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Modeling *in vitro* gas production kinetics: Derivation of Logistic–Exponential (LE) equations and comparison of models

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ABSTRACT

A Logistic–Exponential (LE) model was developed by introducing a shape parameter *d* into the logistic function. The LE models included three mathematical forms of LE_s, LE_0 and LE_{LAG}, where LE_s was a standard LE model, LE_0 was the LE_s model re-parameterized with '$V_0 = 0$', and LE_{LAG} was the LE_0 model with an additional discrete *LAG* time. The LE models generated a wide range of curve shapes, and *d* revealed whether rate of gas production was or was not increased during *in vitro* incubation. The performances of the LE_0 and LE_{LAG} models were evaluated by comparison with published models. For example, one-pool models included exponentials with and without *LAG* (EXP_{LAG} and EXP_0), Gompertz (GOM), Logistic (LOG), generalization of the Mitscherlich (GM) and Michaelis–Menten (MM); while two-pool models included diphase Michaelis–Menten (DGMM), diphase Exponential with *LAG* ($DEXP_{LAG}$), diphase Gompertz (DGOM) and diphase Logistic (DLOG) models. Gas production data consisting of 23 curves, obtained from a wide range of feeds, were used to investigate the fit performance of the models. Statistical criteria used were analysis of residuals (*i.e.*, run test and serial correlation) and goodness-of-fit (*i.e.*, residual mean squares, root mean squared prediction error, akaike's information criterion, observed *versus* predicted regression). Gas production attributes of final asymptotic gas volume (V_F), gas volume at 24 h ($V_{24\,h}$), half-life ($t_{0.5}$) and fractional rate of gas production at half-life ($\mu_{0.5}$) were compared among one-pool models. The LE_0 was better than EXP_{LAG} and EXP_0 models based upon overall statistical criteria, and better than GM and LOG models according to akaike's information criterion. The MM model had higher goodness-of-fit than the LE models, and had the highest V_F and $t_{0.5}$, and lowest $V_{24\,h}$ and $\mu_{0.5}$. The GOM and LOG had higher goodness-of-fit than the LE models, but positive gas volumes at '$t = 0$' were biologically meaningless. Two-pool models could improve the fit to curve, but led to declined robustness. There were no differences of predicted gas production attributes between the LE and GM models. Results indicate that the LE models can be alternatives to describe *in vitro* gas production kinetics.

Abbreviations: ADF, acid detergent fiber; AIC, Akaike's information criterion; CP, crude protein; $DEXP_{LAG}$, diphase Exponential model with discrete *LAG* time; DGOM, diphase Gompertz model; DLOG, diphase logsitic model; DM, dry matter; DMM, diphase Michaelis–Menten model; DW, Durbin–Watson; EXP_0, exponential model without discrete *LAG* time; EXP_{LAG}, exponential model with discrete *LAG* time; GOM, Gompertz model; GM, generalization of the Mitscherlich model; LEs, standard Logistic–Exponential; LE_0, LE_s model with '$V_0 = 0$'; LE_{LAG}, LE_0 model with an discrete *LAG* time; LOG, logistic model; MM, Michaelis–Menten model; MSPE, mean squared prediction error; NDF, neutral detergent fiber; OM, organic matter; RMS, residual mean squares; rMSPE, root mean squared prediction error; SD, standard deviation; $t_{0.5}$, half-life; $\mu_{0.5}$, fractional rate of gas production at half-life; V_F, final asymptotic gas volume; $V_{24\,h}$, gas volume at 24 h.

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1. Introduction

In vitro gas accumulation measurements provide valuable information about the kinetics of feed digestion in rumen fluid. Mathematical models are necessary tools to describe and interpret *in vitro* gas production kinetics and, during the last few decades, a great number of models have been proposed. Most of these models are sigmoidal shapes, except the exponential (EXP) model (France et al., 2000). Among sigmoidal functions, Logistic (LOG) and Gompertz (GOM) are sigmoidal shapes with fixed inflection points (Schofield et al., 1994; Thornley and France, 2005), while generalized Mitscherlich (GM) and Michaelis–Menten (MM) models are sigmoidal shapes without fixed inflection points (Beuvink and Kogut, 1993; France et al., 1993; Groot et al., 1996). Multiple components in a single model has also been employed to improve overall goodness-of-fit (Schofield et al., 1994; Groot et al., 1996), but may bring a decline in model robustness (Motulsky and Ransnas, 1987). New biologically meaningful models are needed to allow modeling of wide ranges of curve shapes with variable inflexion points (France et al., 2000).

The LOG model was firstly developed for *in vitro* gas production kinetic studies by Schofield et al. (1994), based on the assumption that rate of gas production was affected by both current microbial mass and substrate level. However, a LOG model might not be suitable in some cases because of its fixed inflexion point at half of final gas volume (Dhanoa et al., 2000; France et al., 2000). Furthermore, LOG models have positive intercepts, which cause an inherent error in predicting gas volume (Schofield et al., 1994). In the present study, the first objective was to develop new LE models with more flexible logistic functions by introducing a shape parameter d. It was called an LE model, because it could be rewritten as a Logistic–Exponential mixed equation. Three mathematical forms of LE models were further described and biologically interpreted, respectively. The second objective was to evaluate the performance of LE models by comparing them with 6 one-pool and 4 two-pool models. Feedstuffs, 23, were used to provide a wide range of *in vitro* gas production kinetics. Analysis of residuals, goodness-of-fit and gas production attributes were included for model comparison.

2. Materials and methods

2.1. The Logistic–Exponential (LE) models

We assumed that the rate of gas production (ml/h) was proportional to both current microbial mass (M, g) and substrate level (S, g) (Schofield et al., 1994) where:

$$\frac{dV}{dt} = \alpha MS \tag{1}$$

and α is a proportionality constant. When microbial mass and substrate level were converted to gas volume, Eq. (1) was converted to:

$$\begin{cases} \dfrac{dV}{dt} = c\left(\dfrac{V}{V_F} + d\right)\left(1 - \dfrac{V}{V_F}\right) \\ d = \dfrac{\beta M_0/Y_G - V_0}{V_F} \\ c = \dfrac{\alpha V_F^2}{\beta} \\ \beta = \dfrac{V - V_0}{S_0 - S} \\ Y_G = \dfrac{M - M_0}{S_0 - S} \end{cases} \tag{2}$$

where M_0 and S_0 are microbial mass and substrate level with dimensions of 'g' at starting incubation time ($t = 0$); V_0 and V_F are a hypothetical starting and final asymptotic gas volume with dimensions of 'ml'; Y_G is the constant, which links microbial mass and digested substrate; β is the constant with dimension of 'ml/g', which links produced gas volume and digested substrate. When '$d = 0$', Eq. (2) can be expressed in the classic logistic form of '$dV/dt = cV(1 - V/V_F)/V_F$' (Schofield et al., 1994). When '$d \to +\infty$', Eq. (2) can be expressed in the classic exponential form of '$dV/dt = cd(1 - V/V_F)$'. In our study, we assumed that d was not zero to present a more generalized concept because the substrate was not digested at '$t = 0$', and 'V_0/M_0' might, or might not, follow the pattern of 'dV/dM' during incubation.

We called Eq. (2) the Logistic–Exponential (LE) model, because Eq. (2) could be rewritten as '$dV/dt = cV(1 - V/V_F)/V_F + cd(1 - V/V_F)$', where c is a constant with dimension of 'ml/h', and d is a shape parameter

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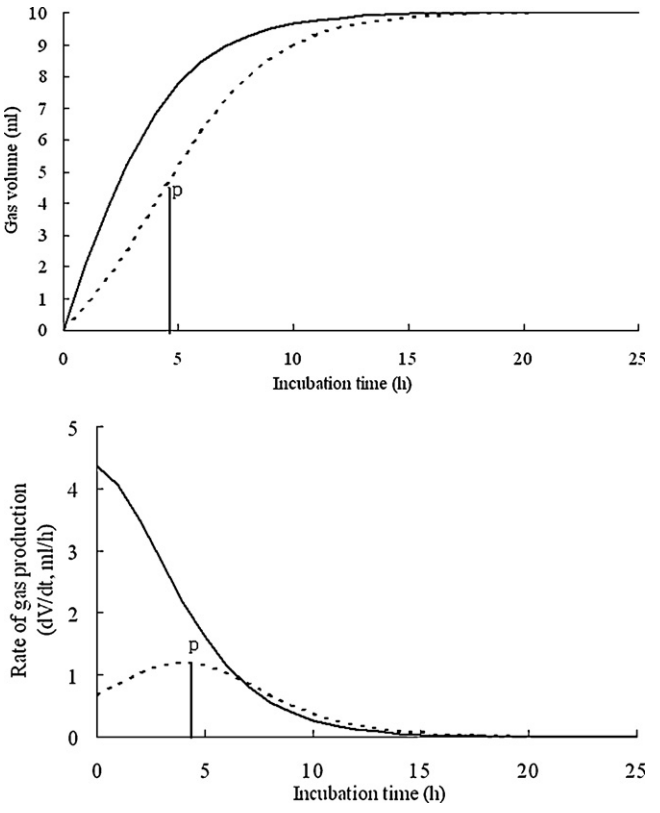

Fig. 1. Behaviours of the Logistic–Exponential model with re-parameterized initial gas volume of zero (LE_0). Solid line: non-sigmoidal curve with $V_F = 10$ ml, $d = 1.2$ and $k = 0.4$/h; broken line: sigmoidal curve with $V_F = 10$ ml, $d = 0.2$ and $k = 0.4$/h. The 'p' is the inflexion point of sigmoidal curve.

without dimension. On integration, Eq. (3) can be expressed as a standard Logistic–Exponential (LE_s) model with the form:

$$\begin{cases} V = V_F\left(\dfrac{1+d}{1+\exp(b-kt)} - d\right) \\ b = \ln\left(\dfrac{1 - V_0/V_F}{V_0/V_F + d}\right) \\ k = \dfrac{c(d+1)}{V_F} \end{cases} \tag{3}$$

when '$V_0 = 0$' was introduced into Eq. (3) to exclude the possibility of a positive intercept, '$b = \ln(1/d)$' was attained. This LE_0 model was expressed as:

$$V = \frac{V_F(1 - \exp(-kt))}{1 + \exp(\ln(1/d) - kt)} \tag{4}$$

where k is the fractional rate of gas production with dimension of '/h', and d is a positive shape parameter. The behaviors of LE_0 model are illustrated by Fig. 1 where '$0 < d < 1$' indicates a sigmoidal shape, while '$d > 1$' indicates a non-sigmoidal shape.

The first derivative of Eq. (3), represented as rate of gas production, can be expressed as:

$$\frac{dV}{dt} = \frac{V_F(1+d)k\,\exp(b-kt)}{(1+\exp(b-kt))^2} \tag{5}$$

and the second derivative of Eq. (3) can be expressed as:

$$\frac{d^2V}{dt^2} = \frac{V_F k^2(1+d)\exp(b-kt)(\exp(b-kt)-1)}{(1+\exp(b-kt))^3} \tag{6}$$

when '$d^2V/dt^2 = 0$', '$t_i = b/k$'. The inflexion of the LE_s model (Eq. (3)) is attained at point ($t_i = b/k$, $V_i = V_F(1-d)/2$) for a sigmoidal curve, where the maximum rate of gas production ($(dV/dt)_{t_i}$, ml/h) occurred. '$b < 0$' and '$b > 0$' indicate sigmoidal and non-sigmoidal shapes, respectively. Using $\ln(1/d)$ to substitute b of LE_s model, the inflexion of LE_0 model is attained at point ($t_i = \ln(1/d)/k$, $V_i = V_F(1-d)/2$) for the sigmoidal curve (Fig. 1).

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Table 1
Chemical composition of the forage (g/kg DM).

	Average	Maximum	Minimum	SD
OM	903	967	777	43.7
CP	118	274	29.0	63.4
NDF	609	795	346	130
ADF	366	503	175	76.0

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; SD, standard deviation.

When a discrete *LAG* time (h) before degradation began was assumed for the LE_0 model, Eq. (7) was developed by introducing *LAG* into Eq. (6), called LE_{LAG} model and expressed as:

$$\begin{cases} V = 0 & t < LAG \\ V = \dfrac{V_F(1-\exp(-k(t-LAG)))}{1+\exp(\ln(1/d)-k(t-LAG))} & t \geq LAG \end{cases} \quad (7)$$

2.2. Data of in vitro gas production

A sample set of 23 *in vitro* 48 h gas production curves was obtained from our previous studies (Tang et al., 2005, 2006, 2008). The 10 types of grass were: *Rumex* K-1, *Lolium perenne* L., *Medicago sativa* Linn., *Trifolium pratense* L., *Secale cereale*, *Sorghum sudanense*, (*Pennisetum purpureum* × *P. americanum*) × *P. purpureum* cv. Mott, *Pennisetum Purpureum Schum.* cv. Mott, *Pennisetum hydridum*, *Hemarthria compressa* (Tang et al., 2005, 2008). The 4 types of straw and stover were: rice straw, wheat straw, maize stover silage and maize stover (Tang et al., 2005, 2008). The 9 botanical fractions of maize stover were: anthotaxy, leaf blade, leaf sheath, whole stem, upper stem, lower stem, cob, husk, and tassel (Tang et al., 2006). The chemical composition of the 23 feedstuffs is in Table 1. The dry matter (DM) content was determined in triplicate by oven drying at 105 °C for 24 h. Organic matter (OM) was determined by ashing at 550 °C for 12 h. The N content was determined according to a Kjeldahl method (*i.e.*, ID 954.01) of AOAC (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using methods of Van Soest et al. (1991), without using sodium sulfite and heat stable amylase, and expressed inclusive of residual ash.

Samples (200 ± 1 mg) of oven dry feedstuffs were accurately weighed into 100 ml glass syringes fitted with plungers. Syringes were filled with 30 mL of medium consisting of 10 mL of rumen fluid and 20 mL of McDougall's buffer solution. Gas volumes were recorded at 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36 and 48 h of incubation. For the incubation procedure details, see our previous papers (Tang et al., 2006, 2008). Gas production profiles are in Fig. 2.

2.3. Models and curve-fitting

One-pool models are presented in Table 2 to describe *in vitro* gas production kinetics. The LE_s of LE model was excluded for curve-fitting, because a positive intercept at $t = 0$ was biologically impossible and contradictory to initial gas volume being zero. Other two LE models (*i.e.*, LE_0 and LE_{LAG}) are used, and illustrated by Eqs. (4) and (7). Exponential equations included exponentials with (EXP_{LAG}) and without discrete *LAG* time (EXP_0) (Schofield et al., 1994). The generalization of the Mitscherlich (GM) model was developed by a modified degradation rate with time, and could be suitable for either

Table 2
Description of one-pool models.

Model	Equation	$t_{0.5}$ (h)	$\mu_{0.5}$ (/h)	Domain
LE				
LE_0	$V = V_F \frac{1-\exp(-kt)}{1+\exp(\ln(1/d)-kt)}$	$\frac{\ln(2+1/d)}{k}$	$\frac{k(d+0.5)}{(1+d)}$	$t \geq 0$
LE_{LAG}	$V = V_F \frac{1-\exp(-k(t-LAG))}{1+\exp(\ln(1/d)-k(t-LAG))}$	$LAG + \frac{\ln(2+1/d)}{k}$	$\frac{k(d+0.5)}{(1+d)}$	$t \geq LAG$
Exponential				
EXP_0	$V = V_F(1-\exp(-kt))$	$\frac{\ln(0.5)}{k}$	k	$t \geq 0$
EXP_{LAG}	$V = V_F(1-\exp(-k(t-LAG)))$	$LAG + \frac{\ln(0.5)}{k}$	k	$t \geq LAG$
GOM	$V = V_F\exp(-\exp(1-k(t-\lambda)))$	$\lambda + \frac{1-\ln(\ln(2))}{k}$	$\ln(2)k$	$t \geq 0$
LOG	$V = \frac{V_F}{1+\exp(2+k(\lambda-t))}$	$\lambda + \frac{2}{k}$	$k/2$	$t \geq 0$
GM	$V = V_F(1-\exp(-k(t-LAG)-d(\sqrt{t}-\sqrt{LAG})))$	$(\sqrt{d^2/(4k^2)+(kLAG+d\sqrt{LAG}-\ln(0.5))/k}-d/2)^2$	$k+\frac{d}{2\sqrt{t_{0.5}}}$	$t \geq LAG$
MM	$V = \frac{V_F t^c}{t^c+K^c}$	K	$\frac{c}{2K}$	$t \geq 0$

LE_0 and LE_{LAG}, Logistic–Exponential (LE) models with Eqs. (4) and (7), respectively; EXP_0 and LE_{LAG}, Exponential model without and with discrete *LAG* time, respectively; GOM, Gompertz model; LOG, Logistic model; GM, Generalization of the Mitscherlich model; MM, Michaelis–Menten model; V_F, final asymptotic gas volume; k, fractional rate of gas production; K, the time at $V_F/2$; λ, derived lag time; *LAG*, discrete *LAG* time; c, and d, shape parameters; $t_{0.5}$, half-life; $\mu_{0.5}$, fractional rate of gas production at half-life.

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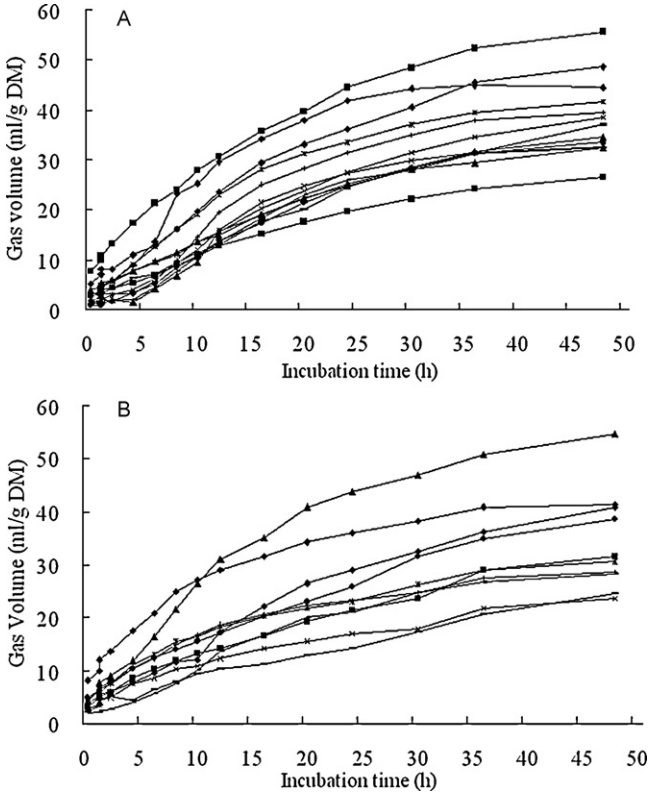

Fig. 2. The 23 in vitro gas production curves. (A) grasses, straws and stover (13): *Rumex* K-1, *Lolium perenne* L., *Medicago sativa* Linn., *Trifolium pratense* L., *Secale cereale*, *Sorghum sudanense*, (*Pennisetum purpureum* × *P. americanum*) × *P. purpureum* cv. Mott, *Pennisetum Purpureum Schum.* cv. Mott, *Pennisetum hydridum*, *Hemarthria compressa*; rice straw; wheat straw; maize stover silage; (B) maize stover and its botanical fractions (10): whole maize stover, anthotaxy, leaf blade, leaf sheath, whole stem, upper stem, lower stem, cob, husk, and tassel.

sigmodial and non-sigmodial shapes (France et al., 2000). The Michaelis–Menten (MM) model was developed in enzyme kinetics and first employed to describe *in vitro* gas production kinetics by Groot et al. (1996). The Gompertz (GOM) and Logistic (LOG) models were developed to describe microbial growth, and first employed to study *in vitro* gas production kinetics by Schofield et al. (1994).

The one-pool model could be extended to the diphasic analysis under the assumption that potential degradable substrate was comprised of two components, being a rapidly and a slowly degradable fraction (Schofield et al., 1994; Groot et al., 1996). For two-pool models, we employed diphase Michaelis–Menten (DMM), diphase Exponential with *LAG* ($DEXP_{LAG}$), diphase Gompertz (DGOM) and diphase Logistic (DLOG) models.

2.4. Statistical analyses

Durbin–Watson (DW) statistic, coefficient of determination (R^2) and residual sum of squares (RSS) were obtained by analyzing the data of gas volume from 0.5 to 48 h with NLREG (Sherrod, 1991). Root mean square prediction error (*rMSPE*) is an indicator of overall deviation between the observed and predicted values, and can be calculated as:

$$\sqrt{\sum_{i=1}^{n}\frac{(VP_i - VO_i)^2}{n}}$$

where VP_i and VO_i are predicted and observed gas volumes respectively, and n is the number of data points defining each individual curve. The MSPE is divided into three components resulting from bias, slope and random variation around the regression line (Bibby and Toutenburg, 1977; Dhanoa et al., 1999), which are calculated as:

$$\begin{cases} \text{bias} = (VP - VO)^2 \\ \text{slope} = (S_{VP} - rS_{VO})^2 \\ \text{random} = (1 - r^2){S_{VO}}^2 \end{cases}$$

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where *VP* and *VO* are average predicted and observed gas volumes, respectively; and S_{VP} and S_{VO} are standard deviations of predicted and observed gas volumes, respectively; and *r* is calculated as:

$$\frac{1}{n}\sum_{i=1}^{n}\frac{(VO_i - VO)(VP_i - VP)}{S_{VO}S_{VP}}$$

Akaike's Information Criterion (*AIC*) was employed to compare goodness-of-fit of models with the same and different numbers of parameters. The *AIC* is calculated as:

$$AIC = n\ln\left(\frac{RSS}{n}\right) + 2(p+1) + \frac{2(p+1)(p+2)}{(n-p-2)}$$

where *RSS* is residual sum of squares; *n* is the number of data points; and *p* is the number of parameters of the model. Residual mean squares (*RMS*) are calculated by *RSS*/*n*. The model with the smaller *AIC* is most likely to be better and provides the standard for pair-wise comparison (Motulsky and Christopoulos, 2003; Calabrò et al., 2005).

A 'run' test is a simple and robust method for determining whether data differ systematically from a theoretical curve (López et al., 1999, 2004). A run is a series of consecutive points with a residual of the same sign (*i.e.*, positive or negative). The number of runs of sign, also called the number of runs associated with a particular fit, was calculated according to Motulsky and Ransnas (1987).

Regressing observed *versus* predicted gas volume is a common approach to evaluate model behavior. The observed (*y*-axis) *versus* predicted (*x*-axis) gas volume for 23 curves and incubation time were pooled to a single regression to estimate the intercept and slope for each model. The significance of the regression parameters were statistically analyzed to test the hypothesis of 'slope = 1' and 'intercept = 0' according to Pineiro et al. (2008).

A number of gas production attributes, including final asymptotic gas volume (V_F), gas volume at 24 h ($V24$ h), half-life ($t_{0.5}$) and fractional rate of gas production at half-life ($\mu_{0.5}$), were compared among one-pool models. The $t_{0.5}$ is the time at which half of the final gas production is generated, and is calculated as the time at '$V = V_F/2$'. The $\mu_{0.5}$ is calculated as the first derivative of each equation by dividing '$V_F/2$' (*i.e.*, $dV/dt|(V_F/2)$), and substituting $t_{0.5}$ for *t* in the '$dV/dt|(V_F/2)$'. The formula of $t_{0.5}$ and $\mu_{0.5}$ are in Table 2 for one-pool models. Statistical analysis used the GLM procedure in SAS (1985). The model was:

$$Y_{ijk} = m + G_i + C_j + e_{ijk}$$

where Y_{ijk} is depended variable, *m* is overall mean, G_i is effect of model, C_j is effect of curves, and e_{ijk} is the residual error. When the model effect was significant (*i.e.*, $P < 0.05$), differences among means were tested with Duncan's multiple comparison test.

3. Results

3.1. Feedstuffs and in vitro gas production profiles

The chemical composition and *in vitro* gas production profiles of the 23 feedstuffs are in Table 1 and Fig. 2, respectively. The feedstuffs exhibited wide variations in chemical composition: CP ranged from 29.0 to 274 g/kg DM; NDF ranged from 346 to 795 g/kg DM, and ADF ranged from 175 to 503 g/kg DM. In addition, Fig. 2 indicated a wide range of *in vitro* gas production profiles among 23 feedstuffs.

3.2. Comparison of the goodness-of-fit

The proportion of the variation explained by one-pool models ranged from 0.973 to 0.993 (Table 3). The GOM and MM had the highest average R^2, while EXP_0 and EXP_{LAG} had the smallest average R^2. A similar tendency occurred for *RMS*. GOM had the smallest average, minimum and maximum *RMS* across 23 curves among one-pool models, while EXP_0 and EXP_{LAG} had the largest average, minimum and maximum *RMS* (Table 3). The LE_0 and LE_{LAG} had intermediate R^2 and *RMS* among one-pool models. When model was extended to a second pool, two-pool models markedly increased R^2 and decreased *RMS* by comparing those of corresponding one-pool models, respectively.

Serial correlation of residuals for the DW statistic is summarized in Table 3, where significant DW statistics indicate serial correlation or autocorrelation of residuals, and a non-significant DW value indicates that serial correlation is small and residuals are distributed randomly around the zero line. The GOM had the largest number of curves with non-significant DW values, while EXP_0 and EXP_{LAG} had the largest number of curves with significant DW values. Both LE_0 and LE_{LAG} had the intermediate number of curves with non-significant DW values among one-pool models. When model was extended to a second pool, two-pool models markedly increased the number of curves with non-significant DW values in comparison to those of corresponding one-pool models, respectively.

Results of the 'run' test are in Table 3. Systematic under- or over- fitting was revealed by a small number of runs of sign. Increased randomness of the residuals, indicating an elevated extent of accuracy for a model to describe the profiles, is revealed by a larger number of runs of sign. The distribution of the 23 curves for each fitted model was illustrated by dividing

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Table 3
Summary of R^2, residual mean squares (RMS), Durbin–Watson (DW) values and run test after fitting models[a].

	One pool								Two pools			
	LE		Exponential		GOM	LOG	GM	MM	$DEXP_{LAG}$	DGOM	DLOG	DMM
	LE_0	LE_{LAG}	EXP_0	EXP_{LAG}								
R^2	0.973	0.974	0.969	0.971	0.993	0.985	0.982	0.992	0.993	0.998	0.998	0.998
RMS												
Average	2.77	2.75	3.35	3.25	0.775	1.78	1.99	0.99	0.966	0.208	0.258	0.238
Min	0.157	0.155	0.564	0.564	0.114	0.542	0.327	0.185	0.146	0.0247	0.0391	0.0366
Max	10.3	9.9	10.9	10.7	1.83	4.03	6.28	2.65	3.67	0.588	0.664	1.07
Distribution of curves according to Durbin–Watson (DW) values[b] (total curve = 23)												
Significant	15	15	22	21	6	18	17	11	6	0	0	0
Non-significant	8	8	1	2	17	5	6	12	17	23	23	23
Distribution of curves according to the number of runs of sign (total curve = 23)												
≤4	10	9	15	17	9	20	11	10	6	0	0	0
5–6	9	11	6	4	12	3	8	7	9	1	2	3
7–8	4	3	2	2	2	0	3	4	4	7	11	7
≥9	0	0	0	0	0	0	1	2	4	15	10	13

[a] LE_0 and LE_{LAG}, Logistic–Exponential (LE) models with Eqs. (4) and (7), respectively; EXP_0 and LE_{LAG}, Exponential model without and with discrete *LAG* time, respectively; GOM, Gompertz model; LOG, Logistic model; GM, Generalization of the Mitscherlich model; MM, Michaelis–Menten model; $DEXP_{LAG}$, Diphase Exponential model with discrete *LAG* time; DGOM, Diphase Gompertz model; DLOG, Diphase Logsitic model; DMM, Diphase Michaelis–Menten model.

[b] Distribution of the curves according to the significant (S, $P < 0.05$) and non-significant (NS, $P > 0.05$) Durbin–Watson values.

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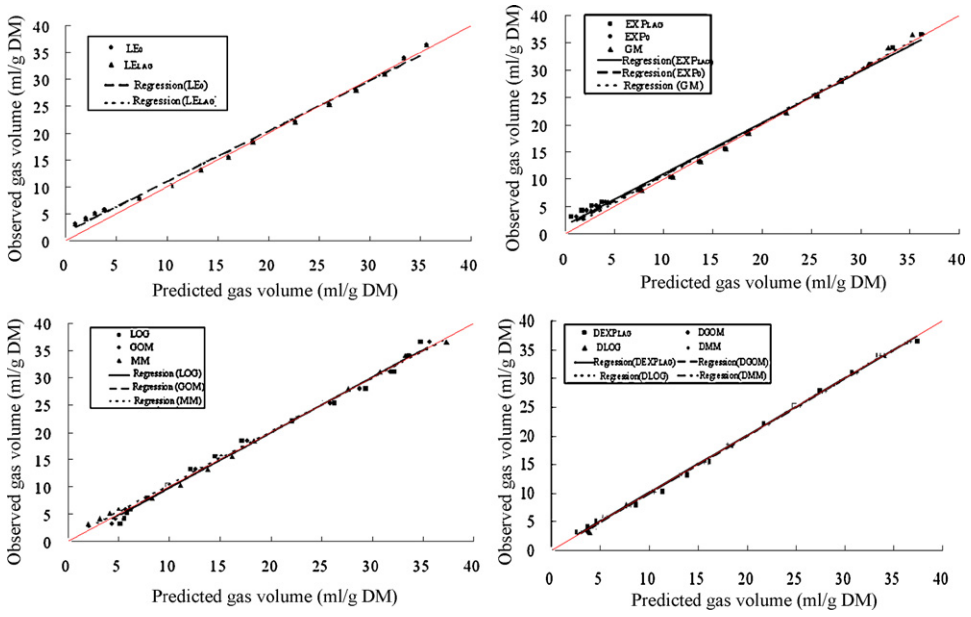

Fig. 3. Scatter plots of observed *versus* predicted regression. The observed and predicted gas volumes are the average of 23 curves at each incubation time. LE_0 and LE_{LAG}, Logistic–Exponential (LE) models with Eqs. (4) and (7), respectively; EXP_0 and LE_{LAG}, Exponential model without and with discrete *LAG* time, respectively; GOM, Gompertz model; LOG, Logistic model; GM, Generalization of the Mitscherlich model; MM, Michaelis–Menten model; $DEXP_{LAG}$, Diphase Exponential model with discrete *LAG* time; DGOM, Diphase Gompertz model; DLOG, Diphase Logsitic model; DMM, Diphase Michaelis–Menten model. A 1:1 line is coloured red for convenience of comparing the regression line.

them into four groups, which were the number of curves with ≤4, 5–6, 7–8 and ≥9 runs of sign, respectively (Table 3). The EXP_0, EXP_{LAG} and LOG had the largest number of curves with ≤4 runs of sign among one-pool models. The LE_0 and LE_{LAG} had 13 and 14 curves with 5 to 8 runs of sign respectively, and were better among one-pool models. Two-pool models of DGOM, DLOG and DMM had the largest number of curves with ≥9 runs of sign among employed models.

The *AIC* and its pair-wise comparison between models are in Table 4. Among one-pool models, GOM had the lowest mean rank of *AIC*, largest number of curves with smallest *AIC*, and largest number of cases with lower *AIC* based on pair-wise comparison. The EXP_{LAG} exhibited the highest mean rank of *AIC*, largest number of curves with largest *AIC*, and largest number of cases with higher *AIC* based on pair-wise comparison. It was accepted that the smaller the *AIC*, the higher the likelihood that the model was appropriate to describe a particular gas production profile. Both GOM and LE_0 were superior and moderate with the AIC criterion to describe *in vitro* gas curves among one-pool models, respectively. When model was extended to a second pool, DMM, DGOM and DLOG models had lower *AIC* compared to corresponding one-pool models.

The *rMSPE* and MSPE components are in Table 5, where *rMSPE* represented the mean deviation between observed and predicted values, and MSPE could be decomposed to bias, slope and random variation. GOM had the lowest *rMSPE* among one-pool models. Components of MSPE analysis indicated that most errors in GOM predictions were due to unexplained variance (98.0%). However, bias and slope had the greatest contribution to errors in LE_0, LE_{LAG}, EXP_0 and EXP_{LAG} predictions. When the model was extended to a second pool, it greatly decreased *rMSPE*, bias and slope variation by compared to those of the corresponding one-pool models.

Results of regressing observed *versus* predicted gas volume are in Table 5 and Fig. 3. Perfect agreement between observations and predictions would be represented by intercept and slope of 0 and 1 respectively, with a regression line of 1:1. The GOM had the closest intercept to 0 and slope to 1 among one-pool models (Table 5). The regression lines of best fit for LE_0, LE_{LAG}, EXP_0, EXP_{LAG}, GM and MM appeared to lie slightly above the 1:1 line, and most of these points occurred at early stages of incubation (Fig. 3). However, only LE_0, LE_{LAG} and EXP_0 had significant slope differences from 1, and LE_0, LE_{LAG}, EXP_0 and EXP_{LAG} had significant intercept differences from 0 (Table 5). Two-pool models greatly increased the extent of intercept to 0 and slope to 1, and had regression lines closer to 1:1 when compared with those of the corresponding one-pool models.

3.3. Derived gas production attributes

The predicted V_F, $V_{24\,h}$, $t_{0.5}$ and $\mu_{0.5}$ of the one-pool models are in Table 6. Different one-pool models affected ($P < 0.001$) predicted V_F, $V_{24\,h}$, $t_{0.5}$ and $\mu_{0.5}$, which ranged from 35.1 to 60.3 ml, 27.6 to 29.3 ml, 12.9 to 25.4 h and 0.0312 to 0.0772/h, respectively. The predicted V_F and $t_{0.5}$ were highest for MM, and lowest for LOG. The $V_{24\,h}$ and $\mu_{0.5}$ were lowest for MM, and highest for LOG. The LE_0, LE_{LAG} and GM had intermediate predicted V_F, $V_{24\,h}$, $t_{0.5}$ and $\mu_{0.5}$ among one-pool models.

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Table 4
Summary of Akaike's information criterion (AIC) after fitting models[a].

	One pool								Two pools			
	LE		Exponential		GOM	LOG	GM	MM	$DEXP_{LAG}$	DGOM	DLOG	DMM
	LE_0	LE_{LAG}	EXP_0	EXP_{LAG}								
Mean rank[b]	6.83	9.22	7.52	9.65	3.70	7.78	9.23	4.57	8.26	3.17	4.48	3.57
Number of curves with smallest AIC[c]	1	0	0	0	7	0	0	3	0	5	5	2
Number of curves with largest AIC	0	8	0	1	0	2	5	0	7	0	0	0
Number of cases where the model was better than others based on according to the pair-wise comparison of AIC (Total case for each model = 253)												
	118	64	104	52	191	98	63	171	86	203	174	194
Number of cases where the model was worse than others based on according to the pair-wise comparison of AIC (Total case for each model = 253)												
	135	189	149	201	62	155	190	82	167	50	79	59

[a] LE_0 and LE_{LAG}, Logistic–Exponential (LE) models with Eqs. (4) and (7), respectively; EXP_0 and LE_{LAG}, Exponential model without and with discrete *LAG* time, respectively; GOM, Gompertz model; LOG, Logistic model; GM, Generalization of the Mitscherlich model; MM, Michaelis–Menten model; $DEXP_{LAG}$, Diphase Exponential model with discrete *LAG* time; DGOM, Diphase Gompertz model; DLOG, Diphase Logsitic model; DMM, Diphase Michaelis–Menten model.

[b] Ranking model according to the AIC, smallest AIC = rank 1, etc. Mean rank was the average value across 23 curves.

[c] Total number of curves = 23.

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Table 5
Summary of root mean squared prediction error (rMSPE), components of MSPE and observed *versus* predicted regression after fitting models[a].

	One pool								Two pools			
	LE		Exponential		GOM	LOG	GM	MM	$DEXP_{LAG}$	DGOM	DLOG	DMM
	LE_0	LE_{LAG}	EXP_0	EXP_{LAG}								
rMSPE	1.49	1.48	1.72	1.71	0.833	1.29	1.32	0.945	0.860	0.422	0.480	0.438
Components of MSPE												
Bias	17.3	17.2	19.5	17.4	1.34	4.39	9.62	5.44	0.178	0.302	0.566	0.108
Slope	20.8	20.8	21.6	21.5	0.65	2.38	10.2	6.76	0.000	0.160	0.313	0.156
Random	61.9	62.0	58.9	61.1	98.0	93.3	80.3	88.0	99.8	99.5	99.1	99.7
Observed (in the *y*-axis) *versus* predicted (in the *x*-axis) regressions												
Intercept	1.52*	1.52*	1.63*	1.20*	−0.120	−0.483	0.733	0.516	0.017	−0.029	−0.047	0.019
SE	0.412	0.413	0.441	0.417	0.298	0.560	0.428	0.321	0.306	0.115	0.151	0.105
Slope	0.940#	0.939#	0.937#	0.952	1.005	1.02	0.979	0.980	0.999	1.001	1.002	0.999
SE	0.020	0.020	0.022	0.020	0.014	0.027	0.021	0.016	0.015	0.006	0.007	0.005

[a] LE_0 and LE_{LAG}, Logistic–Exponential (LE) models with Eqs. (4) and (7), respectively; EXP_0 and LE_{LAG}, Exponential model without and with discrete *LAG* time, respectively; GOM, Gompertz model; LOG, Logistic model; GM, Generalization of the Mitscherlich model; MM, Michaelis–Menten model; $DEXP_{LAG}$, Diphase Exponential model with discrete *LAG* time; DGOM, Diphase Gompertz model; DLOG, Diphase Logsitic model; DMM, Diphase Michaelis–Menten model; SD, standard Deviation.

* Intercept significantly ($P < 0.05$) different from 0.

\# Slope significantly ($P < 0.05$) different from 1.

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Table 6
Comparison of gas production parameters for one-pool models.

Model	V_F (ml)	V_{24h} (ml)	$t_{0.5}$ (h)	$\mu_{0.5}$ (10^{-2}/h)
LE				
LE_0	38.5 abc	28.6 c	13.4 abc	6.70 cd
LE_{LAG}	38.5 abc	28.6 c	13.4 abc	6.70 cd
Exponential				
EXP_0	41.4 c	28.0 b	15.1 bc	5.50 b
EXP_{LAG}	40.8 bc	27.7 ab	15.4 c	5.66 bc
GOM	37.1 ab	28.7 c	13.3 abc	6.70 cd
LOG	35.1 a	29.3 d	12.9 a	7.72 d
GM	39.4 bc	28.1 bc	13.1 ab	6.22 bc
MM	60.3 d	27.6 a	25.4 d	3.12 a
SEM	2.56	1.66	1.18	0.51
P	<0.001	<0.001	<0.001	<0.001

V_F, final asymptotic gas volume; V_{24h}, gas volume at 24 h; $t_{0.5}$, half-life; $\mu_{0.5}$, fractional rate of gas production at half-life; LE_0 and LE_{LAG}, Logistic–Exponential (LE) models with Eqs. (4) and (7), respectively; EXP_0 and LE_{LAG}, Exponential model without and with discrete *LAG* time, respectively; GOM, Gompertz model; LOG, Logistic model; GM, Generalization of the Mitscherlich model; MM, Michaelis–Menten model. Means with different letters within a column differ ($P < 0.05$).

4. Discussion

4.1. Biologically meaningful parameters

Schofield et al. (1994) developed LOG and GOM models to describe *in vitro* gas kinetics with underlying two important assumptions. Firstly, defining rate of gas production as proportional to both current microbial mass and substrate level and; secondly, defining '$V_0/M_0 = dV/dM$'. The second assumption was not always the case, and thus caused an inherent error, because initial microbial mass after inoculation was not zero, and starting gas volume was always zero (Schofield et al., 1994). Our LE models introduced *d* to present a more generalized concept, and thus made the logistic function more flexible. The LE models generated more flexible curves with both non-sigmoidal and sigmoidal shapes when compared with the LOG model. In addition, LE models might be superior to the generalized logistic model, which was developed by introducing the parameter ν into the LOG model (Richards, 1959; Schnute, 1981; Sakanoue, 2007), because the generalized logistic model was unsuitable for constructing empirical curves with inflexion points below 0.368 V_F (V_F/exp(1)) (Birch, 1999).

Non-sigmoidal shapes indicate that rate of gas production decreases continually, while sigmoidal shapes indicate that rate of gas production increased firstly, reached maximum rate and then decreased, which might suggest a close relationship with increased microbial activities during the early stages of incubation. France et al. (1993, 2000) believed that sigmoidal shapes reflected increased substrate accessibility, which might be caused by increased hydration of particles, microbial attachment and microbial numbers at the beginning of incubation. Groot et al. (1996) proposed that sigmoidal shape (*i.e.*, $c > 1$ in MM model) was likely because the microbial population had to multiply and colonize the substrate to form a 'biofilm' before reaching maximum rate of fermentation. Although the assumptions underlying the GM, MM and LE models showed large differences, the biological meaning of *d* might be consistent to that of *d* in GM and *c* in MM. Thus '$d < 1$' indicates that rate of gas production increased first and then declined, while '$d > 1$' indicates that rate of gas production continually decreased.

The LE_s model was a standard LE without re-parameterization. However, the LE_s model might exhibit positive intercept at '$t = 0$', which is biologically impossible, since gas volume is always zero at '$t = 0$'. When the LE_s model was re-parameterized by '$V_0 = 0$', the LE_0 model was obtained to exclude the possibility of exhibiting positive intercept at '$t = 0$' (Fig. 1). Several reports have suggested that a discrete time *LAG* is often observed before gas production begins and that factors affecting it include the nature of the incubated substrate, the inoculated microbe, and the amount of inoculum (Allen and Mertens, 1988; France et al., 1993; Pell and Schofield, 1993). In our study, the LE_{LAG} model was also developed under the assumption that a discrete *LAG* occurred before the start of gas production. In both LE_0 and LE_{LAG} models, *d* was calculated by equation '$\beta M_0/Y_G/V_F$' and had a positive value with an inflexion point below or equal to $V_F/2$. If it is assumed that values of β, Y_G, and V_F are fixed under conditions of certain substrates incubated in ruminal fluid, then the amount of inoculated microbes could have profound effects on shapes of *in vitro* gas curve. Pell and Schofield (1993) indicated that increasing the size of the ruminal fluid inoculum had little effect on maximum gas production, but might affect the curve shape levels, when incoulum was sufficient. Gas production was higher for larger inoculum levels than that of smaller after some incubation times, and substrate seemed to be more easily degraded by microbes with more inoculum (Pell and Schofield, 1993). A positive relationship between *d* and the fractional rate of gas production (Eqs. (2) and (3)) indicated that increasing the amount of inoculated microbes elevated the fractional rate of gas production. It was reported that increasing proportions of rumen fluid reduced the incubation time to half total gas volume (Rymer and Givens, 2000).

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4.2. Statistical performance of models

Exponential functions of EXP_0 and EXP_{LAG} are first-order kinetics. The EXP_{LAG} was developed by introducing a discrete *LAG* into the EXP_0 model. The discrete *LAG* time was added because of hydration, removal of digestion inhibitors and/or attachment of microbes with substrate (Mertens, 1993). The EXP_{LAG} showed potential to decrease the *RMS* and *rMSPE*, compared to the EXP_0 model, which is consistent with many previous studies (Mertens, 1993; Schofield et al., 1994). However, the *AIC* was not decreased for EXP_{LAG} model. Many curves exhibited '*LAG* = 0' after fitting EXP_{LAG} (data not shown), which indicates that a discrete *LAG* might be unnecessary for within exponentials to describe *in vitro* gas data. Others have indicated that some versions of a simple exponential model without *LAG* were adequate to describe gas production kinetics (Ørskov and McDonald, 1979; Blümmel and Ørskov, 1993). The LE_0 model had better goodness-of-fit than EXP models (*i.e.*, EXP_0 and EXP_{LAG}) after data fitting. The superiority of LE to EXP models was that LE models generated both non-sigmoidal and sigmoidal curve shapes, while EXP models could only generate non-sigmoidal curve shapes. In our study, some sigmoidal curves were observed (Fig. 2), consistent with previous studies which reported that non-sigmoidal EXP_0 and EXP_{LAG} were inadequate to describe sigmoidal curves (Dhanoa et al., 2000; Calabrò et al., 2005).

France et al. (1993) proposed a GM model by introducing a modified fractional degradation rate with time in the exponential equation. The increased digestion rate with time might indicate an increase in substrate availability, microbial attachment and/or microbial numbers. The GM model had sigmoidal shape without a fixed inflection point and could generate wide ranges of sigmoidal and non-sigmoidal shapes (France et al., 2000). Dhanoa et al. (2000) concluded that the GM model was better in describing 216 *in vitro* gas production curves covering a wide range of curve shapes. In our study, although the GM model had potential to decrease *RMS*, *rMSPE*, bias and slope variation, extents of intercept close to 0 and slope close to 1 in comparison with EXP models, its *AIC* was not decreased. In addition, the LE_0 model had relatively higher *AIC* than the GM. The '*LAG* = 0', observed for many curves after fitting GM model (data not shown), might greatly affected goodness-of-fit. Calabrò et al. (2005) and Huhtanen et al. (2008) indicated that GM showed intermediate goodness-of-fit among 5 and 11 one-pool models, respectively, but results were not as good as those reported by Dhanoa et al. (2000). Calabrò et al. (2005) attributed inconsistent reports to the different *in vitro* gas recording systems. The small number of data points (*i.e.*, 15), much lower than that of Dhanoa et al. (2000), might impair the goodness-of-fit of GM model in this study. Furthermore, short incubation periods might also contribute to inconsistent goodness-of-fit reports of GM model between this study and Dhanoa et al. (2000) and Calabrò et al. (2005).

The MM model was developed for enzyme kinetics and first employed in describing *in vitro* gas production kinetics by Groot et al. (1996). The MM model showed a wide range of curve shapes without fixed inflexion point. Some studies reported that MM had superior fit with decreased *RMS* (Calabrò et al., 2005; Huhtanen et al., 2008), while others indicated a moderate fit (Dhanoa et al., 2000). In our study, the MM had lower goodness-of-fit than the GOM model, which is inconsistent with that reported by Huhtanen et al. (2008). Manual glass syringe recording systems and small numbers of data points might impair the fit performance of MM model in our study, because it was reported that curves obtained with APES and contained between 60 and 125 points might favor MM model (Calabrò et al., 2005). In addition, the incubation period of 48 h was much shorter than the 72 h of Huhtanen et al. (2008). As the MM model had a clear time-related pattern, and residuals decreased with advancing incubation time (Huhtanen et al., 2008), short incubation periods might also impair the fit performance of the MM model.

Both LOG and GOM models are classic growth functions, and were first employed for modeling *in vitro* gas production curves by Schofield et al. (1994). In our study, the GOM model had a relatively superior fit to the data among the 8 one-pool models. The good fit of the GOM model let to it being solely employed to investigate *in vitro* fermentation characteristics among these feedstuffs in Tang et al. (2005, 2006, 2008). However, the LOG model had only a moderate fit to the data among our one-pool models. Huhtanen et al. (2008) and Dhanoa et al. (2000) indicated that the LOG model was not necessarily a good alternative because the main constraints of LOG and GOM models in fitting raw gas data were fixed inflexion points and positive intercepts at '$t = 0$'. Although LE_0 had lower goodness-of-fit than LOG or GOM, the superiority of LE_0 to LOG and GOM models were flexible curve shape, without fixed inflexion point, and sensible biology as the predicted gas volume at '$t = 0$' was zero.

Models have been extended for multiphasic analysis with the underlying assumption that incubated feedstuffs are not homogeneity (Schofield et al., 1994; Cone et al., 1997), and that incubated feedstuffs are generally comprised of rapidly and slowly degradable components (Schofield et al., 1994; Schofield and Pell, 1995). Many studies indicated that two-pool models fit *in vitro* gas data much better than corresponding one-pool models (Schofield et al., 1994; Schofield and Pell, 1995; Huhtanen et al., 2008). In our study, two-pool models except $DEXP_{LAG}$ generally had lower *AIC* rank, bias and slope variations compared to those the corresponding one-pool models, respectively. However, discrepancies of two-pool models were also observed. Firstly, mathematical division of rapidly and slowly degradable components showed markedly large variation between models (data not shown), which might or might not correspond to chemical entities. Robinson et al. (1986) indicated that expansion to include a second degradable fraction eliminated bias compared to that of one degradable fraction during ruminal *in situ* degradtion curve study, but models that assign all degradable cell wall to either a fast or slow degrading fraction might be biologically unreasonable. Huhtanen et al. (2008) concluded that the division between the rapidly and slowly degradable components was arbitrary based on model structures. Secondly, multiphasic analysis caused the increased number of parameters to lead to lower robustness of the model.

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The LE_0 had intermediate goodness-of-fit among one-pool models, and LE_{LAG} exhibited little potential to decrease *AIC* compared to the LE_0 model. Many curves exhibited '*LAG* = 0' after fitting LE_{LAG} model (data not shown), and its intercept and slope showed little difference to the LE_0 model (Table 5). It seemed that a discrete *LAG* time might be unnecessary for the LE model to describe *in vitro* gas data, which was consistent to that of EXP models in our study. The LE_0, LE_{LAG}, EXP_0, EXP_{LAG}, GM and MM models were slightly above the 1:1 line (Fig. 3), and their predicted gas volumes were lower than observed gas volumes at early stages of incubation. As many curves showed unexpectedly high gas volumes at 0.5 h, these 6 models must correct the relative high gas volumes at initial stages of incubation because gas volume at '$t = 0$' is zero. Although the extent of these corrections differed among these 6 models (Table 5), LE_0 and LE_{LAG} were biologically meaningful, as were the EXP, GM and MM models, and might be potential alterations to better fit gas production kinetics.

4.3. Comparison of derived gas production attributes

The derived gas production attributes were continually analyzed after statistical comparison to reveal possible discrepancies among models. The MM model seemed to be a good alterative to describe *in vitro* gas production kinetics in terms of goodness-of-fit. However, the MM model had the highest V_F and $t_{0.5}$, and the lowest $\mu_{0.5}$ and $V_{24\,h}$, compared to other one-pool models. López et al. (1999) indicated that the MM model had the highest $t_{0.5}$ and lowest $\mu_{0.5}$ among models during an *in situ* degradation curve study. Huhtanen et al. (2008) reported that MM series models (*i.e.*, MM, generalized MM, and two-pool MM models) had the highest final gas volume among 21 models. It seemed that MM model might overestimate V_F and $t_{0.5}$, underestimate $\mu_{0.5}$ and $V_{24\,h}$, when compared with other one-pool models. As the MM model had a positive pattern of time-related residual (Huhtanen et al., 2008), a short incubation time of 48 h might further enhance the extent of overestimating final gas volume, and underestimate fractional rate of gas production.

The LOG model had the lowest V_F and $t_{0.5}$, the highest $\mu_{0.5}$ and $V_{24\,h}$, when compared with other one-pool models. Huhtanen et al. (2008) also reported that the LOG model had the lowest final gas volume among 21 models. It seemed that the LOG model might underestimate V_F and $t_{0.5}$, and overestimate $\mu_{0.5}$ and $V_{24\,h}$, compared to other one-pool models. As the LOG model had an opposite pattern of time-related residuals, short incubation time of 48 h might further enhance the extent of underestimating final gas volume and overestimating fractional rate of gas production.

When '*d*' was introduced into the logistic function, some differences to LOG occurred. The LE_0 and LE_{LAG} models exhibited intermediate values of derived gas production attributes among the employed one-pool models. No significant differences of derived gas production attributes occurred between the LE and GM models. As GM is a widely accepted model to predict gas production attributes (Theodorou et al., 1994; López et al., 1999, 2007), the LE models might be good alterations to predict gas production attributes.

5. Conclusions

The LE models were developed by adding a shape parameter *d* into a logistic function, and included the mathematical forms LE_s, LE_0 and LE_{LAG}. Theoretical investigation indicated that, compared to the LOG, GOM and LE_s models, the LE_0 and LE_{LAG} made more biological sense in describing *in vitro* gas curves, because their initial gas volume was zero and, compared to the EXP_{LAG} and EXP_0 models, the LE_0 and LE_{LAG} models created more flexible curves of both non-sigmoidal and sigmoidal shapes. Statistical analyses indicated that compared with the LE_{LAG}, EXP_{LAG} and GM models, the lower *AIC* for LE_0 and EXP_0 indicated *LAG* time might be unnecessary to describe *in vitro* gas data. The GOM and LOG models exhibited higher goodness-of-fit than LE models, but were incapable of correcting relatively high gas volumes at initial stages of incubation to correctly represent '$V_0 = 0$'. The MM model exhibited higher goodness-of-fit than the LE models, but inaccuracy predicted gas attributes. Two-pool models improved the fit to curve, but led to declined robustness. The LE_0 model had overall intermediate goodness-of-fit among the employed models, and was judged to be an acceptable tool to predict gas production attributes. In summary, our study indicated that LE models could be alternatives to describe *in vitro* gas curves. However, more studies, including longer incubation times, more incubation points, different gas measurement systems and wider ranges of feedstuffs are needed to fully investigate the performance of LE models.

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