimates of the solid phase turnover rate observed in in vivo studies (Faichney, 1986; Zerbini et al., 1995).

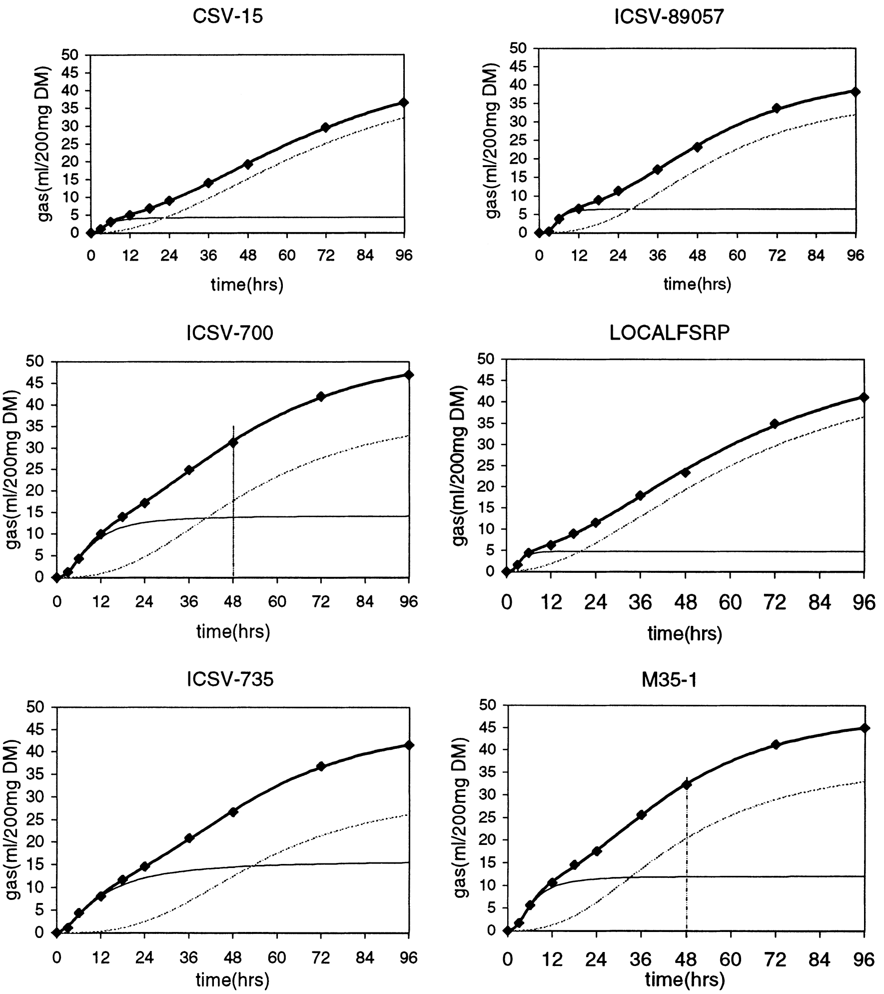

Fig. 2. Observed gas data (■), fitted multiphasic model (———), Phase 1 (—), Phase 2 (············) for the 6 genotypes of sorghum stems.

For sorghum stems, the time at which the rate of fermentation reached its maximum in phase 1 (t_{max1}) was significantly different between genotypes, and, in general, it was consistent with differences observed for t_{m1} and A_1. These parameters showed greater variation in millet stems. In sorghum, t_{max1} was negatively correlated with ADF and lignin ($r = -0.88$, $p < 0.05$ and $r = -0.87$, $p < 0.05$, respectively), whereas for millet only t_{max2} was negatively correlated with lignin ($r = -0.88$, $p < 0.05$).

For both, sorghum and millet genotypes, the maximum rates of gas production in phase 2 (dg/dt_2) were not correlated to any of the fibre components, ash or nitrogen. On the other hand, the maximum rate of gas production in phase 1 (dg/dt_1) was negatively correlated with ash and nitrogen, while its associations with neutral detergent fibre (NDF) and acid detergent fibre (ADF) were weak. The negative correlation of nitrogen with t_{m1} and dg/dt_1 in millet could be related to the non-significant inverse correlation of nitrogen and gas as shown in Table 3.

The degree of variation observed in parameters related to both, phases 1 and 2 seems to suggest that there could be potential for improvement by investigating factors affecting the fermentation of the more soluble as well as the less degradable portion of the roughage substrate. This could depend on the crop studied and perhaps on the important plant parts. For example, Blummel and Becker (1997) reported that 75% of the variation in dry matter (DM) intake was accounted for by in vitro gas production of whole roughage after 8 h of incubation which represented estimates of the highly degradable fraction of that forage.

Important factors contributing to variations in the soluble and the slowly degradable fraction of crop residues are the presence of cell-wall components that resist or inhibit microbial degradation and the physical structure of the cell wall itself. Genetic enhancement of sorghum and millet crops oriented towards the development of dual-purpose breeding material can result in modifications of the structure as well as in the chemical composition of the cell wall of the plant such as in brown mid-rib (bmr) mutants (Degenhart et al., 1995). These differences could affect significantly the utilization of crop residues by ruminants. Identification of the optimal set of parameters that describes genotypic differences will be an essential component of developing improved dual-purpose varieties.

5. Conclusions

The stability of the final values of parameters of the model shown for sorghum stems indicates that the multiphase model can also be applied in cases where only nine observations are available. Especially in sorghum stems, the model was able to differentiate an initial phase, possibly corresponding to the rapid fermentation of the mesophyll cells, and a second phase that could be attributed to the fermentation of the larger component of cell walls composed of bundle sheath cells and sclerenchymal tissue (Akin, 1979; Cheng et al., 1980). On the other hand, for millet stems, only one significant phase could be detected. Analysis of millet stems using rumen fluid from millet-fed animals could provide a better resolution of millet fermentation kinetics, especially during the initial stages of fermentation.

Cumulative gas production at different incubation hours and some kinetics parameters were strongly correlated with fibre components. However, possible substrate differences may not always be reflected in cumulative gas production differences. In such cases, kinetic parameters could be used to identify substrate differences even when genotypes show similar cumulative gas. These results point out the difficulty in finding a single feed quality parameter to be used to rank different crop residues of different genotypes. Additional studies are needed to associate this parameter with in vivo rumen outflow rates with which these phases could be associated.

There is evidence showing associations between cell wall content and cell wall characteristics and animal voluntary intake (Illius and Gordon, 1991; Martens, 1985; Van

Soest, 1965). The strong relationship shown in this study between a number of model parameters and NDF, ADF and lignin is encouraging and has potential for a better evaluation of different genotypes relating in vitro estimates to potential animal performance. However, in vivo experimentation will have to be conducted to establish the quantitative relationships.

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Appendix

SAS macro to fit a multiphase model with two phases. The description of the parameters of this subroutine are indicated below:

DATA=name of the SAS data set containing the gas values of a fermentation profile.
G=name of the variable in the data set DATA holding the gas values
a1=starting value of A1
b1=starting value of B1
a2=starting value of A2
b2=starting value of B2

```
%MACRO FIT(DATA,G,a1,b1,a2,b2);

PROC NLIN DATA=&DATA METHOD=MARQUARDT MAXITER=200 G4SINGULAR
   SMETHOD=halve RHO=2 BEST=1;
PARAMETERS a1=&a1 b1=&b1 c1=2
   a2=&a2 b2=&b2 C2=2;

BOUNDS 1E-2<=a1<100,
      1E-2<=b1<200,
      1<c1<5,
      1E-2<=a2<100,
      1E-2<=b2<200,
      1<c2<5;
a1_ini=&a1;
a2_ini=&a2;
b1_ini=&b1;
b2_ini=&b2;
c1_ini=2;
c2_ini=2;
x1=1+( (b1/t)**c1 );
```

```
y1=−a1/(x1**2);
x2=1+( (b2/t)**c2 );
y2=−a2/(x2**2);

MODEL &G=(a1/(1+(b1/t)**c1))+(a2/(1+(b2/t)**c2));
DER.a1=1/x1;
DER.b1=y1*c1*(b1**(c1−1))/(t**c1);
DER.c1=y1*( (b1/t)**c1 )*LOG(b1/t);
DER.a2=1/x2;
DER.b2=y2*c2*(b2**(c2−1))/(t**c2);
DER.c2=y2*( (b2/t)**c2 )*LOG(b2/t);
ID a1_ini a2_ini b1_ini b2_ini c1_ini c2_ini ;
OUTPUT OUT=NLIN_OUT P=PRE R=RESI SSE=RESI_SS STUDENT=STD_RESI
PARMS=A1 B1 C1 A2 B2 C2;
run;
PROC PRINT DATA=NLIN_OUT;
VAR T &G PRE RESI STD_RESI;
RUN;
%MEND FIT;
```

The above subroutine can be modified for any number of phases.

References

Akin, D.E., 1979. Chemical and biological structure in plants as related to microbial degradation of forage cell walls, pp. 139–157. In: Milligan, L.P., Grovum, W.L., Dobson, A. (Eds.), Control of Digestion and Metabolism in Ruminants. Prentice–Hall, Englewood Cliffs, NJ 07632, 567 p.

Association of Official Analytical Chemists (AOAC), 1980. Official Methods of Analysis, 13th ed. Association of Official Analytical Chemists, Washington, DC.

Beuvink, J.M.W., Kogut, J., 1993. Modeling gas production kinetics of grass silages incubated with buffered ruminal fluid. J. Anim. Sci. 71, 1041–1046.

Blummel, M., Becker, K., 1997. The degradability characteristics of fifty-four roughages and roughage neutral-detergent fibres as described by in vitro gas production and their relationship to voluntary feed intake. Br. J. Nutr. 77, 757–768.

Blummel, M., Bullerdieck, P., 1997. The need to complement in vitro gases production measurements with residue determinations from in sacco degradabilities to improve the prediction of voluntary intake of hays. Anim. Sci. 64, 71–75.

Blummel, M., Orskov, E.R., 1993. Comparison of in vitro gas production and nylon bag degradability of roughages in predicting feed intake in cattle. Anim. Feed Sci. Technol. 40, 109–119.

Cheng, K.J., Fay, J.P., Howarth, R.E., Costerton, J.W., 1980. Appl. Environm. Microbiol. 40: 613–625.

Cone, J.W., van Gelder, A.H., Visscher, G.J.W., Oudshoorn, L., 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. Anim. Feed Sci. Technol. 61, 113–128.

Degenhart, N.R., Werner, B.K., Burton, G.W., 1995. Forage yield and quality of a brown mid-rib mutant in pearl millet. Crop Sci. 35, 986–988.

Doane, P.H., Schofield, P., Pell, A.N., 1997. 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.

Faichney, G.J., 1986. The kinetics of particulate matter in the rumen, pp.173–195. In: Milligan, L.P., Grovum, W.L., Dobson, A. (Eds.), Control of Digestion and Metabolism in Ruminants. Prentice–Hall, Englewood Cliffs, NJ 07632, 567 p.

France, J., Dhanoa, M.S., Theodorou, M.K., Lister, S.J., Davies, D.R., Isac, D., 1993. A model to interpret gas accumulated gas accumulation profiles associated with in vitro degradation of ruminant feeds. J. Theoret. Biol. 163, 99–111.

Getachew, G., Blummel, M, Makkar, H.P.S., Becker, K., 1998. In vitro gas measuring technique for assessment of nutritional quality of feeds: a review. Anim. Feed Sci. Technol. 72, 261–281.

Groot, J.C.J., Cone, J.W., Williams, B.A., Debersaques, F.M.A., Lantinga, E.A., 1996. Multiphase analysis of gas production kinetics for in vitro fermentation of ruminant feeds. Anim. Feed Sci. Technol. 64, 77–89.

Illius, A.W., Gordon, I.J., 1991. Prediction of intake and digestion in ruminants by a model of rumen kinetics integrating animal size and plant characteristics. J. Agric. Sci., Cambridge 116, 145–157.

Khazaal, K., Dentinho, M.T., Ribeiro, J.M., Orskov, E.R., 1993. A comparison of gas production during incubation with rumen contents in vitro and nylon bag degradability as predictors of the apparent digestibility in vivo and the voluntary intake of hays. Anim. Prod. 57, 105–112.

Martens, D.R., 1985. Factors influencing feed intake in lactating cows: from theory to application using neutral detergent fiber. In: Proceedings of Georgia Nutrition Conference, Atlanta, University of Georgia, Athens, p. 1.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Shneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor. J. Agric. Sci. 93, 217–222.

Osuji, P., Nsahlai, I.V., Kalili, H., 1993. Feed evaluation. ILCA Manual 5. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia, 40 p.

Schofield, P., Pitt, R.E., Pell, A.N., 1994. Kinetics of fiber digestion from in vitro gas production. J. Anim. Sci. 72, 2980–2991.

Statistical Analysis Systems Institute, 1989. SAS/STAT User's Guide, version 6, 4th ed., vol. 2. Statistical Analysis Systems Institute Inc., Cary, NC.

Theil, H., 1971. Principles of Econometrics. John Wiley & Sons, Inc., New York, 736 p.

Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48, 185–197.

Van Soest, P.J., Robertson, J.B., 1985. Analysis of forage and fibrous foods. Laboratory Manual for Animal Science. Cornell University, USA, 613 p.

Van Soest, P.J., 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. J. Anim. Sci. 24, 834.

Zerbini, E., Gemeda, T., Gebre Wold, A., Nokoe, S., Demissie, D., 1995. Effect of draught work on performance and metabolism of crossbred cows 2. Effect of work on roughage intake, digestion, and digesta kinetics and plasma metabolites. Anim. Sci. 60, 369–378.