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Evaluation of different mathematical models on fitting the in vitro gas production parameters in beef cattle

Jinze Wang[1,2], Zhiyu Zhou[1,2], Yang Cheng[1], Hao Wu[1], Xin Yi[1], Ziqi Deng[1], Boping Ye[1], Saige Liu[1], Lingyu Ren[1], Ning Li[1], Xiaowen Zhu[1], Wei Li[1], Simeng Yi[1], Qingxiang Meng[1] & Zhenming Zhou[1]✉

In vitro rumen gas production experiment was conducted with 57 kinds of feedstuff, which were categorized into energy feed, protein feed, and roughage, collected within China. Eight mathematical models were employed to describe the kinetics of in vitro rumen gas production. The results found that for energy feeds, protein feeds, and roughages, respectively, Michaelis–Menten (MM) or Logistic-Exponential with lag (LEL), MM, and Mitscherlich (MIT) exhibited the highest or shown no significant difference compared to the highest coefficient of determination (R^2) ($P<0.05$) for all categories of feed. Furthermore, regression estimation of intercept and slope for regression estimates of intercept and slope for Observed versus Predicted of aforementioned models shown no significant difference from 0 and 1, respectively ($P<0.05$), except LEL for energy feed. Mean absolute error (MAE), root mean squared error of prediction (RMSEP), mean squared error of prediction (MSEP) of those models were relatively lower, with minimal systematic bias and regression bias. Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) rankings were higher compared with other models. Given these results, in studies where feedstuff categories are not distinguished or multiple feedstuffs categories are included, the MM model proves to be a good choice. MM or LEL was considered to better fit energy feedstuffs. The MM model was the optimal choice for fitting protein feedstuffs. MIT provided the best accuracy and moderate precision when fitting roughages.

Keywords In vitro gas production, Rumen fermentation, Mathematical model, Model evaluation

In vitro gas production method is commonly used for evaluating ruminant feed and additives[1,2], microbial fermentation process[3,4] and the effect of anti-nutrient factors on microbial activity[5,6]. For better understanding of the rumen fermentation, the kinetic parameters of degradation are crucial, as they not only describe digestion but also characterize the intrinsic properties of substrates.

Mathematical models can effectively parameterize the degradation or fermentation profiles of each category of feedstuff[7]. Exponential models were among the earliest mathematical models applied to the processes of digestion. In 1979, Ørskov and McDonald[8] used exponential model to describe the temporal changes of substrates in the nylon bags during the in situ digestion experiment. Similarly, Krishnamoorthy[9] employed a modified version of the model most commonly used to describe fiber digestion[10], which is now the most widely used exponential model. The exponential model assumed that the digestion rate is proportional to the substrate concentration and independent of microbial biomass. Depending on whether the lag time is considered, exponential models include either 2 (EXP0) or 3 (EXPL) parameters. However, the exponential model suffered from inflexibility issues and failed to adequately describe the gas production process. Schofield[11] first applied the Gompertz (GOM) and Logistic (LOG) models, which originated from microbial growth kinetics, to calculate in vitro rumen gas production parameters. Both models assumed that the rate of digestion is directly proportional to the current microbial population and substrate level, but they differ in the mathematical effect of substrate limitation on microbial growth rate. The LOG model assumes a linear relationship, while the GOM model assumes a logarithmic relationship[11]. However, due to the presence of a positive intercept and a fixed inflection point

[1]State Key Laboratory of Animal Nutrition and Feeding, College of Animal Science and Technology, China Agricultural University, No.2 Yuanmingyuan West Road, Beijing 100193, China. [2] Jinze Wang and Zhiyu Zhou contributed equally to this work. ✉email: zhouzm@cau.edu.cn

occurring at half of the asymptotic gas production, the LOG model may not be suitable in certain situations[12]. Wang et al.[13] introduced a shape parameter into the model, constructing the Logistic-Exponential (LE) model. This model is more flexible and variable, with a non-fixed inflection point, and eliminates the positive intercept on the y-axis. France et al.[14]decomposed the fractional rate of degradation μ into a function related to the square root of time, establishing the Mitscherlich (MIT) model. According to France[12], the relationship between μ and the square root of time may be associated with the diffusion of microbial enzymes. Groot et al.[7] proposed a modified Michaelis–Menten (MM) model based on the Michaelis–Menten equation in enzyme kinetics. The MM model makes more flexible assumptions about fractional degradation rate, which can be continuously decreased over time or increase initially before decreasing[12]. Similar to the MIT model, the increase in the degradation rate reflects the increased accessibility of substrates due to particle hydration, microbial attachment, and an increase in microbial population, while the decrease in degradation rate may reflect the imposition of chemical and structural constraints[12].

Additionally, the gas production process is closely associated with the chemical composition and the physical structure of the substrate. Getachew et al.[15] investigated the relationship between in vitro rumen gas production and chemical nutrients in 12 kinds of feedstuff comprising 38 samples. They found a negative correlation between crude protein (CP) and gas production ($P < 0.001$), while non-fiber carbohydrate (NFC) levels were positively correlated with gas production ($P < 0.01$). It is generally believed that this is because the fermentative capacity of protein is approximately 0.3 times of carbohydrates[16]. Moreover, gas production may decrease due to the reaction between ammonia and carbon dioxide[17]. Different types of carbohydrates produce varying volumes of gas depending on their chemical properties and interactions with other feed components[2]. Compared to the slowly degradable fraction, rapidly degradable carbohydrates produce more propionate, which is a process with lower gas potential compared to acetate[18]. In ruminant nutrition, feedstuff can be simply categorized into energy feed, protein feed, and roughage. Therefore, distinct models should be used based on the nutrient characteristics of each feedstuff.

Though previous studies have investigated the comparison of the goodness of fit of different models, they may suffer from issues such as small sample sizes and undivided feed categories[1,11,13,19–24]. To address these issues, this study aims to compare the performance of various mathematical models in predicting in vitro gas production from different feedstuff categories and to evaluate the impact of sample size and categorization on model reliability. By enhancing the understanding of the relationship between feedstuff characteristics and gas production kinetics, this study will provide researchers with improved modeling tools that facilitate more accurate evaluations of ruminant feed efficiency and fermentation processes. The insights gained from this research could inform more effective feed formulations, ultimately leading to better animal health and productivity in ruminant livestock.

Results

Feedstuffs and in vitro rumen gas production profile

The 57 feedstuff materials were classified as energy feeds, protein feeds, and roughages based on their common use in ruminant production. Descriptive statistics of the nutritional components for these three categories of feedstuff were presented in Table 1, and gas production profiles were depicted in Fig. 1. Energy feeds, which primarily consisted of grains and brans, were characterized by a high starch content (42.31 ± 5.03%) and low levels of structural carbohydrates. Protein feeds exhibited high crude protein (CP) content (33.87 ± 2.4%) and lower carbohydrate proportions. Additionally, due to the inclusion of various by-product from the oil industry, protein feeds had higher levels of crude fat (EE) (6.61 ± 0.77%). Roughages mainly comprised hays and straws, containing significant amounts of structural carbohydrates (67.86 ± 2.59% NDF, 44.78 ± 2.51% ADF, 10.21 ± 1.89% ADL).

Fitting of the gas production profiles

Figure 2 illustrated the fitting of different models for the three categories of feedstuff. Significant differences were observed in the shapes of the fitting curves for the three categories. Gas production from energy feeds was notably higher than that from the other two categories, with both energy and protein feeds exhibited higher gas production rates than roughages during the same period, reaching the plateau phase earlier. The gas production curves for roughages were the most gradual, reaching the plateau phase later and exhibiting relatively lower gas production overall. Among the different models, GOM and LOG models shown sigmoidal curves with similar shapes that almost completely overlap in energy feeds. The LOG model reached the plateau phase earlier than the other models within the same category of feedstuff, while the GOM model exhibited similar characteristics only in energy and protein feed, showing lower gas production after reaching the plateau phase compared to other models. The other models fitted non-sigmoidal curves for all three categories of feedstuff, with the MM model showing the highest final gas production compared to the other models within the same category.

The mean values of Vf, $t_{0.5}$, and $\mu_{0.5}$ predicted by the 8 models for different categories of feedstuff were presented in Fig. 3. The final asymptotic gas production was represented by Vf. The time $t_{0.5}$ was calculated when V = Vf/2. The value $\mu_{0.5}$ was obtained by substituting $t_{0.5}$ into the formula dV/dt/V. Significant differences in gas production parameters were noted among the different categories of feedstuff. The Vf for energy feed (61.98–71.68 ml/200 mg) was much higher than that for protein feed (37.63–45.77 ml/200 mg) and roughage (35.67–46.86 ml/200 mg). Across all categories, the MM model yielded the highest predicted Vf values, while LOG produced the lowest. Predicted $t_{0.5}$ values did not show significant differences among different models for any category. However, both the GOM and LOG models consistently predicted significantly higher $\mu_{0.5}$ values compared to other models across all categories.

Items	Average	Median	Minimum	Maximum	SD
Energy feeds (*N*=24)					
DM	92.88	94.67	82.53	98.79	1.107
CP	14.19	13.95	6.44	21.99	0.794
Starch	42.31	53.77	2.65	81.88	5.026
EE	4.90	3.34	0.25	18.43	1.079
Ash	4.64	3.03	0.17	21.64	1.045
NDF	26.83	22.07	2.09	70.48	3.688
ADF	11.35	11.32	1.47	49.06	2.029
ADL	2.77	2.50	0.13	8.47	0.435
Protein feeds (*N*=22)					
DM	91.80	92.10	79.79	98.35	0.885
CP	33.87	35.26	16.02	55.56	2.400
Starch	4.58	3.83	0.97	13.10	0.710
EE	6.61	5.56	0.70	12.75	0.773
Ash	9.39	6.98	4.32	24.60	1.196
NDF	40.52	38.64	22.11	68.38	2.818
ADF	23.72	20.72	10.46	50.17	2.347
ADL	9.00	7.96	1.72	26.29	1.356
Roughages (*N*=22)					
DM	92.62	92.61	84.02	98.10	0.685
CP	8.76	7.59	3.49	23.61	1.067
Starch	3.86	3.00	0.41	13.05	0.746
EE	2.20	1.49	0.36	8.00	0.406
Ash	10.59	10.24	2.86	17.24	0.865
NDF	67.86	71.39	35.99	83.30	2.588
ADF	44.78	44.45	15.21	68.49	2.507
ADL	10.21	7.44	1.17	32.04	1.893

Table 1. Descriptive statistics of the chemical composition of three categories feedstuff (dry matter basis, %). DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; SD: standard deviation.

Model comparison

The average R^2 values for each category of feedstuff fitted by different models were depicted in Fig. 4, with all models achieving R^2 values above 0.94. Across different groups, a similar ranking of model performance was observed. Within each group, MM consistently exhibited the highest R^2 values, followed by MIT, while LOG consistently showed significantly lower R^2 compared to other models. In the energy feeds and roughages groups, MIT, LEL, and LE0 did not exhibit significant differences compared to MM, whereas in the protein feed group, MM significantly outperformed all other models. Among all feedstuffs, regardless of categorization, the MM model exhibits significantly higher R^2 values compared to the other models.

The results of regressing observed versus predicted gas production were illustrated in Fig. 5 and summarized in Table 2. Among all 8 models, EXPL, MIT and MM exhibited non-significant slopes and intercepts across the three categories of feedstuff. In the energy feeds group, significant differences were observed in the slopes compared to 1 for EXP0, GOM, LOG, LE0, and LEL, with all models except LEL showing significant non-zero intercepts. In the protein feeds and roughages groups, LE0 and LEL also demonstrated good fitting performance. However, EXP0, GOM, and LOG exhibited significantly non-unity slopes, while EXP0 and LOG had intercepts significantly different from 0. In the uncategorized feedstuffs group, only EXPL and MM exhibited non-significant slopes and intercepts.

Error analysis of the 8 models fitting different categories of feedstuff was presented in Table 3. Across the four groups, the MAE, RMSEP, and MSEP of different models were consistently similar, with MM or MIT demonstrating the smallest values, followed by LE0 and LEL. Focusing on the MSEP decomposition parts of these models, it was observed that MIT exhibits no systematic bias across the four groups, showing only a regression bias of 0.01% when fitting roughages. Apart from MIT, LEL and LE0 also demonstrated low system bias in energy feed, with LEL exhibiting lower regression bias as well. In the protein feed, MM showed minimal systematic bias and regression bias other than MIT. However, all models exhibited system bias and regression bias in roughage. In the group comprising all feedstuffs, LE0 also displayed no bias.

The AIC and BIC values were calculated for each model fit, and the models were ranked based on these criteria for each sample, as shown in Table 4. A higher rank indicated better model performance. The MM

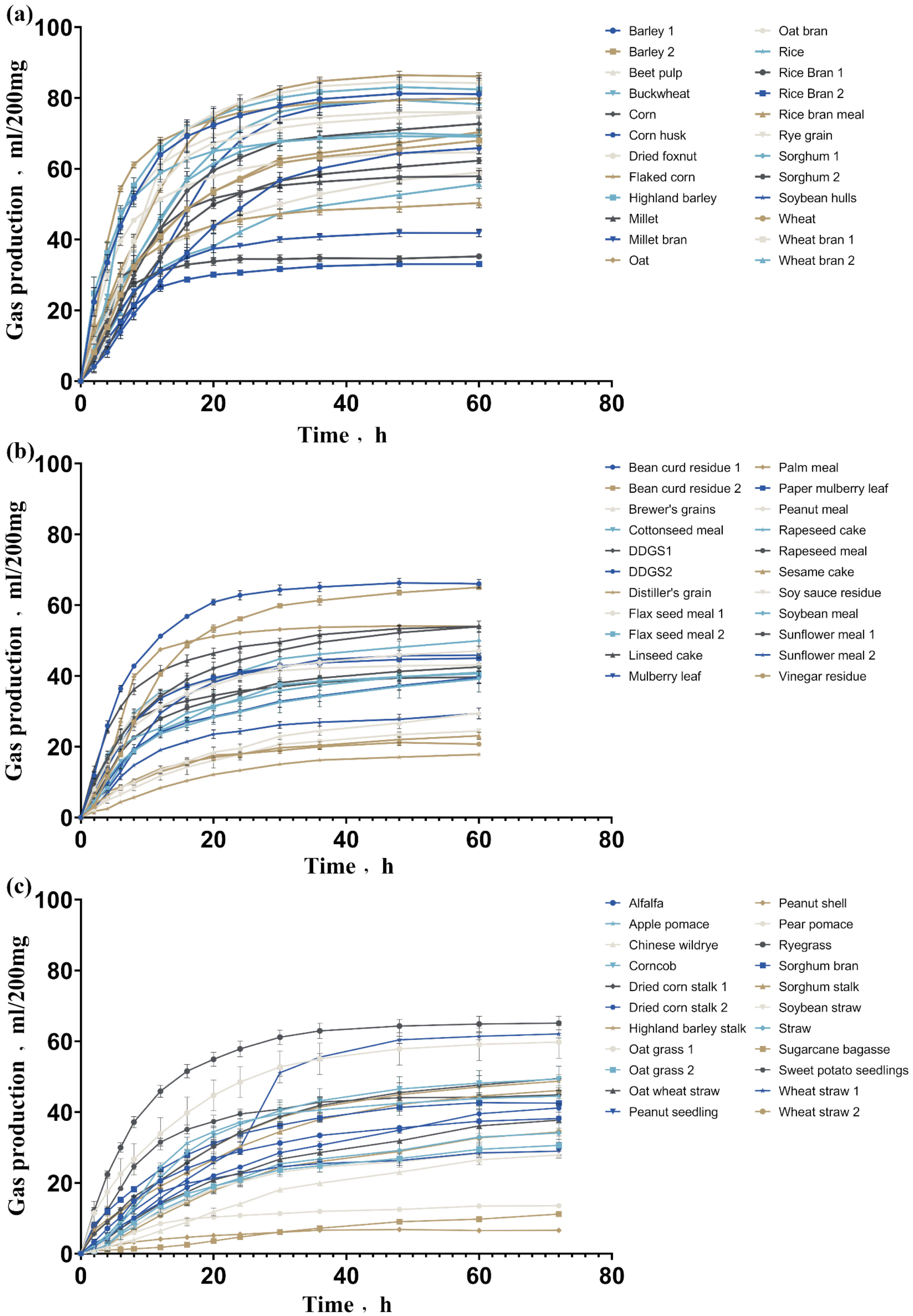

Fig. 1. The in vitro gas production profiles. Fermentation of energy and protein feeds for 60 h, and roughages for 72 h. (a) 24 energy feed samples; (b) 22 protein feed samples; (c) 22 roughage samples.

model consistently ranked highest in terms of AIC (BIC) across all four groups. Among energy feeds, MM (MM) exhibited the highest performance, followed by LE0, LEL, and EXPL (LE0, LEL and EXPL) according to AIC (BIC) criteria. In the case of protein feed, MM (MM) outperformed other models, with EXP0, EXPL, and LE0 (EXP0, EXPL and LE0) following in AIC (BIC) ranking. Regarding roughage, MM (MM) ranked highest, followed by EXPL, MIT, and LE0 (LE0, EXPL and EXP0) in AIC (BIC) ranking. For the entire group of

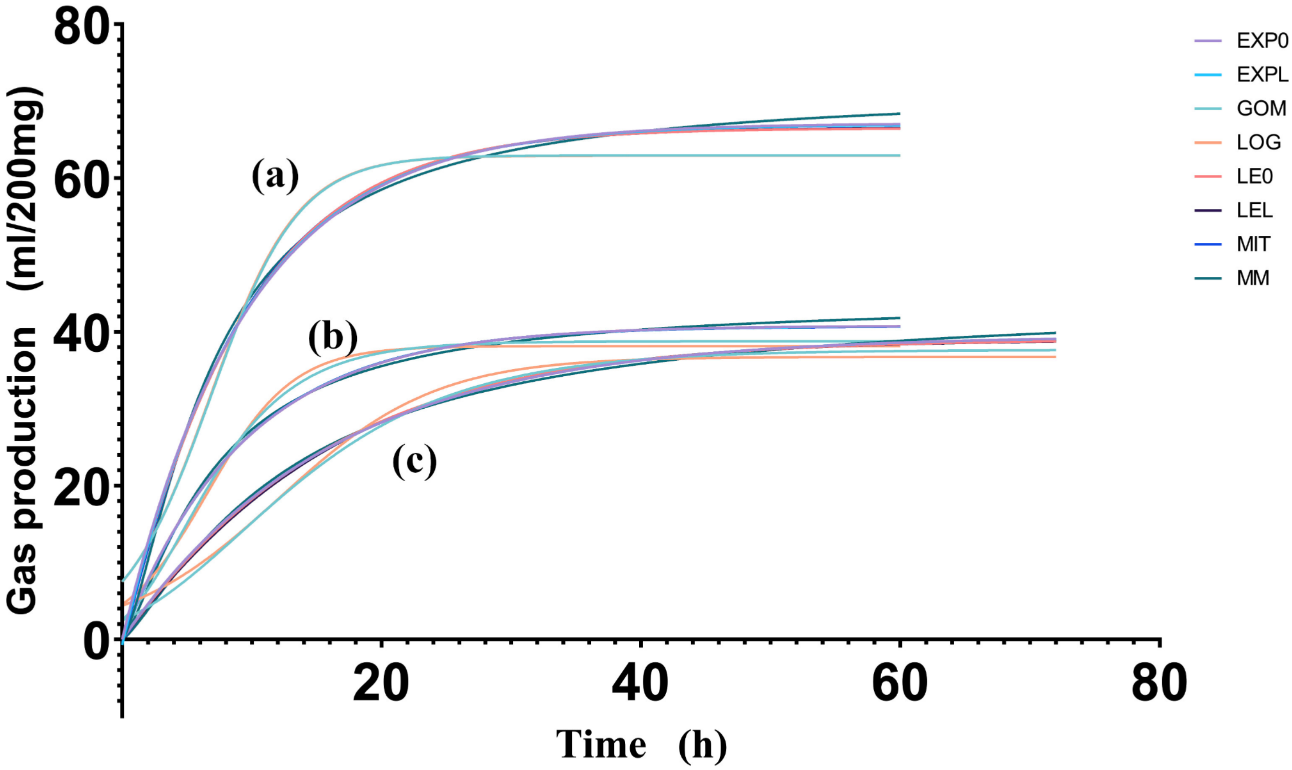

Fig. 2. Fitting profiles of 8 models for 3 categories of feedstuff. The curves of energy and protein feeds for 60 h, and roughages for 72 h. (a)8 curves for energy feeds; (b) 8 curves for protein feeds; (c) 8 curves for roughages. EXP0: exponential without lag phase; EXPL: exponential with lag phase; GOM: Gompertz; LOG: logistic; LE0: logistic-exponential without lag phase; LEL: logistic-exponential with lag phase MIT: Mitscherlich; MM: Michaelis-Menten.

feedstuffs, MM (MM) was superior, with LE0, EXPL, and LEL trailing behind (EXPL, EXP0 and LE0) according to AIC (BIC) criteria.

Discussion

The 68 samples selected for this experiment comprised 57 kinds of feedstuff, categorized into three groups commonly used for ruminants: energy feeds, protein feeds, and roughages. The categorization of feedstuffs in this study was carefully based on chemical nutrients, physical properties, and practical applications rather than their kinetics of in vitro rumen gas production. This deliberate approach was intended to align with the primary objective of the research, ensuring that the nutritional value of the ingredients remained the focal point of analysis. By prioritizing nutritional attributes, we aimed to offer a clearer understanding of feed contributions to overall dietary formulations, thereby promoting more informed decisions in the field of animal nutrition. The nutritional and gas production data exhibit a wide range and substantial quantity, indicating their representativeness.

The chemical composition and the physical structure of feedstuff are closely related to gas production curves. It is generally believed that the main sources of gas in in vitro rumen gas production experiments are carbohydrate degradation and the reaction of volatile fatty acids (VFAs) with substrate buffer solution[25,26]. CP fermentation generated less gas, while EE hardly produces any gas[2]. Structural carbohydrates, being more resistant to degradation, often have a significant impact on both gas volume and gas production rate[27]. This also explains why the final gas production and Vf of most protein and roughage feeds were lower than those of energy feeds. Additionally, roughages typically exhibited flatter gas production curves, longer $t_{0.5}$, and smaller $\mu_{0.5}$ values.

Huhtanen et al.[28], through a comparison of residuals over time, observed that EXP0 and MM tended to overestimate asymptotic gas volume. This trend appeared to be present in both energy feeds and roughages in this experiment. Huhtanen explained the overestimation of asymptotic gas volume by the EXP0 model as its inability to describe lag phenomena[28]. However, this did not explain why EXP0 does not exhibit higher Vf in protein feed, and why there was no significant difference between EXP0 and EXPL in all feedstuff categories. Considering that Huhtanen's samples were roughages, the overestimation of Vf by EXP0 might be due to its exponential mathematical structure being unsuitable for energy and roughage feedstuffs. Additionally, the decreasing fractional degradation rate of MM in the later stages of fermentation might not be reasonable, which led to the overestimation of asymptotic gas volume[21,28]. Schofield et al.[11] found in their model derivation that the maximum rate (i.e., the curve inflection point) of LOG and GOM models occurs at V = Vf/2 and V = Vf/e.

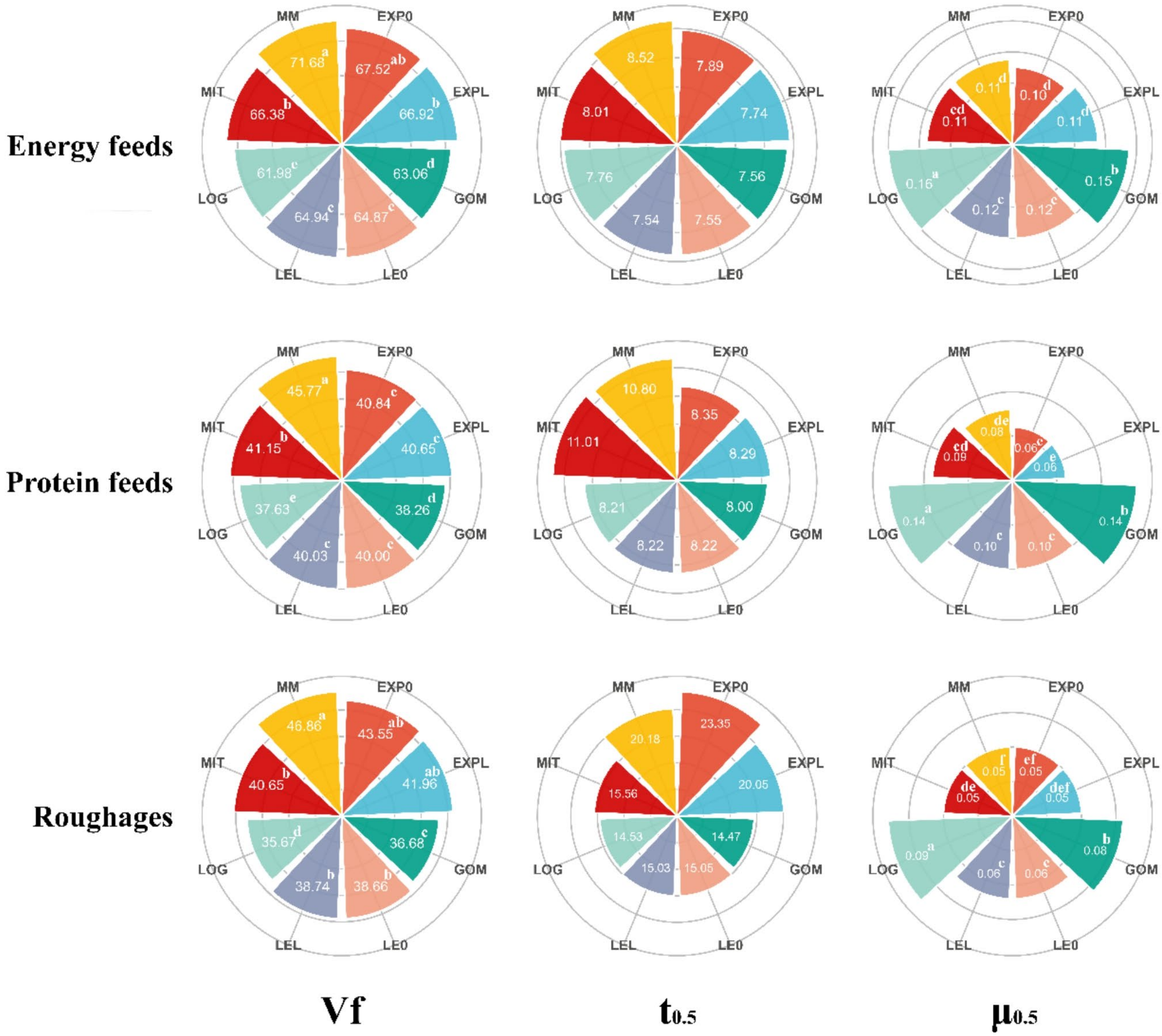

Fig. 3. Comparison of gas production parameters for different models. Vf, $t_{0.5}$, $\mu_{0.5}$ are the average of each category of feedstuff. Means with different letters differ ($P<0.05$). EXP0: exponential without lag phase; EXPL: exponential with lag phase; GOM: Gompertz; LOG: logistic; LE0: logistic-exponential without lag phase; LEL: logistic-exponential with lag phase; MIT: Mitscherlich; MM: Michaelis–Menten; Vf: final asymptotic gas production; $t_{0.5}$: the time at Vf/2; $\mu_{0.5}$: fractional rate of gas production at Vf/2.

This might explain why $\mu_{0.5}$was significantly higher than GOM, while GOM was significantly higher than other models in all feedstuff categories. This was consistent with previous research results[13,28]. Considering that there was no significant difference in $t_{0.5}$ between models within each feedstuff category, different models may not exhibit differently in the fractional rate of gas production when fitting the same category of feedstuff.

The models extended to multi-pool analysis, with the fundamental assumption that the incubated feedstuff was not homogeneous[7,29]. Cone et al.[30] classified gas production into three stages: the initial stage involves the neutral detergent soluble fraction (NDS), the subsequent stage entails NDF fermentation, and the final stage relates to microbial population limitation. Some researchers believe that compared to single-pool analysis, this nonlinear multi-pool analysis increased the number of parameters in the model, potentially compromising its robustness and posing numerous challenges to fitting[1,19,31]. Therefore, this study only selected single-pool models for analysis and comparison.

When comparing mathematical models, no single indicator was enough to fully evaluate their performance. Each indicator highlighted a specific aspect of the comparison[32,33]. Therefore, a combination of various statistical analyses must be employed to assess the adequacy of mathematical models properly, along with a rational analysis of the original conceptualization and development purposes of the mathematical models.

In addition to comparing among the three categories of feedstuffs, this study also included a group containing all feedstuffs to investigate whether it is necessary to differentiate from feedstuff categories when comparing

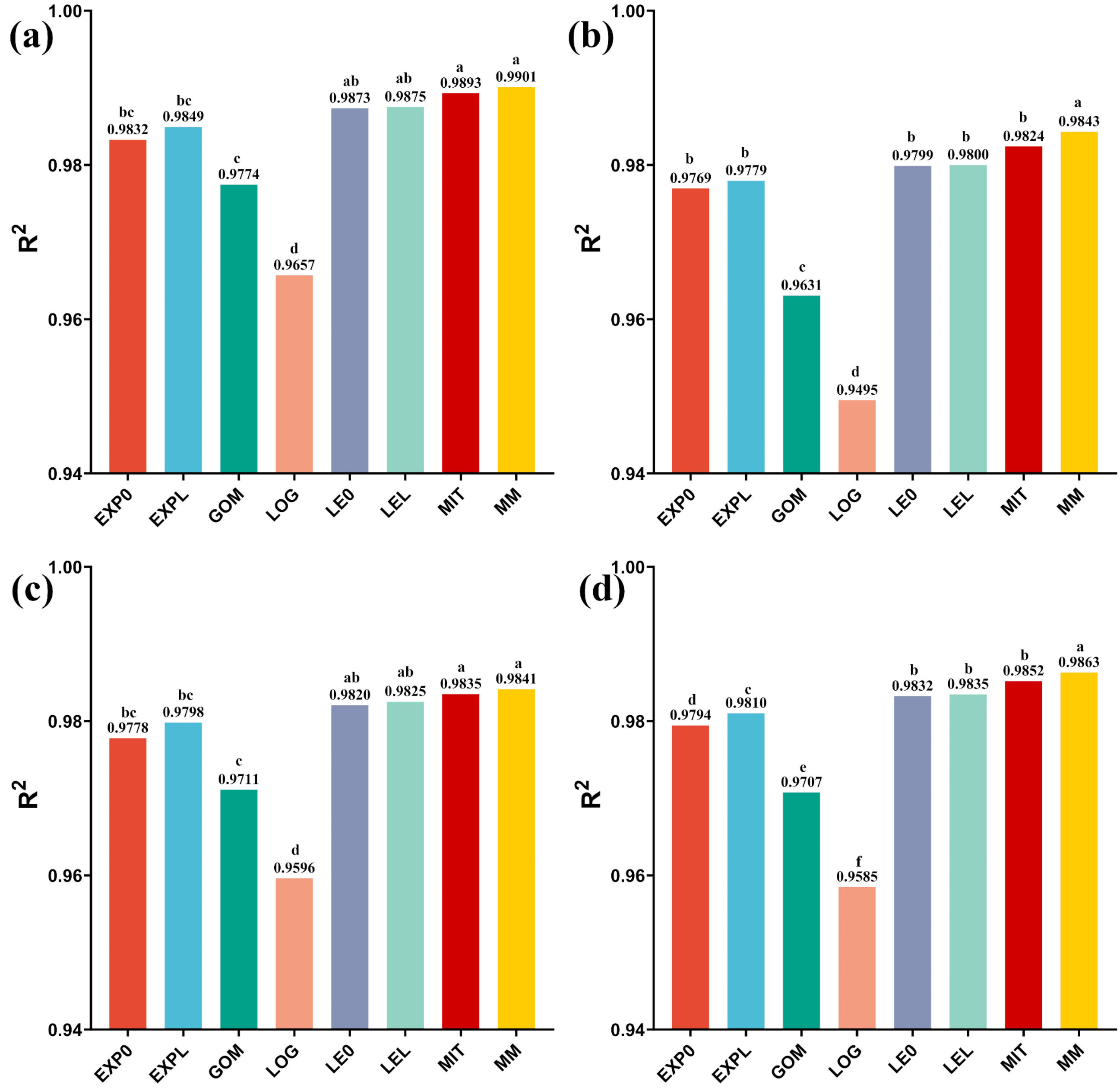

Fig. 4. The R^2 of fitted curves of 8 models for 3 categories of feedstuff. R^2 are the average of each category of feedstuff. (a) energy feeds; (b) protein feeds; (c) roughages; (d) all feedstuffs. Means with different letters differ ($P < 0.05$). EXP0: exponential without lag phase; EXPL: exponential with lag phase; GOM: Gompertz; LOG: logistic; LE0: logistic-exponential without lag phase; LEL: logistic-exponential with lag phase; MIT: Mitscherlich; MM: Michaelis–Menten.

models fitting performance. The results revealed that it exhibited performance distinct from the three groups in terms of Observed versus Predicted (OvP) and error analysis, particularly in relation to changes in the distribution and mean of predicted values. Therefore, using different models for different categories of feedstuff may be necessary, as it would lead to more accurate and realistic results.

The coefficient of determination (R^2) serves as a reliable measure of precision, with higher R^2 values indicating greater precision[32]. The MM model exhibited the highest R^2 across all four groups which surpassed all other models significantly in the protein feeds and the uncategorized group. This finding aligned with the conclusions drawn by most previous studies[1,20,23,31]. After deriving the formulas, France et al.[12] noted that the MM model's assumption regarding gas production rate was the most flexible. Compared to carbohydrates, the energy obtained by rumen microbes from protein fermentation was lower, leading to slower microbial growth[16,34]. Moreover, protein itself yields less gas, and the NH_3 produced during fermentation consumes CO_2[2]. These variations resulted in irregular and challenging-to-fit gas production curves, which the highly flexible MM model can effectively accommodate. A study found that the GOM model outperforms MM when roughages

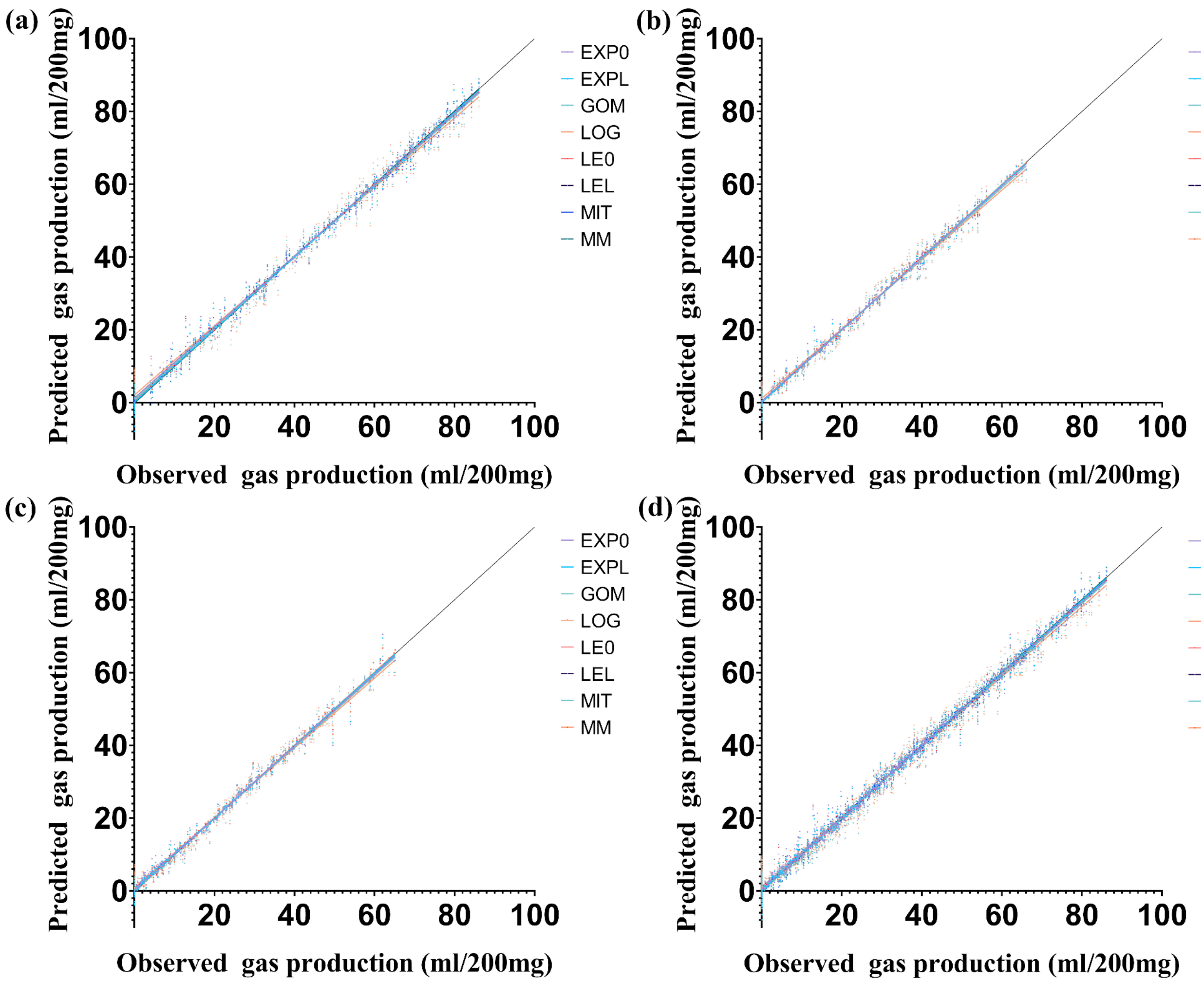

Fig. 5. Scatter plots of observed versus predicted regression. (a) energy feeds; (b) protein feeds; (c) roughages; (d) all feedstuffs. The line-colored black in the figure has y: x = 1:1. EXP0: exponential without lag phase; EXPL: exponential with lag phase; GOM: Gompertz; LOG: logistic; LE0: logistic-exponential without lag phase; LEL: logistic-exponential with lag phase; MIT: Mitscherlich; MM: Michaelis-Menten.

were used as a substrate[13]. Considering MM's characteristic of decreasing residuals over time, this could be attributed to differences in fermentation time between the study (72 h) and the experiments referenced (48 h). Additionally, the MIT, LE0, and LEL models also exhibited high R^2 values when fitting energy and roughage feedstuffs, consistent with a few prior studies[13,22,23,35,36]. These findings further underscore the advantage of employing flexible and adaptable curve shapes to enhance model accuracy.

Generally, accuracy is considered the most important criterion as it measures the model's ability to predict true values[32]. The regression estimates of intercept and slope for OvP values serve as reliable indicators of accuracy, with intercepts and slopes closer to 0 and 1 indicating higher levels of precision[32]. Apart from the flexible and adaptable MIT and MM models, the introduction of lag time in EXPL relative to EXP0 also contributed to higher accuracy. Lag time can be attributed to hydration, removal of digestive inhibitors, or microbial substrate attachment[7,12]. Although lag time may not fully describe the phenomenon of hysteresis, it provides a simple quantitative measure of delayed effects. Allen and Mertens[37] proposed a two-step model describing the digestion process: the first step involves the transformation of substrate from an unavailable to an available form (i.e., the lag pool concept), and the second step represents substrate digestion. While theoretically more accurate than discrete lag models, lag pool models did not fit the data better[28], and were not widely used due to their complexity. The GOM and LOG models inevitably introduce an initial gas volume (V0) during the assumption stage[11], whereas V0 is clearly zero at the start of the experiment. According to Schofield et al.[11], the error in V0 (i.e., intercept on the y-axis) is most severe for GOM and LOG when the digestion rate is low and lag time is short, which evidently leads to poorer accuracy when fitting energy feed. However, the intercept decreased when fitting protein and roughage feedstuffs, which validated the findings of Schofield et al.[11]. The slopes of LE0 and LEL were significantly different from 1 when fitting energy feed. The LE0 and LEL models might underestimate the gas production in the later stages of energy feed fermentation. This was consistent with the significantly lower Vf for LE0 and LEL when fitting energy feeds compared to half of the models.

Items	EXP0	EXPL	GOM	LOG	LE0	LEL	MIT	MM
Energy feeds								
Intercept	1.209*	0.104	0.774*	2.028*	0.417*	0.185	0.132	-0.202
SE	0.260	0.238	0.292	0.379	0.150	0.131	0.188	0.184
P-value	< 0.001	0.664	0.009	< 0.001	0.027	0.317	0.381	0.125
Slope	0.977#	0.996	0.978#	0.952#	0.988#	0.993#	0.996	1.004
SE	0.005	0.005	0.006	0.008	0.003	0.003	0.004	0.004
P-value	< 0.001	0.405	< 0.001	< 0.001	0.002	0.046	0.159	0.179
Protein feeds								
Intercept	0.411*	0.028	0.422	1.135*	0.106	0.014	0.115	0.018
SE	0.163	0.151	0.222	0.275	0.125	0.124	0.101	0.071
P-value	0.012	0.856	0.058	< 0.001	0.398	0.908	0.256	0.795
Slope	0.985#	0.996	0.977#	0.954#	0.992	0.995	0.995	0.999
SE	0.005	0.005	0.007	0.009	0.004	0.004	0.003	0.002
P-value	0.005	0.368	0.002	< 0.001	0.052	0.180	0.098	0.660
Roughages								
Intercept	0.438*	-0.025	0.268	0.818*	0.063	-0.090	0.018	-0.093
SE	0.136	0.123	0.161	0.197	0.089	0.087	0.090	0.085
P-value	0.001	0.839	0.096	< 0.001	0.484	0.300	0.838	0.277
Slope	0.985#	0.998	0.981#	0.961#	0.995	0.999	0.999	1.004
SE	0.005	0.005	0.006	0.007	0.003	0.003	0.003	0.003
P-value	0.002	0.662	0.001	< 0.001	0.118	0.850	0.682	0.214
All feedstuffs								
Intercept	0.563*	0.014	0.377*	1.080*	0.159*	0.017	0.075	-0.076
SE	0.103	0.093	0.121	0.155	0.073	0.072	0.062	0.053
P-value	< 0.001	0.884	0.002	< 0.001	0.030	0.815	0.226	0.152
Slope	0.985#	0.997	0.982#	0.963#	0.992#	0.996#	0.997#	1.002
SE	0.003	0.002	0.003	0.004	0.002	0.002	0.002	0.001
P-value	< 0.001	0.245	< 0.001	< 0.001	< 0.001	0.017	0.037	0.211

Table 2. Observed versus predicted regression of fitting models. EXP0: exponential without lag phase; EXPL: exponential with lag phase; GOM: Gompertz; LOG: logistic; LE0: logistic-exponential without lag phase; LEL: logistic-exponential with lag phase; MIT: Mitscherlich; MM: Michaelis-Menten; SE, Standard Error. * Intercept significantly ($P < 0.05$) different from 0. # Slope significantly ($P < 0.05$) different from 1.

RMSEP and MAE are two standard indicators for evaluating the accuracy of models. Under different error distributions, each has its own advantages and disadvantages[38]. This study presented both indicators to provide a comprehensive assessment. MSEP is perhaps the most common and reliable estimate for evaluating the accuracy of model predictions, and further decomposition of MSEP allows for the analysis of model adequacy by identifying the sources of variation[32]. If there is a significant system bias, the model's predicted mean may be larger or smaller than the observed mean, and if there is significant regression bias, it indicates potential inadequacies in the structural equation of the model in describing the analyzed data[39]. In this study, the results of linear fitting for Observed versus Predicted (OvP) and error analysis were inconsistent in some aspects. This inconsistency might stem from the different principles underlying their analyses, with the former focusing on the distribution of predicted values and the latter on the mean of predicted values. This experiment found that fitting different fermentation substrates does not affect the level of errors but may result in different sources of errors. It is generally believed that the fractional rate of gas production μ of substrates during fermentation is not constant. An initial increase in μ reflects particle hydration, microbial attachment, and an increase in microbial numbers, while a decrease in μ may indicate limitations in chemical nutrients and structure within the substrate[12]. Models like LE0, LEL, MIT, and MM have the ability to allow μ to vary freely over time, leading to smaller errors when fitting the three types of feedstuff[7,13,14]. Camila et al.[1] found that the MM model had the lowest RMSEP when fitting corn silage using various models. Dhanoa et al.[19], in comparing the performance of different models after fitting roughages and silage feedstuffs, found that the residual mean squares of MM and MIT were lower than those of other models. Wang et al.[13] further validated the models and found that the RMSEP of LE0 and LEL was lower than that of most models. The MIT model exhibited near-zero SB and RB in all three categories of feedstuff, while the performance of LE0, LEL, and MM varied depending on the categories of feedstuff being fitted. This may be attributed to the different assumptions underlying LE0 and LEL (microbial growth[13]), MIT (degradation by surface erosion and complete invasion[14]), and MM (formation of microbial film[7]). For energy and roughage feedstuffs, despite its advantages, the MM model exhibited systematic bias and regression bias. Considering the previous discussion, it seems that the MM model may have overestimated

Items	EXP0	EXPL	GOM	LOG	LE0	LEL	MIT	MM
Energy feeds								
MAE	2.28	2.22	2.61	3.27	1.96	1.98	1.81	1.68
RMSEP	3.26	3.05	3.34	4.10	2.79	2.76	2.52	2.42
MSEP	10.63	9.32	11.16	16.84	7.78	7.60	6.34	5.86
SB (%)	0.50	0.02	0.02	0.42	0.01	0.01	0.00	0.08
RB (%)	1.63	0.07	0.05	1.40	0.22	0.00	0.00	0.25
RE (%)	97.87	99.90	99.93	98.18	99.77	99.99	100.00	99.66
Protein feeds								
MAE	1.48	1.46	1.94	2.35	1.29	1.30	1.23	1.10
RMSEP	2.04	1.96	2.45	2.94	1.75	1.74	1.64	1.51
MSEP	4.18	3.85	6.02	8.63	3.05	3.03	2.70	2.29
SB (%)	0.04	0.11	0.02	0.13	0.07	0.14	0.00	0.02
RB (%)	0.11	0.27	0.06	0.34	0.18	0.35	0.00	0.04
RE (%)	99.85	99.62	99.91	99.53	99.74	99.50	100.00	99.94
Roughages								
MAE	1.38	1.34	1.79	2.12	1.18	1.18	1.11	1.13
RMSEP	2.05	1.95	2.36	2.75	1.70	1.69	1.66	1.67
MSEP	4.21	3.79	5.55	7.55	2.90	2.86	2.74	2.77
SB (%)	0.10	0.26	0.23	0.07	0.23	0.55	0.00	0.14
RB (%)	0.18	0.42	0.37	0.11	0.38	0.89	0.01	0.24
RE (%)	99.72	99.32	99.40	99.82	99.38	98.57	99.99	99.62
All feedstuffs								
MAE	1.75	1.71	2.14	2.62	1.51	1.51	1.42	1.32
RMSEP	2.57	2.42	2.79	3.37	2.18	2.16	2.03	1.94
MSEP	6.60	5.87	7.79	11.35	4.77	4.68	4.10	3.78
SB (%)	0.22	0.08	0.01	0.21	0.02	0.11	0.00	0.07
RB (%)	0.45	0.16	0.03	0.44	0.00	0.11	0.00	0.14
RE (%)	99.33	99.76	99.96	99.35	99.98	99.78	100.00	99.79

Table 3. Error analysis of fitting models. EXP0: exponential without lag phase; EXPL: exponential with lag phase; GOM: Gompertz; LOG: logistic; LE0: logistic-exponential without lag phase; LEL: logistic-exponential with lag phase; MIT: Mitscherlich; MM: Michaelis-Menten; MAE: mean absolute error; RMSEP: root mean square error; MSEP: mean square error; SB: system bias; RB: regression bias; RE: random error.

the gas production values[28]. This is an important factor to consider when using the MM model for energy and roughage feedstuffs, as it could impact the accuracy of the results.

In addition to accuracy and precision, complexity directly impacts the performance of the model on both training and unseen data, affecting its interpretability and fitting cost. AIC and BIC serve as indicators that balance complexity and precision. For small datasets, the performance of AIC is similar to or even better than BIC, while BIC becomes the preferred criterion for selecting the correct model, for datasets of moderate size[40]. In this study, both AIC and BIC were chosen to evaluate the complexity and precision of the models, because each sample comprised approximately 40 to 100 data points, falling the range between small and moderate sample sizes[41]. Among the models selected in this study, EXP0 has 2 parameters, while LEL and MIT have 4 parameters, with the rest having 3 parameters. Table 4 revealed that the model with 2 parameters, EXP0, did not exhibit significant advantages, ranking 3.51 (3.10) in AIC (BIC) only for the protein feed where other models struggled to fit. On the other hand, the 3-parameter MM model ensured model precision with a slight increase in complexity. This finding aligned with previous research results[13,23]. The MIT model outperformed other models in roughages, indicating that its theoretical assumptions regarding microbial enzyme diffusion and surface erosion are better suited for difficult-to-degrade roughage[14].

The MM model appears to demonstrate high precision, moderate to high accuracy, and low complexity across all three categories of feed. Therefore, the MM model is a good choice for studies that do not differentiate between feed categories or include multiple categories of feed. However, considering the MM model's overestimation of asymptotic gas production, it is advisable to choose a model based on its specific performance for different feeds. If only accuracy is considered, models such as LEL, MIT and MM for energy feed, MIT and MM for protein and roughage feedstuff have performed well. However, it is unrealistic to rely solely on an accurate but imprecise model for predictions. Therefore, after considering the theoretical assumptions of the models and their accuracy, precision, and complexity in fitting various feedstuffs: (1) For energy feed, MM showed relatively highest R^2 among the 8 models ($P < 0.05$). The slope and intercept in OvP did not significantly differ from 1 and 0 ($P < 0.05$). MAE, RMSEP, and MSE were lowest (1/8), but there was a presence of systematic bias and regression bias. The AIC (BIC) ranking was 2.29 (2.29), exceeding all other models. The LEL model performs worse on certain

Items	EXP0	EXPL	GOM	LOG	LE0	LEL	MIT	MM
Energy feeds								
Mean rank of AIC	4.88	3.96	5.92	7.54	3.08	3.75	4.58	2.29
Number of curves with smallest AIC	1	0	0	0	7	0	1	15
Number of curves with largest AIC	3	0	0	21	0	0	0	0
Mean rank of BIC	4.29	4.00	5.88	7.54	2.92	4.21	4.88	2.29
Number of curves with smallest BIC	1	0	0	0	7	0	1	15
Number of curves with largest BIC	2	0	0	21	0	0	1	0
Protein feeds								
Mean rank of AIC	3.77	3.77	6.45	7.68	3.82	4.77	4.14	1.59
Number of curves with smallest AIC	1	0	0	0	3	0	0	18
Number of curves with largest AIC	3	0	0	19	0	0	0	0
Mean rank of BIC	3.32	3.77	6.45	7.68	3.68	5.00	4.50	1.59
Number of curves with smallest BIC	1	0	0	0	3	0	0	18
Number of curves with largest BIC	3	0	0	19	0	0	0	0
Roughages								
Mean rank of AIC	4.64	3.55	6.09	7.36	4.27	4.45	4.14	2.09
Number of curves with smallest AIC	2	0	1	1	0	0	4	14
Number of curves with largest AIC	3	0	0	18	0	0	1	0
Mean rank of BIC	3.95	3.68	6.09	7.36	3.64	4.64	4.68	1.95
Number of curves with smallest BIC	2	0	1	1	0	0	3	15
Number of curves with largest BIC	3	0	0	18	0	0	1	0
All feedstuffs								
Mean rank of AIC	4.44	3.78	6.12	7.50	3.62	4.24	4.31	2.00
Number of curves with smallest AIC	4	0	1	1	10	0	5	47
Number of curves with largest AIC	9	0	0	58	0	0	1	0
Mean rank of BIC	3.87	3.84	6.10	7.50	3.41	4.62	4.71	1.96
Number of curves with smallest BIC	4	0	1	1	10	0	4	48
Number of curves with largest BIC	8	0	0	58	0	0	2	0

Table 4. AIC and BIC of fitting models. EXP0: exponential without lag phase; EXPL: exponential with lag phase; GOM: Gompertz; LOG: logistic; LE0: logistic-exponential without lag phase; LEL: logistic-exponential with lag phase; MIT: Mitscherlich; MM: Michaelis-Menten; AIC: Akaike information criterion; BIC: Bayesian information criterion. Ranking according to AIC (BIC) of models fitted each feedstuff, the smaller the AIC(BIC), the higher the ranking.

indicators compared with MM, but exhibits no bias, which should be taken into consideration. (2) For protein feed, MM's R^2 was significantly higher than other models ($P<0.05$). The regression estimates of intercept and slope in OvP had no significant difference from 0 to 1, respectively ($P<0.05$). MAE, RMSEP, and MSE were the lowest (1/8) with almost no systematic bias and regression bias. The AIC (BIC) ranking was 1.62 (1.62). (3) For roughage, MIT showed relatively higher R^2 (2/8) with no significant difference from the highest value ($P<0.05$). The intercept and slope in OvP had no significant difference from 0 to 1, respectively ($P<0.05$). MAE, RMSEP, and MSE were low (1/8) with no systematic bias and regression bias. The AIC ranks third only to MM and EXPL.

Conclusions

In summary, due to the involvement of different fermentation processes, the chemical composition and the physical structure of feed, could influence the gas production process, resulting in gas production curves with different characteristics. EXP0 and MM tend to overestimate Vf for energy and roughage feeds. In studies where feedstuff categories are not distinguished or multiple feedstuffs categories are included, the MM model proves to be a good choice. Considering the theoretical assumptions of the models and their accuracy, precision, and complexity in fitting various types of feed, MM or LEL is considered to better fit energy feeds; MM model is the optimal choice for fitting protein feeds; and MIT can provide the best accuracy and moderate precision when fitting roughage feeds. Due to limitations in sample quantity and diversity, the categorization of feeds in this study may not be sufficiently specified or comprehensive. In the future, collecting more extensive and diverse gas production data will enable a better exploration of the suitability of models for different feed categories.

Materials and methods

Animals and diets

All animal procedures used in this study were approved by the Animal Care and Use Committee of China Agricultural University (Approval No.AW81404202-1-9), and the experimental procedures used in this study were in accordance with the university's guidelines for animal research. The rumen fluid used in this experiment was collected from three Angus steers of similar weight (400 ± 50 kg of BW, mean ± SD) with permanent rumen fistulas. The experimental animals had ad libitum access to water and were fed twice daily at 8:00 and 17:00. The diets were formulated based on the body weight and nutritional requirements to meet 1.3 times maintenance needs of beef cattle according to NASEM 2016[42] (Table 5). Prior to the start of the formal experiment, the animals underwent an adaptation period of 7 days. During this time, they were gradually introduced to the experimental diet to ensure they were fully accustomed to it by the time the formal experiment commenced, minimizing the potential for environmental changes to influence the results.

Feed samples preparation and nutrient analysis

A total of 57 types of feedstuffs, commonly used for ruminants, were randomly collected from farms, feed mills, food processing plants, and agricultural fields across China. These feedstuffs were categorized into three groups, with some consisting of two varieties, resulting in a total of 68 samples. Detailed information of the feedstuffs has been provided in the supplementary table. Energy feeds primarily include grains, as well as certain bran and residues with relatively high palatability and digestibility, all characterized by high energy density. Protein feeds mainly consist of oilseeds, oilseed meals, and processing by-products with high protein content. Roughages, with lower energy value, are mainly characterized by a high proportion of structural carbohydrates and include hay, straw, and less digestible residues. According to the above classification criteria, there were 20 types of energy feed, totaling 24 samples, including: barley (2), beet pulp, buckwheat, corn, corn husk, dried foxnut, flaked corn, highland barley, millet, millet bran, oat, oat bran, rice, rice bran (2), rice bran meal, rye grain, sorghum (2), soybean hulls, wheat, and wheat bran (2). For protein feed, there were 18 types totaling 22 samples including: bean curd residue (2), brewer's grains, cottonseed meal, DDGS (2), distiller's grain, flax seed meal (2), linseed cake, mulberry leaf, palm meal, paper mulberry leaf, peanut meal, rapeseed cake, rapeseed meal, sesame cake, soy sauce residue, soybean meal, sunflower meal (2), and vinegar residue. For roughage feedstuffs, there were 19 kinds totaling 22 samples including: alfalfa, apple pomace, Chinese wildrye, corncob, dried corn stalk (2), highland barley stalk, oat grass (2), oat wheat straw, peanut seedling, peanut shell, pear pomace, ryegrass, sorghum bran, sorghum stalk, soybean straw, straw, sugarcane bagasse, sweet potato seedling, and wheat straw (2). The samples were dried in a 65 °C air oven for 48 h, followed by grinding through a 0.5-millimeter sieve, for chemical analysis and in vitro rumen gas production. Dry matter (DM), ash, and starch were determined following the AOAC[43]. Nitrogen content was determined using the Dumas combustion method with nitrogen analyzer (Rapid III, Elementar, Germany), and crude protein (CP) content was calculated as nitrogen content times 6.25. Ether extract (EE) was determined by an automatic fat analyzer (XT15, ANKOM Technology, USA). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined following the method of Van Soest et al.[44] with automatic fiber analyzer (A2000i, ANKOM Technology, USA).

Item	Diet
Ingredient	
Corn silage	29.10
Corn stalks	10.90
Corn	30.00
Jujube powder	8.60
Soybean meal	7.50
Palm kernel meal	7.50
Premix[1]	4.30
Sodium bicarbonate	1.10
Salt	1.10
Chemical composition of diet	
TDN	64.31
CP	11.90
EE	2.80
NDF	45.40
ADF	29.60
Calcium (Ca)	0.40
Total phosphorus (P)	0.30

Table 5. Composition of experimental diet offered to steers (dry matter basis, %). TDN: total digestible nutrition; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber. TDN(%) = $0.98 \times (100\text{-}NDF_N\text{-}CP\text{-}Ash + IADICP\text{-}EE) + 0.93\times CP + 2.25\times(EE\text{-}1) + 0.75\times(NDF_N\text{-}ADL)\times[1\text{-}(ADL/NDF_N)^{0.667}]\text{-}7$. Premix: Provides per kg: Vitamin A acetate 150,000 to 450,000 IU, Vitamin D3 40,000 to 120,000 IU, DL-α-tocopheryl acetate 400 mg, Copper 250 to 750 mg, Iron 1000 to 5000 mg, Manganese 1000 to 3000 mg, Zinc 1500 to 3700 mg, 10 to 25% Calcium, 0.D3% Total phosphorus, 15 to 30% Sodium chloride.

Model	Equation	$t_{0.5}$ (h)	$\mu_{0.5}$ (/h)
EXP0	$V = Vf(1 - exp(-k * t))$	$\frac{ln(2)}{k}$	k
EXPL	$V = Vf(1 - exp(-k * (t - lag)))$	$lag + \frac{ln(2)}{k}$	k
GOM	$V = Vfexp(-exp(1 - ek(t - lag)))$	$lag + \frac{1-ln(ln(2))}{ek}$	$ln(2)ek$
LOG	$V = \frac{Vf}{1+exp(2+4k(t-lag))}$	$lag + \frac{1}{2k}$	$2k$
LE0	$V = \frac{Vf(1-exp(-kt))}{1+exp(ln(\frac{1}{d})-kt))}$	$\frac{ln\left(2+\frac{1}{d}\right)}{k}$	$\frac{k(d+0.5)}{d+1}$
LEL	$V = \frac{Vf(1-exp(-k(t-lag)))}{1+exp(ln(\frac{1}{d})-k(t-lag))}$	$lag + \frac{ln\left(2+\frac{1}{d}\right)}{k}$	$\frac{k(d+0.5)}{d+1}$
MIT	$V = Vf(1-exp(-k(t - lag) - d(\sqrt{t}-\sqrt{lag})))$	$(\sqrt{\frac{d^2}{4k^2}+\frac{\left(klag+d\sqrt{lag}+ln(2)\right)}{k}}-\frac{2}{d})^2$	$k + \frac{d}{2\sqrt{t_{0.5}}}$
MM	$V = \frac{Vft^c}{t^c+K^c}$	K	$\frac{c}{2K}$

Table 6. Description of models considered in this study. EXP0: exponential without lag phase; EXPL: exponential with lag phase; GOM: Gompertz; LOG: logistic; LE0: logistic-exponential without lag phase; LEL: logistic-exponential with lag phase; MIT: Mitscherlich; MM: Michaelis-Menten. Vf: final asymptotic gas production; k: fractional rate of gas production; c and d: shape parameters; K: the time at Vf/2; lag: lag time; $t_{0.5}$: the time at Vf/2; $\mu_{0.5}$: fractional rate of gas production at Vf/2.

In vitro rumen gas production

In vitro rumen gas production was conducted following the method outlined by Menke et al.[45]. Accurately weighing 0.200 g (DM) air-dried feedstuff sample, and placing at the bottom of a glass syringe. Evenly apply an appropriate amount of Vaseline to the front one-third of the piston of the culture tube, then place the assembled culture tube in a 39 °C incubator for preheating. All preparation work should be completed the day before the formal experiment. In the morning of the experiment day, prior to feeding, the rumen contents and fluid were uniformly collected from the steer. The samples were obtained from the ventral sac, dorsal sac, and atrium of the rumen to ensure a representative mix. The collected samples were then thoroughly mixed to obtain a homogenous solution for subsequent analyses. After filtering through four layers of cheesecloth, the mixed rumen fluid was placed in a preheated thermos bottle at 39 °C. The rumen fluid was mixed with CO_2-saturated rumen buffer solution at a ratio of 1:2 to prepare rumen inoculum. Then, 30 mL of the inoculum was added to the glass syringe using an automatic pipette and placed in a 39 °C water baths shaker with 60 revolutions per minute. Each sample is prepared in triplicate, and three blank tubes without feed are set as controls. At 0, 2, 4, 6, 8, 12, 16, 20, 24, 30, 36, 48, 60 (and 72) hours of incubation, remove the culture tubes and quickly record the piston scale reading (mL). Fermentation of energy and protein feeds for 60 h, and roughages for 72 h. If the reading exceeds 75 mL at any time point, release the gas immediately after recording and note the scale value after venting.

Models and curve-fitting

Table 6 presented 8 commonly used single-pool models describing in vitro rumen gas production kinetics. The exponential model included two types: EXP0 (without lag phase) and EXPL (with lag phase), which were among the most used models in in vitro rumen gas production. The Gompertz (GOM) and Logistic (LOG) models, initially proposed in microbiology, were first applied to in vitro rumen gas production kinetics by Schofield et al.[11]. Wang et al.[13] introduced the shape parameter d to the Logistic (LOG) model, developing a more flexible Logistic-Exponential (LE) model. France et al.[14]incorporated the concept of degradation by surface erosion and by complete invasion into mathematical formulas, resulting in the Mitscherlich (MIT) model. The Michaelis-Menten (MM) model originated in enzymatic kinetics, and Groot et al.[7] were the first to apply it to describe in vitro rumen gas production kinetics.

Table 6 provided the time ($t_{0.5}$) and fractional rate of gas production ($\mu_{0.5}$) for each model at V = Vf/2. Here, the final asymptotic gas production was represented by Vf. The time $t_{0.5}$ was calculated when V = Vf/2. The value $\mu_{0.5}$ was obtained by substituting $t_{0.5}$ into the formula dV/dt/V.

Statistical analysis

In R version 4.3.2 (R Core Team, Vienna, Austria), each sample was fitted using the nonlinear least squares method via the NLS function. The stats package was used to compute the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) for the fits. Gas production attributes (Vf, $t_{0.5}$, $\mu_{0.5}$) for each category feedstuff were compared across different models using repeated measures one-way ANOVA with Greenhouse-Geisser correction. Bar charts were plotted using the ggplot2 package. The determination coefficient (R^2) for each feedstuff category was calculated separately, and comparisons were made using repeated measures one-way ANOVA with Greenhouse-Geisser correction. R^2 was calculated as:

$$R^2 = 1 - \frac{\sum(y_i - \widehat{y_i})^2}{\sum(y_i - \bar{y})^2}$$

Here, y_i represents the actual value of the i -th observed data, $\widehat{y_i}$ represents the predicted value of the model for the i -th observed data, $\bar{y}$ denotes the mean value of the observed data.

Using GraphPad Prism 9.5.0 (GraphPad Software, San Diego, CA, USA), linear regression was performed on the observed gas production values (x-axis) versus the predicted values (y-axis) for each feedstuff category. The intercepts and slopes of each model were evaluated, and fitted equations were plotted. Statistical analysis was conducted to test the significance of the regression parameters, examining the hypotheses "slope = 1" and "intercept = 0".

Error analysis refers to the systematic analysis and interpretation of the differences between model predictions and actual observed values. The following methods were used to assess the errors of the model: Mean Absolute Error (MAE), Root Mean Squared Error of Prediction (RMSEP), and Mean Squared Error of Prediction (MSEP). MSEP is further decomposed into system bias (SB), regression bias (RB), and random error (RE)[32,46]. The calculation formulas were as follows:

$$SB = \left(\frac{\sum y_i}{n} - \frac{\sum \widehat{y_i}}{n} \right)^2$$

$$RB = (S_{\widehat{y}} - r \times S_y)^2$$

$$RE = (1 - r^2) \times S_y$$

$$r = \frac{\sum \frac{(y_i - \frac{\sum y_i}{n}) \times (\widehat{y_i} - \frac{\sum \widehat{y_i}}{n})}{S_y \times S_{\widehat{y}}}}{n}$$

Where n represents the number of data points, S_y denotes the variance of the observed data, and $S_{\widehat{y}}$ represents the variance of the model-predicted data.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

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Author contributions
JW designed the study, analyzed the data, and wrote the manuscript. ZZ and YC performed the experiments and sample analyses.BY, SL, LR, NL, XZ and WL conducted the trial. XY, ZD and SY assisted with the manuscript preparation. ZZ, HW and QM revised the manuscript and provided experimental guidance. All authors read and approved the final manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
All animal procedures used in this study were approved by the Animal Care and Use Committee of China Agricultural University (Approval No. AW81404202-1-9).

Additional information
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Correspondence and requests for materials should be addressed to Z.Z.

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