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PROPOSAL OF A NON-LINEAR MODEL TO ADJUST IN VITRO GAS PRODUCTION AT DIFFERENT INCUBATION TIMES

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Abstract

This work aims to propose a new model named Gompertz-Von Bertalanffy bicompartmental (GVB), a combination of the models Gompertz and Von Bertalanffy. The GVB models is applied to fit the kinetic curve of cumulative gas production (CGP) of four foods (SS – sunflower silage; CS – corn silage; and the mixtures 340SS – 660 gkg^{-1} of corn silage and 340 gkg^{-1} of sunflower silage; and 660SS – 340 gkg^{-1} of corn silage and 660 gkg^{-1} of sunflower silage). The GVB fit is compared to models Logistic-Von Bertalanffy bicompartmental (LVB) and bicompartmental logistic (BL). All the process studied employed the semi-automatic "*in vitro*" technique of producing gases used in ruminant nutrition. The gas production readout was performed at times 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 48, 72, and 96 h. The data generated were used to estimate the models' parameters by the least squared method with the iterative Gauss-Newton process. The data fit quality of the models was verified using the adjusted coefficient of determination criterion ($R^2_{aj.}$), mean residual square (MRS), Akaike information criterion (AIC), and mean absolute deviation (MAD). Among the analyzed models, the LVB model presented the best quality of fit evaluators for CS. In contrast, the GVB model showed better quality of fit to describe CGP over time for 340SS, 660SS, and SS, presenting the highest values of ($R^2_{aj.}$) and the lowest values of MSR, AIC, and MAD.

Keywords: Alternative foods. Degradation kinetics. Non-linear models. Silage. Suggested model.

1. Introduction

Several studies have been conducted seeking to evaluate the cumulative gas production (CGP), agronomic characteristics, bromatological composition, fermentation pattern, and nutritional value of single sunflower silage (SS) or single corn silage (CS) (Leite et al. 2006; Wanderley et al. 2012; Martins et al. 2014; Aragadvay-Yungán et al. 2015; Leite et al. 2017; Santos et al. 2019; Maidana et al. 2020). However, few studies on CGP of both SS and CS are found as a single roughage or their mixtures.

Mathematical growth functions that satisfactorily describe CGP are critical to understanding the nutritional value of diets provided to ruminants (Santos et al. 2020). In the literature, there are various mathematical functions, many times a simplified version of the analyzed growth phenomenon, that can be used to model the CGP. It is up to the researcher to identify the most suitable non-linear function for the modeling. Meantime, among the various existing mathematical functions to model the in vitro gas production curve, we highlight the bicompartmental logistic model proposed by Schofield et al. (1994).

According to Oliveira et al. (2017), mathematical models used in evaluating food for ruminants have favorable and unfavorable adjustment factors depending on the experimental conditions and the considered food. Therefore, a prior evaluation of several models in different conditions is necessary. In general, there is not a single optimal model for all food types in the most varied conditions, it will exist a specific model appropriated for each situation.

Due to the range of models present in the literature to describe the same phenomenon and the variation in composition among foods, it is not prudent to use a single model for an adjustment (France et al. 2005). The authors also report that it is necessary to choose a suitable model for each substrate. However, few studies using sigmoid bicompartmental models have selected the best model to be used for each situation (Santos et al. 2019; Santos et al. 2020).

Therefore, it is essential to indicate which mathematical model best interprets each forage's degradation profile to be studied. Consequently, it seems that using a single model for the substrates variety evaluation is not adequate, and it is obliged to evaluate the suitability of different mathematical models in adjusting the data for each type of substrate. Thus, the present study aimed to propose a new bicompartmental model and identify the best model among the following ones, LVB and BL in SS, CS, and their mixtures.

2. Material and Methods

Data Used

Four diets with different increasing proportions of sunflower silage were evaluated to replace corn silage as roughage food: treatment 1 - corn silage as a single roughage plus concentrate (CS); treatment 2 - 660 g·kg-1 of corn silage and 340 g·kg-1 of sunflower silage (34SS); treatment 3 - 340 g·kg-1 of corn silage and 660 g·kg-1 of sunflower silage (66SS); and treatment 4 - sunflower silage as single roughage plus concentrate (SS), in a roughage ratio of 56:44% based on a dry matter. The pressure readouts of the gases produced during the fermentations were taken at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 48, 72, and 96 hours after the start of the incubations. More details in Leite et al. (2006).

Suggested model

In the 1990s, many authors proposed several models for the biological interpretation of in vitro gas production kinetics, among them (France et al. 1993; Schofield et al. 1994; Groot et al. 1996; France et al. 2000). Schofield et al. (1994) proposed the bicompartmental logistic model to study in vitro gas production kinetics. They assumed that the gas production kinetics rate was affected by the microbial mass and the level of the substrate. The bicompartmental logistic model has been the most used in the literature.

The idea of combining non-linear growth models has received considerable attention in the literature (Schofield et al. 1994; Santos et al. 2019; Santos et al. 2020). In this sense, combined multicompartmental models describe a characteristic behavior of more than one phase, which allows the curve to be divided into several parts. It is necessary to adopt multicompartmental growth models, as they favor exclusive parameters for each compartment (Koops 1986). Therefore, a single model consisting of two or more submodels is considered so that each one explains a particular phase of growth (Koops 1986; Thornley and France 2005).

More recently, Santos et al. (2019) developed a model-generating method by combining existing models, the different ways of combining these models being called constructor methods. In addition to the construction methods, the authors generated a bicompartmental growth model considering phases. Thus, the proposed model developed was the result of combining the models Gompertz Unicompartimental and Von Bertalanffy, whose formulas are expressed, respectively, by:

$$W_I(t)=\alpha_1\, e^{-\beta_1 e^{-k_1 t}} + \varepsilon \quad (1)$$
$$W_{II}(t)=\alpha_2(1-\beta_2 e^{-k_2 t})^3 + \varepsilon. \quad (2)$$

From the product of Equations (1) and (2) (see Santos et al., 2019) and considering $\alpha = \alpha_1\alpha_2$ we can write the following model

$$W(t)=W_I(t)\, W_{II}(t) + \varepsilon = \alpha\, e^{-\beta_1 e^{-k_1 t}}\, (1-\beta_2 e^{-k_2 t})^3 + \varepsilon. \quad (3)$$

Therefore, Equation (3) constitutes our proposed model, called the Gompertz - Von Bertalanffy Bicompartimental model, denoted by GVB. Thus, in addition to the proposed model, the kinetics of cumulative gas production was fitted using the non-linear bicompartmental models LVB proposed by Santos et al. (2020) for being a new proposal and BL proposed by Schofield et al. (1994), for being a consensus of researchers in the use, represented, respectively, by the following expressions:

$$W(t)=\alpha_1\left\{1+e^{[2-4k_1(t-\lambda)]}\right\}^{-1}+\alpha_2\left\{1-\beta e^{-k_2(t-\lambda)}\right\}^{3}+\varepsilon, \quad (4)$$
$$W(t)=\alpha_1\left\{1+e^{[2-4\,k_1(t-\lambda)]}\right\}^{-1}+\alpha_2\left\{1+e^{[2-4\,k_2(t-\lambda)]}\right\}^{-1}+\varepsilon \quad (5)$$

where W(t) is the volume of gas accumulated (ml) in time t (incubation time); α_1 is the maximum volume of gases produced from the fast-digesting fraction of non-fibrous carbohydrates (NFC) ml/g; α_2 is the maximum volume of gases produced from the fraction of slow degradation of fibrous carbohydrates (FC) ml/g; β is the shape parameter, without biological interpretation; k_1 is the rate of degradation of the fast-digesting fraction (NFC) h-1; k_2 is the rate of degradation of the slow-digesting fraction (FC) %.h-1; λ is the bacteria colonization time (h); t is the fermentation time; e is the natural exponential function; and ε is the random error associated with each observation with normal distribution, zero mean and constant variance.

Criteria for selecting models

The quality of the fit obtained by the models was assessed based on the following criteria, adjusted coefficient of determination ($R^2_{aj.}$), mean residual square (MRS), Akaike information criterion (AIC), and mean absolute deviation (MAD), defined as:

$$R^2_{aj.} = 1-\frac{(1-R^2)(n-1)}{n-p} \quad (6)$$
$$MRS=\frac{\sum_{i=1}^{n}(y_i-\hat{y}_i)^2}{n-p} \quad (7)$$
$$AIC = -2.logL + 2(p+1) \quad (8)$$
$$MAD=\frac{\sum_{i=1}^{n}\left|y_i-\hat{y}_i\right|}{n} \quad (9)$$

where R^2 is the coefficient of determination, n is the number of observations, p the number of parameters, y_i is the observed volume, $\hat{y}_i$ is the estimated volume, and L is the logarithm of the likelihood function.

Residue analysis

Given an adjusted model, it is crucial to analyze the residues to investigate the meeting veracity of the model's assumptions, where most of the existing works despise it (Santos et al. 2020). The statistical test usually used to verify the assumption of residual normality is that of Shapiro-Wilk (Shapiro and Wilk 1965), where the null hypothesis indicates normality. The Durbin-Watson (Durbin and Watson 1950) test is used to verify independence. The Breusch-Pagan (Breush and Pagan 1979) test is usually used to verify the heteroscedasticity of the residues. The null hypothesis is that the residues are homoscedastic. If such actions are left out in the adjustment process, biased estimates may occur (Pasternak and Shalev 1994) and underestimate the parameters' variances (Souza 1998).

All the computational implementations involved here were done using free software R, version 3.5.1 (R DEVELOPMENT CORE TEAM 2018). They included the analysis of the residues, construction of graphs, fit quality criteria, the parameters estimation obtained through the least squares method with the iterative process of Gauss Newton (implemented in the Nonlinear Least Square function (nls) of the package Stats).

3. Results

Table 1 shows the average values for the p-values for the Shapiro-Wilk, Breusch-Pagan, and Durbin-Watson tests for the CS, 340SS, 660SS, and SS treatments. As for the normality of the residues, the models did not present problems. However, to verify independence, the BL model presented a problem for all Durbin-Watson test treatments, while the GVB model presented a problem for corn silage. The LVB model, on the other hand, offers waste independence for all treatments. For the Breusch-Pagan test, with the exception of the LVB model for the CS, 340SG, and 660SG treatments, it is perceived for all treatments in all studied situations that the estimated residues for the GVB and BL models are homoscedastic.

Table 1. Statistical values of the Shapiro-Wilk, Durbin-Watson, and Breusch-Pagan tests, with the respective p-values, applied to LVB and BL model residuals adjusted to CGP for CS, 340SS, 660SS, and SS.

Treatment	Shapiro-Wilk	p-value	Durbin-Watson	p-value	Breusch-Pagan	p-value
CS_{GVB}	0.9097	0.1150	1.0989	0.0109	1.2859	0.2568
CS_{LVB}	0.9674	0.7950	1.8455	0.2677	6.0622	0.0138
CS_{BL}	0.9419	0.3730	1.1824	0.0185	0.7354	0.3911
$340SS_{GVB}$	0.9536	0.5495	1.7554	0.2095	0.4993	0.4798
$340SS_{LVB}$	0.9646	0.7453	1.9083	0.3124	10.454	0.0012
$340SS_{BL}$	0.9389	0.3366	1.0983	0.0109	0.3366	0.5618
$660SS_{GVB}$	0.9536	0.5493	1.7553	0.2095	0.4894	0.4842
$660SS_{LVB}$	0.9792	0.9574	1.8699	0.2848	8.8269	0.0029
$660SS_{BL}$	0.9358	0.3014	1.1512	0.0153	0.7187	0.3966
SS_{GVB}	0.9535	0.54kg	1.7553	0.2096	0.4829	0.4871
SS_{LVB}	0.9488	0.4709	1.6103	0.1323	2.5644	0.1093
SS_{BL}	0.9145	0.1374	1.1428	0.0146	0.5289	0.4670

The accumulated volume of the CGP (ml·kg of CS-1) obtained from the observed data and adjusted to the treatments for the GVB, LVB, and BL models are shown in Figure 1.

The BL model presents useful fitted to the curves in the initial incubation times; however, it was less effective in the exponential and asymptote phases. The GVB and LVB models were effective in fitted the curves in all phases of the treatment CGP. The volume of gases produced by the NFC fraction was higher than the gas volume from the FC's fermentation for all treatments. The kinetic parameters of degradation estimated by the different models and the other fit quality evaluators are shown in Table 2. The SS variety showed the highest volume of NFC gases (258.18; 168.79; and 167.14 ml/g); while, the CS treatment promoted the lowest volume of NFC gases (175.32; 113.86; and 95.78 ml/g) for the GVB, LVB, and BL models, respectively. It was probably due to SS containing the highest levels of NFC. Regarding the volume of PCG resulting from the fermentation of FC, except for treatment 660SS, no significant variations were observed between the different treatments for the LVB and BL models.

The PCG rates of NFC (and FC) ranged from 0.02 to 0.09 (0.02 to 0.20) ml/g, respectively (Table 2). Regarding the colonization time, the shortest lag time was for the 660SS treatment with a value of 2.00 and the longest for the CS treatment 3.18 for the LVB model, and lag time for the BL model of (lowest) and highest value for treatment CS (SS) of 4.56 (5.79), respectively.

The treatment with 100% of CS inclusion was the one with the lowest potential of final total gas volume produced by the sum of the NFC and/or FC of the GVB, LVB, and BL models, with a value of 175.32; 167.65 and 165.60, respectively. As the SG was included, there was an increase in the maximum gas production, observed in the treatments with (340SS) and 660SS of SS inclusion, which presented a maximum production of (205.95; 200.38 and 197.09) and 233.38; 227.93 and 223.42 for models GVB, LVB, and BL, respectively. Thus, the treatment with 100% inclusion of SS was the most significant potential for gas production. The production reaches its plateau, getting the value of 258.18, 247.66, and 245.77 for the GVB, LVB, and BL models, respectively.

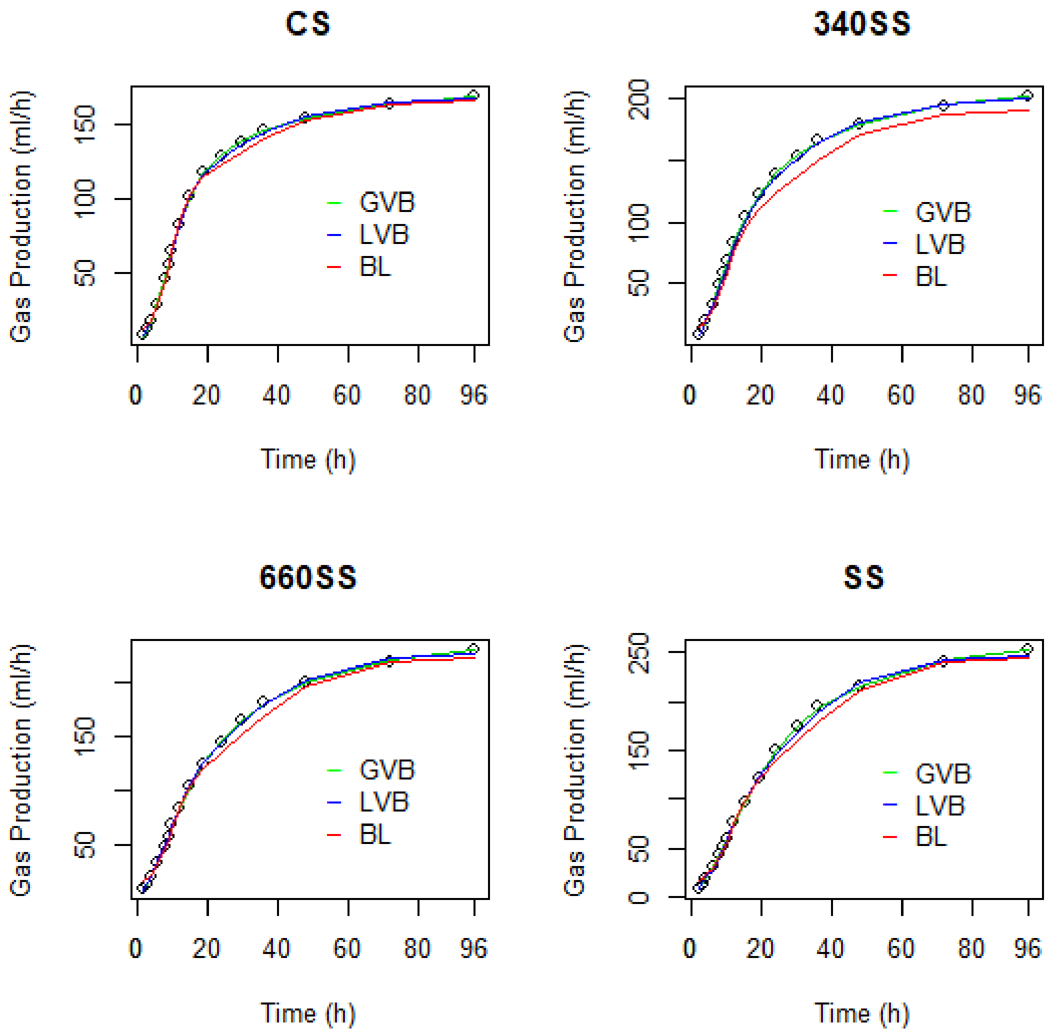

Figure 1. Cumulative gas production curves for corn silage (CS), sunflower silage (SS), and associations (340SS and 660SS), over the incubation time, from the data observed (represented by open diamonds) and adjusted by the GVB (green line), LVB (blue line) and BL (red line) models.

Table 2. Estimated parameter values (α_1, α_2, k_1, k_2, β, and λ) and criteria used to select the most suitable nonlinear model for the GVB, LVB, and BL models adjusted for CS, 340SS, 660SS, and SS data.

		Parameters							Measurers			
Levels	Models	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{k}_1$	$\hat{k}_2$	$\hat{\beta}_1$	$\hat{\beta}_2$	$\hat{\lambda}$	R^2_{aj}	RMS	AIC	MAD
CS	GVB	175.32		0.03	0.19	0.16	4.25		99.95	2.56	66.46	1.15
	LVB	113.86	53.79	0.08	0.06	1.25		3.18	99.97	1.56	59.08	0.77
	BL	95.78	69.82	0.09	0.02			4.56	99.87	5.86	79.71	1.63
340SS	GVB	205.95		0.04	0.19	0.27	3.44		99.97	1.61	59.03	0.92
	LVB	118.33	82.05	0.07	0.06	1.35		2.36	99.96	2.67	67.63	0.96
	BL	109.05	88.04	0.02	0.09			4.62	99.80	12.93	92.36	2.51
660SS	GVB	233.38		0.04	0.19	0.36	2.90		99.97	2.15	63.63	0.96
	LVB	118.98	108.95	0.07	0.06	1.37		2.00	99.94	5.32	78.62	1.43
	BL	143.05	80.37	0.02	0.10			5.01	99.73	21.96	100.8	3.36
SS	GVB	258.18		0.04	0.15	0.37	2.67		99.97	2.95	68.74	1.13
	LVB	168.79	78.87	0.02	0.20	1.25		2.90	99.85	17.39	97.58	2.83
	BL	167.14	78.63	0.02	0.09			5.79	99.71	29.82	105.7	3.89

For the levels under study, the proportion of variation ($R^2_{aj.}$) explained by the evaluated models ranged from 99.71 to 99.97% (Table 2), with the BL model presenting the lowest values for all tested levels. The highest value of R^2_{aj}, and the lowest values for MRS, AIC, and MAD were observed for the levels of 340SS, 660SS, and SS using the GVB model. For these same criteria, the highest and lowest values for the CS level were observed using the LVB model, showing the best quality of fit for these models, compared to the BL model, having been the different models considered more efficient in the respective substrates. Therefore, the BL model presented the worst values of $R^2_{aj.}$, MRS, AIC, and MAD for all treatments reported about the other models.

4. Discussion

These tests the dispersion of residues are usually neglected in most studies. If such attention is ignored in the adjustment process or if any of these tests are not met, the model is considered inadequate. This deviation must be corrected or considered in the model (Fernandes et al. 2014).

These results agree with the studies by Santos et al. (2020) that found problems of homoscedasticity and independence when they evaluated the adjustment of the LVB and BL models, respectively, to data from SC, 340SS, 660SS, and SS.

To verify the dispersion of residues in the Brody and France models, Mello et al. (2008) used graphical methods. They observed that the models had identical dispersion in sunflower silages and close dispersion in corn silage, with positive residues and different to the patterns of other models in the first hours of incubation. The authors also found that the Gompertz, Von Bertalanffy, Modified Logistics, and Logistics models followed the same dispersion trend in both substrates. They found greater residual dispersion up to 60, 72-hours post-incubation in SS and CS, respectively, possibly due to greater microbial activity during that period. These authors also observed that the residues were more homogeneous in the 60-to-144-hour interval in SS and the 72-to-144-hour interval in CS, doubtless due to the lower microbial activity during these intervals.

The curves of in vitro gas production generate a sigmoid curve, divided into three phases, namely: initial phase with slow gas production, exponential phase of rapid gas production, and lastly, asymptotic phase with a decrease in the gas production rate, reaching zero (Santos et al. 2021).

These results of the curves adjustments corroborate those of Santos et al. (2020), in which the authors report a better adjustment of the LVB model compared to the adjustment of the BL model for the SS, 340SG, 660SG, and CS treatments. The authors above report that the LVB and BL models differ in terms of the second compartment.

Mello et al. (2008) mention that the von Bertalanffy, Gompertz, Logistics, and Modified Logistics models overestimated the transition between the final exponential phase and the beginning of the asymptotic phase, most notably between the 36-to-72-hour post-incubation intervals in CS and 24-to-60-hour post-incubation in SS. The authors also report that all models underestimated the asymptotic phase after 144 hours of incubation on both substrates.

Santos et al. (2020) evaluated the ruminal degradation kinetics parameters of the CS, 340SS, 660SS and SS treatments, using the in vitro cumulative gas production technique for the LVB and BL models, found values of α_1, α_2, k_1, k_2 and λ, identical to our findings.

Corroborating the results discussed above, Santos et al. (2020) observed a reduction in the total volume of gas when the inclusion of CS was made. According to Sá et al. (2011), diets and/or foods with higher protein contents result in lower CGP.

Aragadvay-Yungán et al. (2015) worked with 100% CS and SS; their mixtures in the proportions of 25, 50, and 75% found more outstanding cumulative total gas production for corn silage. The authors recommended as an alternative to a mixture of sunflower silage and corn silage up to 25%, as being similar to 100% corn silage.

Whether as single roughage or mixed roughage diets (CS and SS), the production conclusions obtained in digestibility studies on the use of SS are inconstant (Santos et al. 2020). SS concerning CS contains less quality of its fibrous fraction, which leads to a reduction in its nutritional value for ruminants

(Pereira et al. 2016). Martins et al. (2011) observed weight loss and lower DM consumption in crossbred cows receiving SS 100%, attributed to the low digestibility of roughage fiber.

In work evaluating the sunflower and corn silage in the diet of dairy cows, Leite et al. (2006) observed that the partial replacement of CS by SS (34SS) did not affect CS consumption than CS as a single roughage. Leite et al. (2017) observed that SS compared to diets containing CS, provided the highest nutrient consumption and maintenance of milk production in dairy cows.

R^2_{aj} indicates the accuracy of the model used. Thus, by not evaluating the biological coherence of the data, Santos et al. (2020) recommends that this coefficient should not be used as the sole criterion for evaluating the models. Another important observation for this criterion is the values of $R^2_{aj.}$, for all tested models, were reduced with the increase of SS levels. This observation is corroborated by Santos et al. (2020), who observed a decrease in the total volume of gases with SS inclusion. Krueger et al. (2010) observed in alfalfa samples incubated in vitro a decrease in the volume of gases and in the rate from fibrous carbohydrates when glycerol was included.

As commented by Üçkardeş and Efe (2014), the same model can present different performance when using other substrates. Evaluating the bicompartmental models LVB and BL to describe the volume of gases from CS, SS, and their mixtures, Santos et al. (2020) concluded that the best model was the LVB for presenting the highest values of $R^2_{aj.}$ and the lower values of MRS, MAD, AIC, and Bayesian information criterion (BIC), besides obtained relative efficiency (RE) greater than one, indicating superiority in comparison to BL.

Comparing seven non-linear models to CGP data in CS and SS, Mello et al. (2008) concluded that the BL model was statistically the best to describe CGP. To compare the models in this study, the authors used the coefficient of determination, mean square of the residue, graphical analysis of observed and estimated curves, graphical analysis of the dispersion of studentized residues, average percentage error, relative efficiency, and the number of iterations of the models adjusted for the different substrates. According to the authors, the France model presented RE greater than one in all CS comparisons, while the BL model was more efficient in all comparisons for SS. Therefore, as Oliveira (2016) stated, the CGP data interpretation must be careful, requiring models that correctly interpret each food's degradation profiles.

5. Conclusions

The study's results show that the modeling is dynamic and that a new model's power can generate of easy biological interpretation parameters and generate more accurate estimates. Thus, a selection of models is presented as a fundamental step for PCG kinetics. Therefore, the GVB model can be an alternative for better in vitro gas production kinetics in sunflower silages and their mixtures 340SG and 660SG. Meanwhile, the LVB model is indicated for studying the accumulated gas production kinetics for corn silage, having been the different models considered more efficient in the substrates. Thus, the GVB model is recommended as an alternative for the study of PCG kinetics. However, more studies, including short and longer incubation times, are needed to fully investigate the performance of GVB new model.

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