Animal Nutrition 3 (2017) 300–308

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Rumen liquor from slaughtered cattle as inoculum for feed evaluation

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ARTICLE INFO

Article history:
Received 19 April 2017
Received in revised form
19 June 2017
Accepted 20 June 2017
Available online 15 July 2017

Keywords:
In vitro gas production technique
Model comparison
Model selection
Nonlinear mathematical models
Rhodes grass hay
Rumen liquor

ABSTRACT

Use of nonlinear mathematical models has been majorly based on *in vitro* gas production (GP) data generated when substrates are incubated with rumen liquor from fistulated steers. However, existing evidence suggests that rumen liquor from slaughtered cattle of unknown dietary history also generates quantifiable *in vitro* GP data. Fitting and description of GP data obtained from 4 diets incubated with rumen liquor from slaughtered cattle was evaluated using single-pool exponential model with discrete lag time (EXPL), logistic (LOG), Groot's (GRTS) and Gompertz (GOMP) models. Diets were formulated by varying proportions of Rhodes grass (*Chloris gayana*) hay and a concentrate mixed on dry matter basis to be: 1,000 g/kg Rhodes grass hay (RGH) and 0 of the concentrate (D1), 900 g/kg RGH and 100 g/kg concentrate (D2), 800 g/kg RGH and 200 g/kg concentrate (D3), 700 g/kg RGH and 300 g/kg concentrate (D4). Dietary kinetics for the models were determined by measuring GP at 2, 4, 8, 10, 18, 24, 36, 48, 72, 96 and 120 h. Model comparison was based on derived GP kinetics, graphical analysis of observed versus predicted GP profiles plus residual distribution and goodness-of-fit from analysis of root mean square error (RMSE), adjusted coefficient of determination (Adj-R^2) and Akaike's information criterion (AIC). Asymptotic GP, half-life and fractional rate of GP differed ($P < 0.001$) among the 4 models. The RMSE, Adj-R^2 and AIC ranged from 1.555 to 4.429, 0.906 to 0.984 and 2.452 to 15.874, respectively, for all diets compared across the 4 models. Based on the goodness-of-fit statistical criterion, GP profiles of D1 were more appropriately fitted and described by GRTS and GOMP than the EXPL and LOG models. The GRTS model had the lowest AIC value for D2 (2.452). Although GRTS model had the most homogenous residual dispersion for the 4 diets, all the 4 models exhibited a sigmoidal behavior. Therefore, rumen liquor from slaughtered cattle of unknown dietary history can be used to derive nutritionally important feed parameters, but choice of the most appropriate model should be made based on fitting criteria and dietary substrates incubated.

1. Introduction

Over the last 2 decades, *in vitro* gas production (GP) technique using graduated syringes has gained popularity in developing countries as a tool for evaluating ruminant feeds and studying the kinetics of rumen fermentation (López et al., 2007). This is because of the strong relationship between *in vivo* digestibility and digestibility predicted from *in vitro* GP using the glass syringes (Dhanoa et al., 2000; France et al., 2005).

The availability of useful data on digestion kinetics associated with fermentation of soluble, slowly degradable and the undegradable fractions of ruminant feedstuffs from *in vitro* GP measurements has created a need for compartmental models, which are inherently nonlinear functions of GP throughout duration of incubation (Dijkstra et al., 2005). Mathematical description of GP profiles allows for the estimation of nutritionally important feed

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.aninu.2017.06.010

parameters (Dijkstra et al., 2005; Kebreab et al., 2008) and comparison of substrates based on the kinetics of fermentation. Availability of such data on soluble and slowly degradable fractions for ruminant feeds and characteristics of the fermentation environment (Groot et al., 1996) provide useful information needed in formulation of well-balanced diets for improved ruminant production.

Various mathematical models have been used to link and describe *in vitro* GP data to biological processes occurring *in vivo* (France et al., 2005; López et al., 2007, 2011; Piquer et al., 2009; Jay et al., 2012). The commonly used models to describe and fit *in vitro* GP data include: 1) the simple exponential model, which is based on first-order kinetics and assumes a constant fractional rate of fermentation of the substrate (Ørskov and McDonald, 1979), and 2) the non-exponential (sigmoidal) models including the logistic, Korkmaz-Ückardes (Korkmaz and Üçkardes, 2014), Gompertz (Lavrenčič et al., 1997) and the logistic-exponential (Wang et al., 2011), which assume that fractional rate of fermentation of the substrate varies with time (Tedeschi et al., 2008) and may have either variable or fixed inflection points (Peripolli et al., 2014). These nonlinear mathematical models are either single or multi-compartmental in nature with one, two or more 'pools' from which GP occurs (France et al., 2005; Savian et al., 2007; Huhtanen et al., 2008; López et al., 2011).

Use of nonlinear mathematical models to describe *in vitro* GP profiles has been majorly based on data generated when feed substrates are incubated with rumen liquor from fistulated steers of known feeding regimens. However, existing literature has shown higher GP when samples of grass forages were incubated with rumen liquor from slaughtered cattle of unknown dietary history compared to when the same samples were incubated with a suspension of sheep faeces (Borba et al., 2001). Besides, anecdotal evidence suggests that use of rumen liquor from slaughtered cattle generates quantifiable data that can be analyzed using mathematical models to derive biologically important parameters. Similarly, Chaudhry and Mohamed (2012) reported higher *in vitro* dry matter (DM) and crude protein (CP) degradation of rapeseed meal and grass nuts when incubated with fresh rumen liquor from slaughtered cattle compared to when the frozen and thawed rumen liquor was used. Use of rumen liquor from such slaughtered cattle to evaluate ruminant feeds by *in vitro* GP technique based on glass syringes has been accepted especially in animal nutrition laboratories found in developing countries, which do not have adequate capacity for surgical preparations and maintenance of fistulated animals (Mutimura et al., 2013). Besides, the site of fistulated animals is often offensive to society and provocative to animal welfare activists (Mould et al., 2005). Therefore, the *in vitro* GP technique using glass syringes inoculated with rumen liquor from slaughtered cattle appears to be gaining wide reputation in developing countries where maintenance of fistulated steers and use of automated systems are still a challenge (Singh et al., 2010).

Even though rumen liquor from slaughtered cattle generates typical digestion kinetics of feeds (Denek et al., 2006; Yánez-Ruiz et al., 2016), there are many different single-pool and multi-compartmental mathematical models that can describe GP profiles with a meaningful biological interpretation (López et al., 2011). This results in difficulties when selecting the more appropriate model to fit the GP data.

Multi-pool models such as those with a 6-parameter equation are always likely to predict the rate of GP better than one with only 2 or 3 parameters. The difficulty with a multi-parametric equation is that it becomes impossible to predict the values of each parameter for given roughages; hence the equation can be used only to describe the rate of GP for a particular roughage. Similarly, while multi-pool models fit *in vitro* GP data better than single-pool models due to the underlying assumption that incubated feedstuffs are not homogenous and are, therefore, comprising the relative contributions of the rapidly and slowly degradable pools, and increased number of parameters in multi-pool models reduce model robustness (Huhtanen et al., 2008). Besides, mathematical division of the rapidly and slowly degradable components of feed substrates have been reported to show markedly large variations between models, which may be inconsistent with the chemical entities of the substrates that determine the shape of GP profile obtained by fitting a particular model (Nathanaelsson and Sandström, 2003). Similarly, whereas Robinson et al. (1986) contends that expansion of a model to include the 2 pools eliminates bias compared to when a single pool model is used in ruminal *in situ* degradation studies, Huhtanen et al. (2008) argue that models that assign all degradable cell wall components to either rapidly or slowly degrading pools might be biologically unreasonable since the degradable components are arbitrarily assigned based on model structures. Biologically meaningful single pool mathematical models are, therefore, commonly used to fit and describe a wide range of GP profiles and assume that the rate of GP is affected by the microbial mass and substrate level. However, the choice of an appropriate model to fit GP data is still complicated by a paucity of information that can inform the selection process of the most appropriate single pool model that is capable of describing GP profiles to allow analysis of GP data obtained when different dietary substrates are incubated with rumen liquor from slaughtered cattle. It is thus hypothesized that the commonly used single-pool nonlinear mathematical models such as the exponential model with a discrete lag time (EXPL), logistic (LOG), Groot's (GRTS) and Gompertz (GOMP) differ in their suitability to fit and describe GP profiles of different diets incubated with rumen liquor from slaughtered cattle of unknown dietary history. Moreover, the variations in the GP profile shapes, from steep diminishing returns (exponential) to highly sigmoidal, requires cautious selection of a model capable of fitting and describing GP data (Peripolli et al., 2014). The objective of this study was, therefore, to compare the ability of 4 commonly used single-pool mathematical models to fit and describe GP profiles obtained when 4 diets formulated by varying the proportions of Rhodes grass (*Chloris gayana*) hay and a concentrate were incubated with rumen liquor from slaughtered cattle of unknown dietary history.

2. Materials and methods

2.1. Study site and diet formulation

In vitro GP and chemical analysis of the diets were performed at the animal science laboratory of the department of agricultural production, Makerere University. Four diets were formulated by mixing varying proportions of milled Rhodes grass hay (RGH) and a homemade concentrate (HMC) mixed to be [g/kg DM]: (D1) 1,000:0; (D2) 900:100; (D3) 800:200 and (D4) 700:300 of RGH:HMC composite. A commercial hay farmer produced the grass hay from primary growth of Rhodes grass swards harvested at different stages of maturity over a period of 3 years. Fresh grass was harvested and sun dried in the field for 2 to 3 days. Small hay bales measuring 85 cm × 55 cm × 45 cm and on average weighing 18 to 20 kg were made. Hay materials were sampled from different bales and homogenized to make a composite hay sample. The concentrate was composed of 877 g/kg DM maize bran, 83 g/kg DM cotton seedcake, 30 g/kg DM Pharma mineral lick and 10 g/kg DM common salt premixed prior to mixing with the different proportions of milled hay. The Pharma mineral lick was composed of macro and micro nutrients that included: 6,000 mg Mn, 5,000 mg Zn, 4,000 mg Fe, 800 mg Cu, 100 mg I, 30 mg Co, 20 mg Se, 7,000,000 IU

vitamin A, 2,000,000 IU vitamin D_3, 35,000 mg choline, 10,000 mg vitamin E, 200 mg vitamin K_3, 300 mg vitamin B_1, 800 mg vitamin B_2, 400 mg vitamin B_6, 2 mg vitamin B_{12}, 3,000 mg niacin, 75 mg biotin, 100 mg folic acid, 1,000 mg pantothenic acid and 20,000 mg antioxidant.

2.2. Chemical analyses

Samples of the 4 diets were oven dried at 70 °C for 48 h and subsequently ground to pass a 1-mm screen for use in chemical analyses and *in vitro* GP assays while the laboratory DM content of dietary samples was determined by oven-drying at 100 °C for 24 h. Crude protein (CP) and ash were analyzed according to standard methods 976.06 and 942.05, respectively, of AOAC (2000). Neutral detergent fiber (NDF), exclusive of residual ash (NDFom), was determined without addition of α-amylase or sodium sulphite (Van Soest et al., 1991). The sample was sequentially analyzed for acid detergent fiber exclusive of residual ash (ADFom), and lignin (sa) was determined by solubilization of cellulose with sulfuric acid (6 mol/L) (Van Soest et al., 1991).

2.3. In vitro gas production assay

In vitro GP was determined according to Menke and Steingass (1988) with modifications by Blümmel and Ørskov (1993). Rumen liquor was obtained from 4 cattle freshly slaughtered (approximate live weight 300 kg) at a local abattoir (Gayaza slaughter house, Wakiso district, Uganda) on 4 separate occasions. Each collection occasion was separated by a period of 28 days to cover dietary regimens in both dry and wet seasons. Although contact of the respective cattle owners could not be established for purposes of ascertaining the age, breed and feeding history, information about the sex, live body weight and geographical origin of the cattle was obtained from the abattoir authority. In order to minimize effects of possible variations in rumen environment due to different geographical regions of origin, 4 donor animals each got from a different region were randomly selected as sources of rumen liquor collected on 4 different days spread over a period of 4 months. About 15 min after slaughter, the middle part of the rumen of each animal was incised and samples of fresh rumen content were scooped and filled into pre-warmed thermos flasks leaving no head space. The flasks were tightly sealed and transported to the animal science laboratory within 1 h of collection. Upon arrival, the contents of the 4 animals were pooled and macerated to form uniform rumen slurry using a Waring blender. The slurry was filtered through 3 layers of cheesecloth into 250-mL glass bottles and flushed with carbon dioxide (CO_2) to produce rumen liquor. The bottles were kept at 39 °C in a gently shaking water bath at all times. The strained rumen liquor from the slaughtered cattle was poured into the buffer mixture of artificial saliva in a ratio of 1:2 (vol/vol) to constitute the inoculum according to Osuji et al. (1993).

Samples (200 ± 1 mg) of the 4 different oven dried dietary substrates were weighed into graduated syringes (100 mL) fitted with lightly greased plungers. Three syringes per dietary sample were then filled with 30 mL of the inoculum. The syringes were placed in a gently shaking water bath maintained at 39 °C. Triplicates of cumulative gas volumes were read manually at 2, 4, 8, 10, 12, 18, 24, 36, 48, 72, 96 and 120 h of incubation for each of the 4 dietary samples. Triplicate syringes containing 30 mL of inoculum with no substrate (blanks) were also incubated alongside each of the dietary samples to correct for GP, organic matter (OM) disappearance and volatile fatty acid (VFA) production. Net gas volumes for the incubated dietary feed samples were determined by subtracting the average blank gas volumes from the cumulative gas volumes of each sample.

2.4. Model selection, comparison and statistical analysis

Four commonly used single-pool nonlinear mathematical models were selected to evaluate their ability to fit and describe the GP data of the 4 diets incubated with rumen liquor inoculum from slaughtered cattle. Comparisons were made on the exponential model with discrete lag time (EXPL), logistic (LOG), Groot's (GRTS) and Gompertz (GOMP). The basis for selection of the above models was their ability to link rate of GP to the rate of substrate degradation in the rumen and how frequently they are used in literature to describe the kinetics of GP (France et al., 2005; Wang et al., 2011; Üçkardes et al., 2013; Peripolli et al., 2014). The functional equations for each model are presented in Table 1.

Root mean square error (RMSE), adjusted coefficient of determination (Adj-R^2) and Akaike's information criterion (AIC) (Calabrò et al., 2005; Symonds and Moussalli, 2011; Chai and Draxler, 2014; Üçkardes and Efe, 2014) were used as the goodness-of-fit test statistics. Model behavior was evaluated by analyzing the graphical representation of the observed versus predicted GP profiles and distribution of the residuals to determine if any of the models underestimated or overestimated certain sections of the GP profile. Three *in vitro* GP parameters namely asymptotic GP (A), half-life ($t_{½}$) and fractional rate of GP (k) were also compared among the single-pool models. The $t_{½}$ is the time (h) to generate 50% of total GP, and was calculated for each model as the time at $Y = A/2$, where Y = GP (mL) at time 't', A = asymptotic GP, mL/200 mg DM.

The 4 models were fitted to net gas volume data using the algorithm of Levenberg Marquardt employed in the NLIN procedure of SAS (2002). Several possible starting values were selected for each of the parameters used in the models in order to reach convergence within a reasonable number of iterations. Data for each of the diets were analyzed using GLM procedure of SAS (2002) to test whether RMSE, Adj-R^2, AIC and some derived GP parameters were statistically different from each other for the 4 single-pool nonlinear mathematical models. Differences between least square means for the various parameters were compared using the

Table 1
Details of single-pool mathematical models.

Model	Equation	$t_{½}$, h	Reference
EXPL	$Y = A\{1 - \exp[-k(t - \text{lag})]\}$	$\text{lag} + \{\ln(0.5)/k\}$	Tedeschi et al. (2008)
LOG	$Y = A/[1 + \exp(2 - 4kt)]$	$1/(2k)$	Rodrigues et al. (2009)
GRTS[1]	$Y = A/\{1 + (B/t)^C\}$	B	Groot et al. (1996)
GOMP[2]	$Y = A\exp[-k\exp(-ct)]$	$\{\ln[\ln(2)]\}/ck$	Lavrenčič et al. (1997)

EXPL = exponential model with discrete lag time; LOG = logistic model; GRTS = Groot's model; GOMP = Gompertz model; Y = gas production (mL) at time 't'; A = asymptotic gas production, mL/200 mg DM; k = fractional rate of gas production, /h; lag = discrete lag time, h; $t_{½}$ = half-life, h.

[1] B = half-life, h; C = sharpness of the switching characteristic for the profile. The fractional rate of gas production (k, /h) and the time at which it occurs (T_k, h) were calculated according to the following equations (Rodrigues et al., 2014): $k = A(B^C)C[T_k^{(-C-1)}]/\{1 + (B^C)[T_k^{(-C)}]\}^2$ and $T_k = B[(C - 1)/(C + 1)]^{1/C}$. Fractional rate of dietary substrate degradation (R_D) was calculated using the equation of Groot et al. (1996) as: $R_D = (CT_k^{(C-1)})/(B^C + T_k^C)$.

[2] c, constant factor of microbial efficiency; k, fractional rate of gas production, /h; $t_{½}$, was calculated as $\ln(\ln(2))/ck$.

probability of difference options of SAS (2002). Probability values less than 0.001 were expressed as ($P < 0.001$).

3. Results

3.1. Chemical composition of diets

Considerable variations were observed in the chemical composition of the 4 diets (Table 2). Crude protein content increased ($P < 0.001$) at a decreasing rate with increasing proportions of the concentrate ranging from 47.8 g/kg DM of D1 to 74.7 g/kg DM of D4. The NDFom and lignin (sa) of the diets decreased ($P < 0.001$) at a decreasing rate with increasing proportions of the concentrate ranging from 641.0 to 495.1 g/kg DM and 76.9 to 67.9 g/kg DM, respectively. The ash content of diets followed a similar trend, with D4 having the lowest value of 76.9 g/kg DM.

3.2. Gas production profiles fitted with the four single-pool mathematical models

All the models fitted to data sets exhibited a sigmoidal behavior and no fitting problems were encountered (Fig. 1). Gas production profiles differed ($P < 0.05$) in both rate and extent of fermentation among diets (Table 3) for the EXPL, LOG, GRTS and GOMP models. The asymptotic GP (i.e., A, mL/200 mg DM) of the 4 models exhibited both positive linear and quadratic responses ($P < 0.001$), increasing at a decreasing rate as the proportion of concentrate increased in the diets. The discrete lag time (lag, EXPL) and the time at which maximum rate of GP (T_k, GRTS) occurred decreased linearly with increasing proportions of the concentrate ($P < 0.001$). Time of incubation at which half of the asymptotic gas volume was produced (B, GRTS) also exhibited a quadratic response ($P < 0.001$) with increasing level of concentrate. The highest and lowest ($P < 0.001$) fractional rate of GP (k) for the models was obtained with D3 and D1, respectively, but generally increased curvilinearly ($P < 0.001$) as the proportion of the concentrate in the diet increased with the exception of the GOMP model.

3.3. Comparison of models using some derived gas production parameters

The asymptotic GP (A), half-life ($t_{½}$) and fractional rate of GP (k) of the 4 single-pool models are presented in Table 4. The A, $t_{½}$ and k values differed ($P < 0.001$) among the 4 single-pool models, and ranged from 28.4 to 50.6 mL, 1.2 to 42.1 h and 0.015 to 5.374/h, respectively, for the 4 diets. The highest ($P < 0.001$) asymptotic GP (A) and half-life ($t_{½}$) values of 50.6 mL and 42.1 h were obtained with the EXPL model, while the lowest ($P < 0.001$) values of 28.4 mL and 1.2 h were obtained with the GOMP model for all the diets. Conversely, the GOMP model had the highest ($P < 0.001$) fractional rate of GP (k), and the LOG model had the lowest ($P < 0.001$). The GRTS model generally had intermediate values for A, $t_{½}$ and k among the four single-pool models evaluated for the diets.

Table 2
Chemical composition (g/kg DM) of diets varying in proportions of Rhodes grass hay and a concentrate.

Chemical composition	Diets				*P*-value		
	D1	D2	D3	D4	SE	Lin	Quad
DM	928.3	925.9	924.4	920.6	0.677	<0.001	<0.001
CP	47.8	56.6	68.9	74.7	1.549	<0.001	0.005
NDFom	641.0	611.4	543.6	495.1	10.875	<0.001	0.014
ADFom	355.8	330.1	307.7	284.3	4.070	<0.001	0.421
Lignin (sa)	76.9	76.9	67.9	68.4	2.612	0.001	<0.001
Ash	118.0	111.1	109.9	76.9	3.660	<0.001	<0.001
OM	810.3	813.9	814.6	843.7	1.980	<0.001	0.001

D1 = diet 1 (1,000 g/kg Rhodes grass hay and 0 of the concentrate); D2 = diet 2 (900 g/kg Rhodes grass hay and 100 g/kg concentrate); D3 = diet 3 (800 g/kg Rhodes grass hay and 200 g/kg concentrate); D4 = diet 4 (700 g/kg Rhodes grass hay and 300 g/kg concentrate); SE = standard error of the means; lin = linear effect; quad = quadratic effect; DM = dry matter; CP = crude protein; NDFom = neutral detergent fiber expressed exclusive of residual ash; ADFom = acid detergent fiber expressed exclusive of residual ash; lignin (sa) = lignin determined by solubilization of cellulose with sulfuric acid; OM = organic matter.

3.4. Comparison of models using goodness-of-fit test

Goodness-of-fit test statistics criteria (RMSE, Adj-R^2 and AIC) of the different diets compared across the 4 models are presented in Table 5. The root mean square error (RMSE) of D1, D2, D3 and D4 ranged from 1.555 to 3.189, 2.785 to 4.429, 2.164 to 3.826 and 1.820 to 3.142, respectively. The EXPL, GRTS and GOMP had the lowest ($P < 0.05$) but comparable RMSE values, while LOG had the highest RMSE for all the diets. The LOG model had the lowest adjusted proportions of variance that is explained by the single-pool model (Adj-R^2) while the GRTS model had the highest ($P < 0.001$) for D1, D2, D3 and D4 with values ranging from 0.906 to 0.978, 0.906 to 0.945, 0.946 to 0.981 and 0.950 to 0.984, respectively. Conversely, for the AIC, the LOG model had the highest ($P < 0.001$) value while the GRTS model had the lowest ($P < 0.001$) for D1, D2, D3 and D4 with values ranging from 3.33 to 11.05, 2.45 to 21.38, 7.54 to 15.87 and 4.13 to 11.10, respectively.

3.5. Model comparison using graphical analyses

The time course for the actual and predicted GP of D1, D2, D3 and D4 incubated with rumen liquor from the abattoir-slaughtered cattle and fitted to EXPL, LOG, GRTS and GOMP models are presented in Fig. 1. Generally, the observed GP profiles for D1, D2, D3 and D4 coincided well with the predicted GP profiles obtained when the GOMP and GRTS models were fitted to the data throughout the incubation time periods. However, during the early periods of incubation (0 to 8 h) for all the diets, the EXPL and LOG models provided poor relationship between the observed and predicted GP; with the LOG model tending to overestimate while the EXPL model underestimating GP. The EXPL model yielded negative values of GP, which are biologically unrealistic. However, the predicted GP profiles obtained using EXPL and LOG models coincided well with the observed GP profiles during incubation period beyond 18 h for all the diets.

The dispersion plots of residuals against incubation time obtained from the differences between observed and predicted GP for the 4 diets as determined by the EXPL, LOG, GOMP and GRTS models are presented in Fig. 2. Residual dispersion provided further evidence for the fitting and descriptive ability of each of the models being evaluated. The widest residual dispersion occurred during the initial phase of incubation (0 to 18 h) when the EXPL (A) and LOG (B) models were used to estimate the residuals. The GRTS model presented the most homogenous residual dispersion throughout the incubation period for the 4 diets when incubated with rumen liquor from the abattoir.

4. Discussion

Although use of mathematical models to fit GP profiles has been majorly based on data generated when feed substrates are inoculated with rumen liquor from fistulated steers of a known feeding regimen (López et al., 2007; Wang et al., 2011), results in this study showed that the 4 models fitted GP data sets for all the

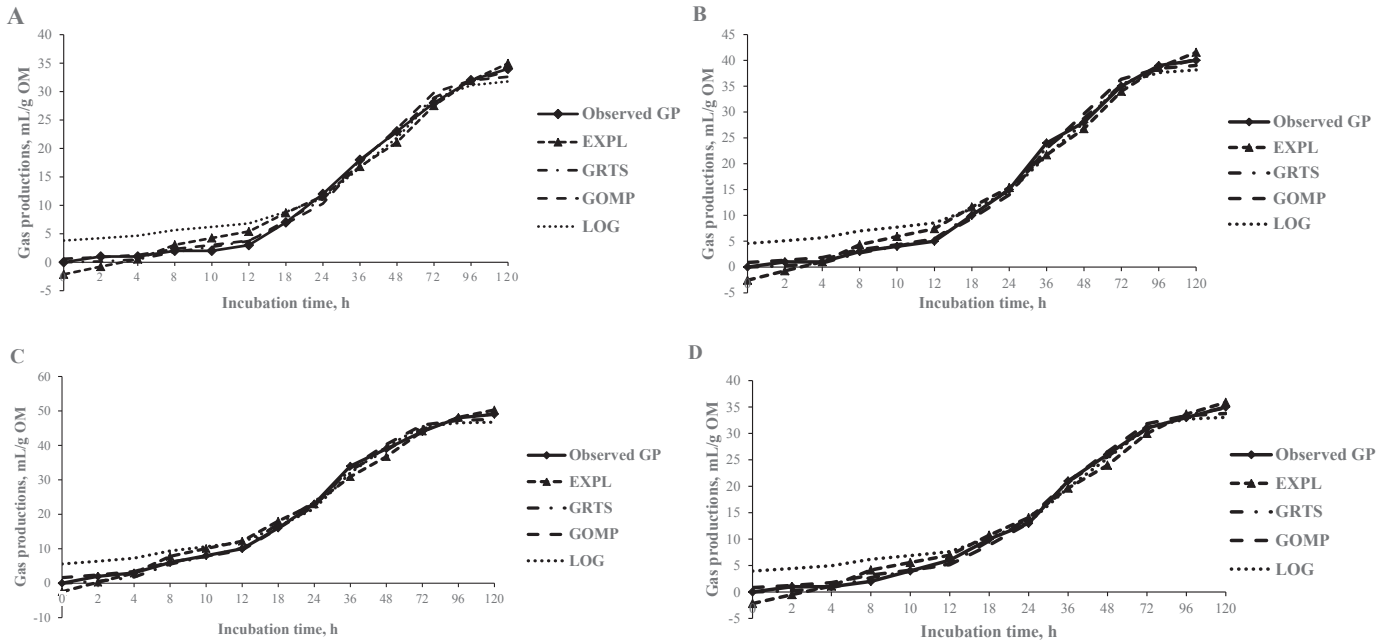

Fig. 1. Observed and predicted gas production profiles of diets (A) D1, (B) D2, (C) D3 and (D) D4 as determined by the exponential with discrete lag time (EXPL), logistic (LOG), Groot's (GRTS) and Gompertz (GOMP) models. D1 = diet 1 (1,000 g/kg Rhodes grass hay and 0 of the concentrate); D2 = diet 2 (900 g/kg Rhodes grass hay and 100 g/kg concentrate); D3 = diet 3 (800 g/kg Rhodes grass hay and 200 g/kg concentrate); D4 = diet 4 (700 g/kg Rhodes grass hay and 300 g/kg concentrate).

diets incubated with rumen liquor from slaughtered cattle. Moreover, models exhibited a sigmoidal behavior with no fitting problems. These results support existing literature, which suggests that GP data for several feedstuffs incubated with rumen liquor from slaughtered cattle is also a viable option for feed evaluation (Chaudhry and Mohamed, 2012; Mutimura et al., 2013). The observed sigmoid behavior is perhaps due to the preponderance of microbial population within the inoculum promoted by the unknown dietary diversity of slaughtered cattle and the ability of these microbes to colonize, ferment and utilize the substrate for their growth (Hidayat et al., 1993; Singh et al., 2010). Furthermore, observed sigmoidal behavior across the 4 models is perhaps the reason why use of slaughtered cattle as sources of inoculum for *in vitro* feed evaluation has gained acceptance among poorly developed countries like those in Sub Saharan Africa that are usually challenged with the high costs of maintaining fistulated animals (Jones and Barnes, 1996; Mutimura et al., 2013).

Table 3
Parameters of gas production profiles of the diets as described by the 4 single-pool mathematical models.

Models	Parameters[1]	Diets				*P*-value		
		D1	D2	D3	D4	SEM	Lin	Quad
EXPL	*A*	37.1	34.4	51.2	35.5	1.7190	<0.001	<0.001
	k	0.018	0.080	0.027	0.020	0.0020	<0.001	<0.001
	lag	3.90	3.61	2.93	1.85	0.6130	<0.001	<0.001
LOG	*A*	23.7	32.1	30.4	27.0	3.2480	<0.001	<0.001
	k	0.011	0.015	0.018	0.015	0.0030	<0.001	<0.001
GRTS	*A*	27.9	36.5	50.1	29.8	0.0002	<0.001	<0.001
	B	41.2	39.4	40.1	39.8	0.0003	<0.001	<0.001
	C	2.18	2.27	2.19	2.01	0.0871	<0.001	<0.001
	T_k	28.92	30.95	27.77	23.59	2.3992	<0.001	<0.001
	k	0.442	0.511	0.837	0.477	0.0091	<0.001	<0.001
	R_D	0.026	0.029	0.025	0.023	0.0014	<0.001	<0.001
GOMP	*A*	25.3	28.4	31.6	28.2	1.1950	<0.001	<0.001
	k	5.956	5.052	4.872	4.320	0.3090	<0.001	<0.001
	c	0.04	0.06	0.07	0.19	0.0450	<0.001	<0.001

D1 = diet 1 (1,000 g/kg Rhodes grass hay and 0 of the concentrate); D2 = diet 2 (900 g/kg Rhodes grass hay and 100 g/kg concentrate); D3 = diet 3 (800 g/kg Rhodes grass hay and 200 g/kg concentrate); D4 = diet 4 (700 g/kg Rhodes grass hay and 300 g/kg concentrate); EXPL = exponential model with discrete lag time; LOG = logistic model; GRTS = Groot's model; GOMP = Gompertz model.

[1] *A* = asymptotic gas production, mL/200 mg DM; *k* = fractional rate of gas production, /h; lag = discrete lag time, h; *B* = half-life, h; *C* = sharpness of the switching characteristic for the profile; T_k = time at which maximum rate of gas production occurs, h; R_D = fractional rate of dietary substrate degradation, /h; *c* = constant factor of microbial efficiency.

Table 4
Comparison of derived *in vitro* gas production parameters for the diets across the four single-pool models.

Parameters[1]	Diets	Models				SEM	*P*-value
		EXPL	LOG	GRTS	GOMP		
A	D1	35.4^{a}	29.3^{c}	31.9^{b}	28.4^{c}	0.468	<0.001
	D2	40.6^{a}	32.4^{c}	36.5^{b}	29.6^{d}	0.305	<0.001
	D3	50.6^{a}	33.8^{b}	49.8^{a}	30.9^{c}	0.454	<0.001
	D4	36.1^{a}	29.7^{c}	34.1^{b}	28.7^{c}	0.278	<0.001
$t_{½}$	D1	42.1^{a}	32.0^{c}	37.2^{b}	2.1^{d}	0.479	<0.001
	D2	41.5^{a}	32.9^{c}	36.9^{b}	1.3^{d}	0.029	<0.001
	D3	27.8^{b}	26.4^{b}	32.8^{a}	1.2^{c}	0.822	<0.001
	D4	37.5^{a}	32.0^{c}	35.0^{b}	1.4^{d}	0.472	<0.001
k	D1	0.018^{c}	0.016^{c}	0.539^{b}	5.374^{a}	0.051	<0.001
	D2	0.018^{c}	0.015^{c}	0.587^{b}	4.727^{a}	0.037	<0.001
	D3	0.027^{c}	0.019^{d}	0.833^{b}	4.307^{a}	0.062	<0.001
	D4	0.020^{c}	0.016^{c}	0.544^{b}	4.125^{a}	0.023	<0.001

D1 = diet 1 (1,000 g/kg Rhodes grass hay and 0 of the concentrate); D2 = diet 2 (900 g/kg Rhodes grass hay and 100 g/kg concentrate); D3 = diet 3 (800 g/kg Rhodes grass hay and 200 g/kg concentrate); D4 = diet 4 (700 g/kg Rhodes grass hay and 300 g/kg concentrate); EXPL = exponential model with discrete lag time; LOG = logistic model; GRTS = Groot's model; GOMP = Gompertz model.

[a,b,c,d] Within a row, means with different letters differ $P < 0.05$.

[1] *A* = asymptotic gas production, mL/200 mg DM; $t_{½}$ = half-life, h; *k* = fractional rate of gas production, /h.

Table 5
Goodness-of-fit test statistics of the different diets compared across the 4 models.

Item	Diets	Models				SEM	*P*-value
		EXPL	LOG	GRTS	GOMP		
RMSE	D1	2.156[b]	3.189[a]	1.609[b]	1.555[b]	0.234	<0.001
	D2	3.400[ab]	4.429[a]	2.893[b]	2.785[b]	0.447	0.060
	D3	2.920[ab]	3.826[a]	2.164[b]	2.240[b]	0.381	<0.012
	D4	2.071[b]	3.142[a]	1.820[b]	1.844[b]	0.286	0.320
Adj-R^2	D1	0.936[b]	0.906[b]	0.978[a]	0.976[a]	0.013	<0.001
	D2	0.937	0.906	0.945	0.944	0.019	0.440
	D3	0.971[a]	0.946[b]	0.981[a]	0.981[a]	0.006	<0.001
	D4	0.978[a]	0.950[b]	0.984[a]	0.982[a]	0.006	0.024
AIC	D1	5.341[b]	11.052[a]	3.325[b]	3.873[b]	0.928	<0.041
	D2	14.148[b]	21.382[a]	2.452[c]	12.082[b]	2.452	<0.041
	D3	13.237[ab]	15.874[a]	7.542[b]	7.575[b]	1.978	<0.008
	D4	5.630[b]	11.100[a]	4.132[b]	4.646[b]	1.6293	0.039

EXPL = exponential model with discrete lag time; LOG = logistic model; GRTS = Groot's model; GOMP = Gompertz model; RMSE = root mean square error; Adj-R^2 = adjusted coefficient of determination; AIC = Akaike's information criterion.
[a,b,c,d] Within a row, means with different letters differ $P < 0.05$.

In Fig. 1, all the models fitted GP data well exhibiting a highly sigmoidal shape that could be distinguished into 3 different phases: 1) initial phase being characterized by slow or no GP and occurred during the first 6 to 8 h, 2) exponential phase being characterized by rapid GP, 3) asymptotic phase in which the rate of GP slows and finally reaches zero. Low GP observed during the early hours of incubation for all diets across the 4 models was possibly due to restricted microbial fermentation caused by the limited hydration and colonization of the substrate by rumen microbes (Groot et al., 1996). However, during the asymptotic phase, most easily degradable part of the substrate was possibly degraded leaving an increasingly non-degradable fraction of the substrate hence explaining why the observed fractional rate of GP tended to reach zero as earlier observed by Cone et al. (1997).

The observed sigmoidicity of the EXPL model was possibly due to the high proportions of the slowly degrading cell wall contents of Rhodes grass hay contained in the diets (Robinson et al., 1986; López et al., 2011) and the discrete lag time parameter that was selectively added to it to increase its robustness in fitting GP profiles (Dhanoa et al., 2000). However, GP profile shapes presented by the LOG, GRTS and GOMP models were not affected by the lag time parameter and is perhaps a reflection of the different phases in the profiles as influenced by the differences in substrate fermentation rates (Tedeschi et al., 2008), which in turn heavily depends on proportions of the concentrate in diets. Nonetheless, the exclusion of discrete lag time from the LOG, GRTS and GOMP models, was based on the capability of these models to describe both GP profile shapes with no inflection point and those in which the inflection point may be variable or fixed (Tedeschi et al., 2008). Based on the sigmoidal behavior, it can be inferred that the GRTS and GOMP models seem equally suited to describe the profiles throughout the incubation period (120 h) because the predicted GP obtained with these models coincided well with the observed GP (Fig. 1). However, the EXPL and LOG underestimated and overestimated GP in the early phase of incubation, respectively. This is in agreement with Peripolli et al. (2014) who earlier observed similar results for the two models. The poor estimation of GP during the initial phases observed for the EXPL and LOG models could be related to differences in structures of the two models (Huhtanen et al., 2008). The EXPL model assumes that rate of GP only depends on the substrate available for fermentation with no consideration for microbial population while the LOG model assumes that at early incubation stages, GP is limited by microbial population but not by substrate concentration (Schofield et al., 1994; López et al., 2011). Therefore,

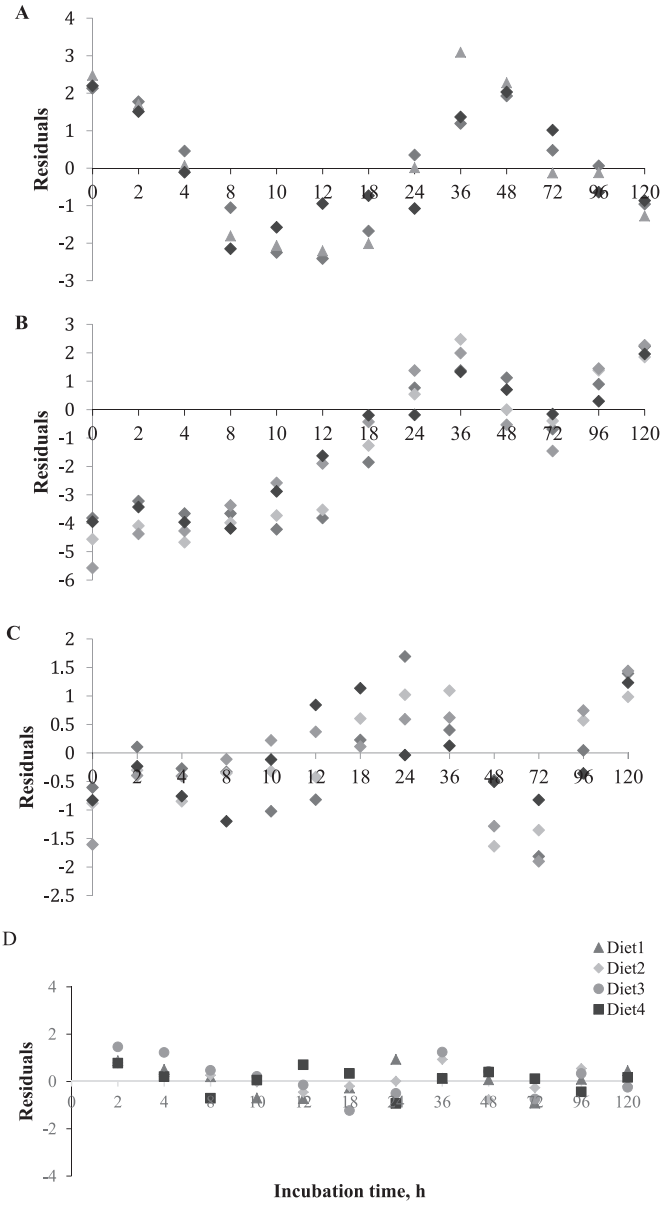

Fig. 2. Dispersion of residuals measured from differences between observed and predicted gas production of the 4 diets during the incubation period as determined by (A) EXPL, (B) LOG, (C) GOMP and (D) GRTS models. Diet 1, 1,000 g/kg Rhodes grass hay and 0 of the concentrate; Diet 2, 900 g/kg Rhodes grass hay and 100 g/kg concentrate; Diet 3, 800 g/kg Rhodes grass hay and 200 g/kg concentrate; Diet 4, 700 g/kg Rhodes grass hay and 300 g/kg concentrate.

the suitability of these two models to evaluate *in vitro* fermentation of highly soluble components of diets (Wang et al., 2013) during early-stages is likely to be compromised irrespective of rumen liquor source. While the LOG model presented a positive intercept on the *y*-axis, indicating that there was instant GP from substrate fermentation in the syringe, which is inexplicable (López et al., 2011), the curve for the EXPL model crossed the *x*-axis at an early incubation time (3 h) resulting in a negative *y*-intercept even when a discrete lag time was included in the equation. Moreover, López et al. (2011) stated that the model function domain should be restricted to the first quadrant where both cumulative GP (Y) and incubation time (t) are positive rather than the biologically unrealistic negative values.

The increasing GP at a decreasing rate in later phases of fermentation is related to degradation of the more recalcitrant but degradable cell wall components. Therefore, the relative proportions of the soluble, insoluble but degradable, and the undegradable fractions of the diets influenced the kinetics of GP irrespective of the model used as observed in Fig. 1.

The use of mathematical models allows analysis of data, comparison of substrate or fermentation environments and can provide useful information concerning the feedstuff or dietary substrate composition and the fermentability of soluble and slowly fermentable components of the feeds (Getachew et al., 1998; Do Cabo, 2012). In order to compare models based on fitting criteria, GP profiles obtained from fermentation of the 4 diets varying in proportions of concentrate with rumen liquor from slaughtered cattle were fitted to 4 single-pool mathematical models. The values of the final asymptotic gas volumes of the 4 diets across the 4 models ranged between 23.7 and 51.2 mL/200 mg DM and were comparable to values ranging between 35.1 and 60.3 mL/200 mg for rumen liquor obtained from fistulated cattle earlier reported by Wang et al. (2011). However, McAllister et al. (1994) and Groot et al. (1996) observed lower values of maximum GP when substrates with high cell wall contents were fermented with rumen liquor from fistulated cattle. This could be due to the fact that easily fermentable and highly soluble fractions of the substrate are rapidly degraded in early stages of fermentation while the less digestible or totally indigestible fractions (Beuvink and Kogut, 1993) contribute to decreasing rate of GP during exponential and asymptotic phases of fermentation.

Similarities and differences among the candidate models in the study were determined on the basis of the derived GP parameters, goodness-of-fit test statistics assessment and residual analysis (Motulsky and Ransnas, 1987; López et al., 2004). However, Üçkardes et al. (2013) emphasized the necessity to use few criteria to determine the differences between models. Derived kinetics of GP were used to compare the models for each of the diets (Table 4). The kinetics of GP obtained for the 4 models provide valuable information needed in formulation of well-balanced diets for improved ruminant production and can also be used in modeling animal responses. The significant differences that occurred among the 4 candidate models for the derived asymptotic GP (A), time taken to produce half of the asymptotic gas volume ($t_{½}$) and fractional rate of GP (k) suggest differences in suitability of models to estimate nutritionally important feed parameters directly related to improved animal production. The EXPL model had the highest asymptotic GP and half-life compared to other models. According to López et al. (1999) and Huhtanen et al. (2008), higher asymptotic GP and half-life parameters is an indication of the model underestimating the fractional rate of GP. Furthermore, observed $t_{½}$ for the EXPL model were higher than those of the simple exponential model reported by Sahin et al. (2011b) and Wang et al. (2011) yet the feed substrates had comparable ranges of NDFom of 346.0 to 795.0 g/kg DM and 495.0 to 641.0 g/kg DM. Nevertheless, the range of $t_{½}$ values of 1.2 to 42.1 h for the 4 models were in the same range of 1 to 50 h reported for *in vitro* GP studies for ruminant feeds elsewhere (Wang et al., 2013). The differences in $t_{½}$ values revealed variations in the ability of different models to determine the nutritional value of the different feeds.

Exhibition of the lowest $t_{½}$ and the highest k values for the GOMP model is an indication of its superiority in determining the nutritional value of the feeds among the tested models. This observation is in agreement with Sahin et al. (2011b) who stated that the fractional rate of GP is inversely proportional to estimated partial GP times ($t_{½}$). Moreover, any changes in k are related to the differences in particle hydration and microbial attachment (France et al., 2000), which are directly related to $t_{½}$ (Wang et al., 2013). Furthermore, based on the direct relationship between the fractional rate of GP and the rate at which the substrate is degraded, the lower k value obtained with the GOMP model emphasized the mathematical effect of the substrate limitation on the growth rates of rumen microbes (Schofield et al., 1994) on GP. The differences in the k values are also indicative of how different models describe GP profiles and detect variations in rate of GP as influenced by alteration in particle hydration, microbial attachment and microbial numbers during the incubation (Wang et al., 2013). Differences in rates of GP across models are also indicative of the ability of such models to detect dietary effects on microbial population.

The root mean square error (RMSE), adjusted proportion of variance accounted for by the model (Adj-R^2) and AIC values ranged from 1.555 to 4.429, 0.906 to 0.984 and 2.452 to 15.874, respectively, for all the diets compared across the 4 models (Table 5). The Adj-R^2 for D1, D2, D3 and D4 that ranged from 0.906 to 0.978, 0.906 to 0.945, 0.946 to 0.981 and 0.950 to 0.984 is an indication of strong fitting of all the models to the GP data. However, the RMSE, Adj-R^2 and AIC of the GRTS and GOMP models were found to be more appropriate in fitting GP data than the LOG model. The small but comparable RMSE and AIC values of the GRTS and GOMP models indicate that these models fitted the data better and were more appropriate in describing the GP profiles of the diets (Huhtanen et al., 2008). The EXPL model showed a relatively high RMSE than GRTS and GOMP when fitted to GP data because it assumes that after a discrete lag time the feed is fermented instantaneously at maximum rate (Singh et al., 2010). The poor ability of the LOG model to fit the data according to RMSE and AIC was possibly due to its fixed inflexion point and intercept at '$t = 0$' (Wang et al., 2011). However, the Adj-R^2 value criterion suggests that the 4 models evaluated fitted the data well and the Adj-R^2 values of the LOG and EXPL models were found to be comparable to those reported by Üçkardes and Efe (2014). Therefore, based on the goodness-of-fit statistical criterion, GP profiles of the diets were more appropriately fitted and described by GRTS and GOMP than the EXPL and LOG models, in that order.

Assessing the distribution of residuals estimated from the differences between observed and estimated values indicated that the GRTS model was more appropriate in predicting GP in all phases of incubation compared to the EXPL, LOG and GOMP models (Fig. 2). The wide residual dispersion, which occurred in both the initial and intermediate hours of incubation for the EXPL and LOG models, is explained by the differences in model structure as earlier described (López et al., 2011). These results are consistent with the findings of Sahin et al. (2011a) who found that the EXPL model underestimated GP during the initial phase. Similarly, our results agreed with the findings of Posada and Noguera (2007) who observed greater residuals dispersion and greater tendency of the LOG model to overestimate GP during the entire time course of fermentation. According to the visual inspection of the estimated residual distribution graphs, the GRTS model had the lowest and most homogenous residual dispersion as a function of fermentation time for the 4 diets. This indicates that the GRTS model fitted the GP data more appropriately when diets of Rhodes grass hay with varying proportions of concentrate were incubated with rumen liquor from slaughtered cattle.

5. Conclusion

Differences among the 4 models in describing the 3 phases of the GP profile reflected variances in the ability of selected single-pool models to describe the biological behavior of *in vitro* fermentation processes. In our study, GRTS and GOMP models were more appropriate than the EXPL and LOG models in fitting and describing GP profiles of the 4 diets across the entire incubation

period. Furthermore, our study indicated that the EXPL model had the highest asymptotic gas volume and the longest half-life across the entire incubation period. Therefore, the EXPL model may not be suitable for the mathematical description of GP profiles of highly fibrous ruminant feeds. However, according to derived GP parameters, GRTS and GOMP models were more appropriate for describing GP profiles for the diets. Although the GRTS model presented the most homogenous residual dispersion throughout the incubation period for the 4 diets, all models exhibited a sigmoidal behavior and are considered acceptable. Therefore, rumen liquor from slaughtered cattle of unknown dietary history can be used to derive nutritionally important feed parameters, but the choice of the most appropriate model should be made based on fitting and description criteria and dietary substrates used.

Conflict of interest declaration

The authors declare that there are no actual or potential conflicts of interest associated with this work.

Acknowledgment

We are sincerely grateful to the Livestock and Fisheries Programme (LFP 04) of the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA- RC12_LFP-04 under the auspices of the World Bank for providing financial support to undertake this research. Much appreciation is also extended to Mr. Abasi Kigozi, Abubakar Sadik Mustafa and Mr. Emmanuel Mutebi of the Animal science laboratory, Makerere University, Kampala, for facilitating laboratory analysis.

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