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MODELING OF IN VITRO GAS PRODUCTION

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Abstract: In this study, *in vitro* gas production values (hour/ml) of standard, alfalfa and corn plants at 3, 6, 9, 12, 24, 36, 48, 72 and 96 hours in Kahramanmaras Sutcu Imam University Animal Science Department Feeds and Animal Nutrition Laboratory.) used cubic piecewise regression, Richard, Logistics, Gamma, Gompertz, Orscov, Sigmoaidal and Quadratic Piecewise Regression models were used. In the modeling study, mean squares of error, determination coefficient, Akaike information criterion and Durbin-Watson autocorrelation values were taken into account for each model of in vitro gas production values. As a result of the study, it was concluded that Logistic and Gompertz models had the best results in corn and alfalfa, standard, while the Gamma model had the worst results in all feeds in terms of the comparison criteria, mean squares of error, coefficient of determination, Akaike information criterion and Durbin-Watson Autocorrelation values.

Keywords: Feed, *In vitro*, Gas production, Stomach

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1. Introduction

In vitro gas production method is one of the most widely used methods in feed evaluation in ruminants (France and al., 2000; Canbolatandal., 2013; Menkeand al., 1988; Rymerand al., 2005; Van, 1994). In the *in vitro* evaluation of feeds with gas production method, the amount of rumen fluid is affected by the amount of feed used, the feed/rumen fluid ratio and the volume of the incubation medium. These factors are the factors that directly affect the amount of gas production. For this reason, it will be possible to make an accurate interpretation of the gas production method in *in vitro* gas production method, by choosing the right model (Canbolat and al., 2007; Karabulut and al., 2006; Özturk and al., 2006; Çölkesen and al., 2005; Gülboy and Önder, 2018). Many different mathematical equations are used to better model and interpret gas production curves (Orskov and McDonald, 1979). Commonly used models in in-vitro gas production method can be listed as exponential, Cubic, Richards, Logistics, France, Gompertz and Groot models. However, the fact that gas production curves are polynomial and a sigmoidal curve makes model selection very difficult. Gas production in the first stage of fermentation is very low. This situation shows a stable increase until it reaches the asymptote. The important thing here is to be able to choose a model that includes sigmoidal structures with and without bends in the gas production curve. Gas production curves correlate with digestion and microbial density. Therefore, the curves obtained by the in-vitro gas production method will show slight differences each time without disturbing the general structure of the curve. In this study, it was aimed to model in vitro gas production values (hr/ml) of standard, corn and alfalfa in nine different time periods (3, 6, 9, 12, 24, 36, 48, 72 and 96 hours) by using Cubic, Gompertz, Logistics, Gamma, Richard, Cubic Piece, Orscov and Sigmoaidal models.

2. Material and Methods

2.1. Materials

In this study, *in vitro* gas production at 3, 6, 9, 12, 24, 36, 48, 72 and 96 hours for 3 different groups belonging to standard, corn and alfalfa in Kahramanmaras Sutcu Imam University Feeds and Animal Nutrition Laboratory values (hr/ml) were obtained. Four measurements were made for each hour from each feed sample and the averages of these measurements were used in the modeling.

2.2. Methods

In modeling of in-vitro gas production values, cubic piecewise regression, Richard, Logistic, Gamma, Gompertz, Orskov, Sigmoaidal and Quadratic Piecewise Regression models were used. Obtaining the curves and estimating the model parameters were made in the SAS (7.0) package program. The equations and expansions of these models are as follows (equation 1, 2, 3, 4, 5, 6, 7 and 8);

Cubic Piecewise Regression,

$$W_t=\beta_0+\beta_1 t+\beta_2 t^2+\beta_3 t^3+\beta_4(t-a)^3+\beta_5(t-b)^3 \quad (1)$$

Logistic,

$W_t = \beta_0/(1+\beta_1 e(-\beta_2 t))$ (2)

Gompertz,

$W_t = \beta_0 e(-\beta_2 e(-\beta_3 t)$ (3)

Gamma,

$W_t = \beta_0 t^{\beta 1(-\beta 2t)}$ (4)

Orskov,

$W_t = \beta_0(1-e^{-ct})$ (5)

Richard,

$W_t = 1/(\beta_0 + \beta_1 e(\beta_2 t)^{(-\beta_3)}$ (6)

Sigmoidal,

$W_t = \beta_0/(1+(\beta_1/t)^{\beta 2}$ (7)

Quadratic Piecewise Regression,

$W_t = \beta_0 + \beta_1 t + \beta_2 t^2$ (8)

is in the form. Here, Wt:t. gas production over time, β0, β1, β2, β3, β4, and β5: constants defined for the models, a and b; In the piecewise regression, it represents the node points, e: 2.7182, t: time (hour) (Rodrigues, 2009; Çetinkaya. 2015).

2.2.1. Model comparison criteria

In curve modeling studies in the biological field, comparison criteria such as coefficient of determination, corrected coefficient of determination, mean squared error, Durbin-Watson autocorrelation coefficient, BIC, AIC and root mean square error are taken into account. All of these criteria are equations created to determine how adequate or insufficient the model is to represent the point distribution. These equations test how close the point distribution is to the curve created, whether there is a relationship between the error terms, how close the values obtained with the estimation equations and the values obtained, and whether they are within the statistically acceptable error limits while doing these. In this study, the coefficient of determination, mean square error, Durbin-Watson and AIC were taken into account in the comparison of the conformity of different models of the values obtained with the in vitro gas production technique to the point distribution.

Equality of the coefficient of determination (equation 9),

$R^2 = 1-(RSS/SST)$ (9)

is in the form. In the equation, RSS: Resudual the sum of squares, SST: Sum of square total. The R^2 value indicates how much of the total variation in the data set can be expressed by the model fitted to the nocturnal distribution. It takes values in the range of $0<R^2<1$. A high coefficient of determination means that the obtained model has a high fit to the point distribution.

Equality of mean squared error (equation 10),

$$MSE = \Sigma\left(Y_i - \widehat{Y}_i\right)^2/n \quad (10)$$

is in the form. The low mean of squares of error is a strong indication that the model is well suited to the point distribution. Therefore, it is widely used in model comparisons.

The Akaike information criterion is a widely used criterion to choose the statistically most appropriate one among the equations created. As a rule, the model with the smallest Akaike information criteria (AIC) value is considered to be the most appropriate model. Equality of the AIC (equation 11),

$$AIC = nxln\left(\frac{HKT}{n}\right) + 2k \quad (11)$$

is in the form (Üçkardeş and al.,2013; Üçkardeş and Efe, 2014).

Durbin-Watson autocorrelation test is a test to test whether the error terms of the predicted model are related. The fact that the value obtained with this test is around 2 is a strong indication that there is no autocorrelation. Durbin Watson test statistics where e_i = error term, t = time (equation 12),

$$DW = \frac{\sum_{t=2}^{n}(e_t - e_{t-1})^2}{\sum_{t=1}^{n} e_t^2} \quad (12)$$

is in the form. The Durbin Watson value always lies between 0 and 4. If the DW value is 2, it is considered that there is no autocorrelation.

3. Results and Discussion

For the standard; it was concluded that the logistic, Gompertz and Quadratic Piecewise Regression models had the best results, while the Gamma model had the worst results in terms of means of error squares, coefficient of determination, Akaike information criterion and Durbin-Watson Autocorrelation values. For corn, as in the standard, when all comparison criteria are taken into account, it has been determined that the Logistic and Gompertz models have the best results, and the Gamma model has the worst results, as in the standard (Table 1, 2 and 3). In terms of comparison criteria for alfalfa, it was concluded that the logistic and Gompertz models had the best results, while the Gamma model had the worst results, as in standard and maize (Figure 1, 2 and 3).

As a result of the study, it was concluded that the logistic and Gompertz models had the best results in standard, corn and alfalfa feed groups in terms of comparison criteria, while the Gamma model had the worst results in all feeds. On the other hand, it was determined that the quadratic model gave values close to these models. These results are in line with the results obtained from the modeling studies of in vitro gas production values (Rodrigues et al., 2009; Wang et al., 2011; Üçkardeş et al., 2013; Çetinkaya and Erdem, 2015;) are in agreement.

Table 1. Gas values obtained for standard; Mean Squared Error, Coefficients of Determination, Akaike Information Criteria and Durbin-Watson Autocorrelation values of all models

Models	MSE	R^2	AIC	DW
Cubic piecewise regression	39.99	0.9910	-233.5	2.33
Richard	53.04	0.9975	-243.9	2.86
Logistic	37.24	0.9986	-412.6	2.09
Gamma	339.5	0.9625	-29.6	1.12
Gompertz	37.28	0.9986	-429.3	1.92
Orskov	208.8	0.9873	-196.5	2.45
Sigmoidal	62.65	0.9971	-313.7	0.96
Quadratic Piecewise Regression	28.47	0.9909	-429.9	2.11

Table 2. Gas values obtained for corn; Mean Squared Error, Coefficients of Determination, Akaike Information Criteria and Durbin-Watson Autocorrelation values of all models.

Models	MSE	R^2	AIC	DW
Cubic piecewise regression	28.03	0.9894	-213.5	2.43
Richard	42.98	0.9974	-263.1	2.96
Logistic	23.84	0.9982	-429.5	2.11
Gamma	653.1	0.9832	-51.6	1.22
Gompertz	24.90	0.9982	-431.5	1.98
Orskov	189.3	0.9880	-181.5	2.47
Sigmoidal	50.80	0.9969	-343.2	0.81
Quadratic Piecewise Regression	18.87	0.9883	-318.5	2.61

Table 3. Gas values obtained for alfalfa; Mean Squared Error, Coefficients of Determination, Akaike Information Criteria and Durbin-Watson Autocorrelation values of all models

Models	MSE	R^2	AIC	DW
Cubic piecewise regression	37.6	0.9911	-259.1	2.35
Richard	53.85	0.9984	-296.3	2.01
Logistic	50.29	0.9981	-441.5	2.04
Gamma	699.7	0.9731	-72.4	1.33
Gompertz	43.36	0.9983	-422.5	1.97
Orskov	233.1	0.9895	-174.6	2.49
Sigmoidal	43.69	0.9981	-402.6	1.09
Quadratic Piecewise Regression	37.72	0.9867	-208.7	2.98

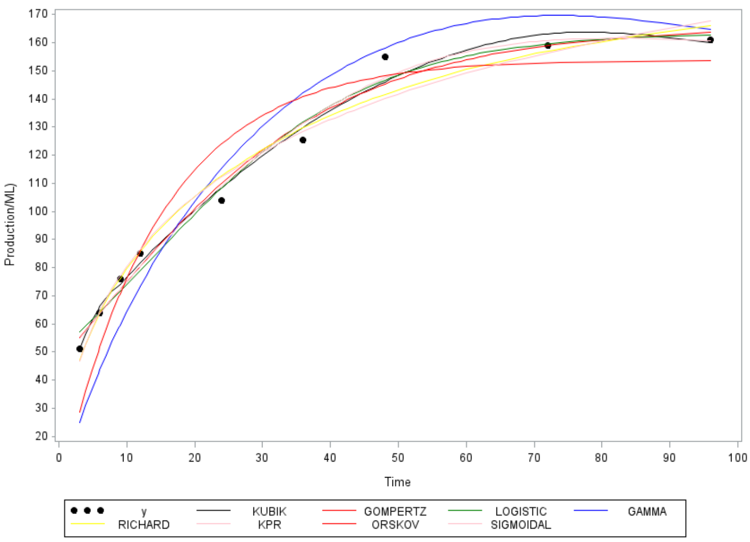

Figure 1. For the standard; gas production curves of cubic piecewise regression, Gompertz, Logistic, Gamma, Richard, Orscov, Sigmoaidal and Quadratic Piecemeal Regression.

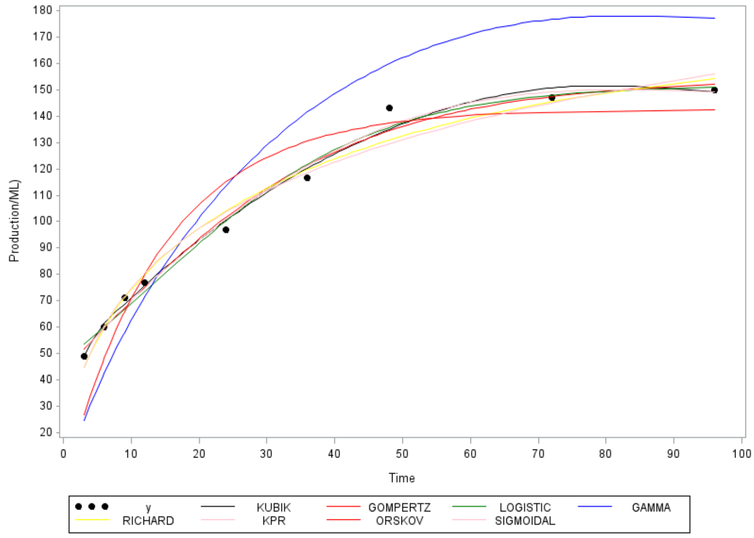

Figure 2. For Corn; gas production curves of Cubic Piecewise Regression, Gompertz, Logistic, Gamma, Richard, Orscov, Sigmoaidal and Quadratic Piecemeal Regression.

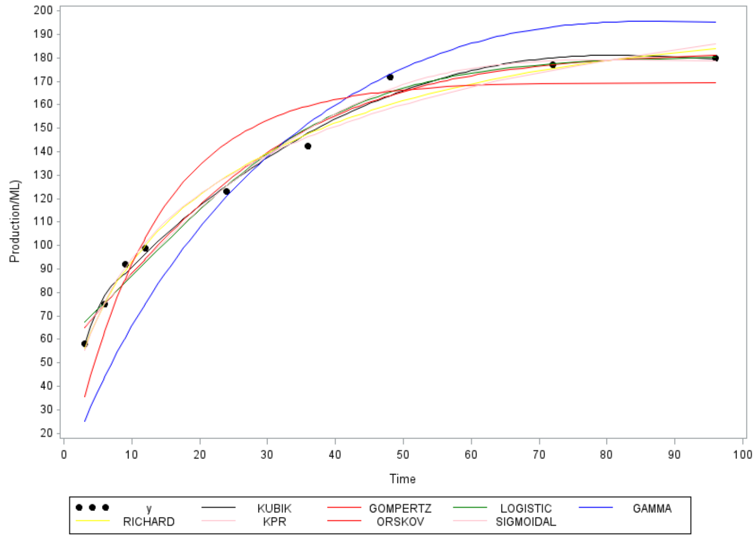

Figure 3. For Alfalfa; gas production curves of cubic piecewise regression, Gompertz, Logistic, Gamma, Richard, Orscov, Sigmoaidal and Quadratic Piecemeal Regression.

4. Conclusion

The point that should not be forgotten here and must be taken into account is the fact that the in-vitro evaluation of feeds by gas production method is affected by the amount of rumen fluid, the amount of feed used, the feed/rumen fluid ratio and the volume of the incubation environment. These factors are the factors that directly affect the amount of gas production. For this reason, accurate interpretation of the gas production method in in-vitro gas production method will only be possible with the selection of the right model. Because of the variability of these factors in each study, the shape of the gas curves obtained, that is, the polynomial structure of the curves, will be slightly different. For this reason, in the modeling of in-vitro gas production curves, it will be the most accurate method to consider more than one model and to consider the biological interpretability, advantages and disadvantages of the models in the evaluation.

Author Contributions

M.B. (34 %), M.Ş. (33%) and T.T. (33%) design of study. M.B. (34 %), M.Ş. (33%) and T.T. (33%) data acquisition and analysis. M.B. (34 %), M.Ş. (33%) and T.T. (33%) writing up. M.B. (33 %), M.Ş. (33%) and T.T. (34%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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