



# **Research Article**

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# DETERMINATION OF THE MOST APPROPRIATE REGRESSION METHOD TO ESTIMATE METABOLIC ENERGY VALUES BY IN VITRO GAS PRODUCTION TECHNIQUE

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**Abstract**: Estimation of metabolic energy values of feeds using in vitro gas production values is an essential issue in practice. It is determined that estimation of metabolic energy values with lonely use of in vitro gas production was not achieved in previous studies. In this study, to determine the most fitted regression model for estimating metabolic energy with use of gas production in certain incubation periods and parameters of gas production equation, models were set taking into account distributions of data and models were compared with the criteria of determination coefficient and mean square error. It is indicated that in vitro gas production values have long tailed, skewed, distributions such as Burr, Dagum and Generalized Pareto distribution. As a result, five different models with high coefficient of determination were set to estimate metabolic energy from in vitro gas production using normalized data. The model which estimates the metabolic energy with use of only gas production at 24 hours was determined as the best model from these five. Models were simplified to provide convenience to users when they use models to estimate metabolic energy.

Keywords: Gas production values, Metabolizable energy, Regression, Distribution

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## 1. Introduction

Gas production technique has been acknowledged as a routine method to evaluate feeds after the study of Menke et al. (1979) which set there is a high relation between in vitro gas measurement and in vivo digestibility (Kamalak, 2005; Kılıç and Sarıçiçek, 2006). In vitro gas production can be calculated with equation 1 as mentioned by Ørskov and Mcdonald (1979).

$$Y = a + b(1 + e^{ct}) (1)$$

where; Y: produced gas at time "t", a: the gas production from the immediately soluble fraction (mL), b: the gas production from the insoluble fraction (mL), c: the gas production rate constant for the insoluble fraction (mL  $h^{-1}$ ) and t: incubation time (h) (Aydın et al., 2010; Sarıçiçek and Kılıç, 2011; Canbolat, 2012). The estimation of a, b and c parameters were solved by using equation 1 with eight different incubation time from 3h to 96h (3, 6, 9, 12, 24, 48, 72and 96) (Abaş et al., 2005; Kılıç and Sarıçiçek, 2006; Şahin et al., 2011;

Kiliç et al., 2011). Amount of gas production for 24 h can be used to calculate metabolizable energy (ME), net energy, lactation net energy and organic matter digestibility of feed. But, these estimated values generally conflict with declared values of NRC (National Research Council) (Boga et al., 2014). The main reason of this condition can be regarded to non-normal distribution of data (Beuvink and Kogut, 1993).

The aim of this study is to set the best regression model to estimate ME with use of gas production. For this aim the most important criteria is the structure of the data as in all statistical methods (Arı and Önder, 2013; Zhang et al., 2014).

## 2. Material and Methods

### 2.1. Material

Data of this study was taken from a PhD thesis of Ünal Kılıç (2005) where gas production values was taken from rumen fluid of three ruminal cannulas healthy Sakız x Karayaka crossbreed ram. Corn silage, alfalfa hay and vetch hay were used as feed material for gas production with 475 records. Gas production values of eight time point (3h, 6h, 9h, 12h, 24h, 48h, 72h and 96h) were used in this study. Additionally, a, b, c and (a+b) values obtained from gas production equation and metabolizable energy value were used. For analysis 30 days trial version of Easy Fit (URL1) for distributions and SPSS for model building were used with the license of Ondokuz Mayıs University.

### 2.2. Method

The distribution of the variables was determined by using EasyFit software, to decide the distribution Anderson-Darling test was used (Razali and Wah, 2011). Additionally, curve estimation of explanatory variables on ME was examined.

For the model building data normalized with own distribution parameters which refer to more sophisticated adjustments where the intention is to bring the entire probability distributions of adjusted values into alignment. Simplification of regression model was evaluated as;

$$Y_i = \beta_0 + \beta X_i \tag{2}$$

$$\beta X_i = \beta \left( \frac{X_i - \mu}{\sigma} \right) \tag{3}$$

here:

$$Y_i = \beta_0 + \beta \left( \frac{X_i - \mu}{\sigma} \right) \tag{4}$$

$$Y_i = \beta_0 + \frac{\beta X_i - \beta \mu}{\sigma} \tag{5}$$

$$Y_i = \beta_0 + \frac{\beta X_i}{\sigma} - \frac{\beta \mu}{\sigma} \tag{6}$$

$$Y_{i} = \left(\beta_{0} - \frac{\beta \mu}{\sigma}\right) + \left(\frac{\beta}{\sigma}\right) X_{i} \tag{7}$$

$$\beta_0^* = \beta_0 - \frac{\beta \mu}{\sigma} \text{ and } (8)$$

$$\beta^* = \frac{\beta}{\sigma} \tag{9}$$

hence,

$$\hat{Y}_i = \beta_0^* + \beta^* X_i$$

was obtained.

The parameters  $\mu$  and  $\sigma$  were belongs to original distribution of data. The aim of simplification was to provide for researchers to use original values for explanatory variables without any transformation.

## 3. Results and Discussion

The curve estimations of explanatory variables on ME were given in Table 1.

**Table 1.** The curve estimations of explanatory variables on ME.

	Shape	$\mathbf{R^2_c}$
3 h	Quadratic	0.919
6 h	Quadratic	0.921
9 h	Quadratic	0.939
12 h	Quadratic	0.957
24 h	Linear	0.960
48 h	Linear	0.856
72 h	Linear	0.821
96 h	Linear	0.798
a	Quadratic	0.624
b	Linear	0.347
c	Quadratic	0.429
a+b	Quadratic	0.776

ME = metabolizable energy

Shape of the explanatory variables on ME which is response variable were linear and quadratic, other shapes such as cubic could not observed. The distributions of quadratic variables were examined after squaring of original data. Distributions of data and relevant parameters were given in Table 2 and Table 3.

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 $\textbf{Table 2.} \ \ \textbf{Distributions and related parameters of original data}.$ 

	Distribution	Significance	Parameters
3 h	Wakeby	0.59745	$\alpha$ =8.9576 $\beta$ =0.41768 $\gamma$ =0 $\delta$ =0 $\xi$ =6.2263
6 h	Wakeby	0.42388	$\alpha$ =13.32 $\beta$ =0.40098 $\gamma$ =0 $\delta$ =0 $\xi$ =9.865
9 h	Wakeby	0.48406	$\alpha$ =22.047 $\beta$ =0.73162 $\gamma$ =0 $\delta$ =0 $\xi$ =12.505
12 h	Wakeby	0.47905	$\alpha$ =25.154 $\beta$ =0.83665 $\gamma$ =0 $\delta$ =0 $\xi$ =16.947
24 h	Wakeby	0.67753	$\alpha$ =31.421 $\beta$ =1.1024 $\gamma$ =0 $\delta$ =0 $\xi$ =28.17
48 h	Power Function	0.76427	α=1.2147 a=34.759
			b=64.34
72 h	Kumaraswamy	0.67968	$\alpha_1$ =1.1278 $\alpha_2$ =0.74654 $\alpha_3$ =37.158 b=66.4
96 h	Generalized Extreme Value	0.57171	k=-0.39762
			σ=9.677 μ=53.016
a	Pearson 5 (3P)	0.33277	$\alpha$ =6.3067 $\beta$ =47.325
			γ=-5.1939
b	Dagum	0.37874	$k=0,19317 \alpha=33.521$
			β=59.061
c	Error	0.90959	k=1.8459 σ=0.01469
			μ=0.0619
a+b	Generalized Extreme Value	0.46214	k=-0.46452
			$\sigma$ =9.3961 $\mu$ =52.969
(3 h) <sup>2</sup>	Wakeby	0.72078	$\alpha$ =159.55 $\beta$ =0.08216 $\gamma$ =0 $\delta$ =0 $\xi$ =31.33
$(6 h)^2$	Generalized Extreme Value	0.37341	$k$ =0.43432 $\sigma$ =0.44273 $\mu$ =0.38347
(9 h) <sup>2</sup>	Generalized Pareto	0.6303	k=-0.41394 $\sigma$ =822.76 $\mu$ =118.71
(12 h) <sup>2</sup>	Wakeby	0.61205	$\alpha$ =1180.0 $\beta$ =0.5465 $\gamma$ =0 $\delta$ =0 $\xi$ =244.2
(a) <sup>2</sup>	Burr	0.39466	k=31.855 $\alpha$ =0.50741 $\beta$ =13289.0
(c) <sup>2</sup>	Burr	0.92221	k=3.2347 $\alpha$ =2.8171 $\beta$ =0.00635
(a+b) <sup>2</sup>	Generalized Pareto	0.26464	k=-1.1709 $\sigma$ =3691.6 $\mu$ =1426.9
ME	Wakeby	0.73211	$\alpha$ =5.1611 $\beta$ =1.1642 $\gamma$ =0 $\delta$ =0 $\xi$ =6.5376

ME = metabolizable energy

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**Table 3.** Descriptive statistics of variables.

	Distribution	μ	$\sigma^2$	σ	Skewness	Kurtosis
3 h	Wakeby	12.545	21.752	4.664	0.70031	-0.31776
6 h	Wakeby	19.373	50.167	7.0829	0.73004	-0.24761
9 h	Wakeby	25.237	65.811	8.1124	0.26368	-1.0331
12 h	Wakeby	30.643	70.165	8.3765	0.15218	-1.1269
24 h	Wakeby	43.115	69.695	8.3484	-0.08514	-1.2141
48 h	Power Function	50.983	67.413	8.2105	-0.16571	-1.1173
72 h	Kumaraswamy	54.691	71.563	8.4595	-0.35475	-1.0828
96 h	Generalized Extreme Value	55.757	84.497	9.1922	-0.35199	-0.14966
a	Pearson 5 (3P)	3.7239	18.466	4.2972	2.5104	19.152
b	Dagum	51.656	55.775	7.4683	-0.96947	1.2309
С	Error	0.0619	2.1580*10-4	0.01469	0	0.17268
a+b	Generalized Extreme Value	55.283	76.818	8.7646	-0.53572	0.08158
(3 h) <sup>2</sup>	Wakeby	178.76	18669.0	136.63	1.5891	3.1826
$(6 h)^2$	Generalized Extreme Value	0.9681	4.8584	2.2042		
(9 h) <sup>2</sup>	Generalized Pareto	700.6	$1.8524*10^{5}$	430.4	0.70688	-0.30248
(12 h) <sup>2</sup>	Wakeby	1007.2	2.7817*105	527.42	0.49713	-0.72207
(a) <sup>2</sup>	Burr	31.003	5401.0	73.492	7.9934	141.69
(c) <sup>2</sup>	Burr	0.00405	3.4307*10-6	0.00185	0.95013	2.1582
(a+b) <sup>2</sup>	Generalized Pareto	3127.4	8.6534*105	930.4	-0.13843	-1.2133
ME	Wakeby	8.9224	1.7088	1.3072	-0.13333	-1.2136

ME = metabolizable energy

Obtained distributions showed that both explanatory and response variables were a member of long tailed distribution families. These results supported the study of Beuvink and Kogut (1993) that non-normality causes the unreliable models to estimate ME from these variables.

Table 3 showed that every variable except c were skewed. To determine the best model stepwise variable selection method (Kleinbaum et al., 1998) was used. Corrected determination coefficient ( $R_d^2$ ) and mean square error (MSE) values of the models were given in Table 4.

These five models' corrected determination coefficients were found very high and MSE values were very low. Hence, these five models could be used to estimate ME values with great reliability. Estimated models and their parameters were given in Table 5.

**Table 4.** MSE and  $R_d^2$  values of models

Model	MSE	$R_d^2$
Model 1	0.069	0.960
Model 2	0.042	0.976
Model 3	0.024	0.986
Model 4	0.018	0.989
Model 5	0.016	0.991

After the simplification obtained models were found as given in Table 6.

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**Table 5.** Models and their parameters

Models	Parameters	Beta	Std. Error	t	Sig.
1	Constant	8.922	0.040	220.344	<0.001
	24 h	1.284	0.041	31.349	< 0.001
	Constant	8.922	0.032	282.109	<0.001
2	24 h	0.793	0.100	7.891	< 0.001
	12 h	0.519	0.101	5.154	< 0.001
	Constant	9.362	0.084	111.255	< 0.001
	24 h	0.633	0.082	7.750	< 0.001
3	12 h	0.983	0.114	8.590	< 0.001
	$(6 \text{ h})^2$	-0.008	0.001	-5.448	< 0.001
	Constant	9.624	0.103	92.995	< 0.001
	24 h	0.654	0.072	9.148	< 0.001
4	12 h	0.996	0.100	9.973	< 0.001
	$(6 \text{ h})^2$	-0.012	0.002	-6.924	< 0.001
	a	0.196	0.054	3.594	0.001
	Constant	10.153	0.240	42.379	< 0.001
	24 h	0.695	0.069	10.022	< 0.001
	12 h	0.649	0.172	3.777	0.001
5	$(6 \text{ h})^2$	-0.022	0.004	-5.151	< 0.001
	a	0.170	0.052	3.248	0.003
	6 h	0.732	0.303	2.415	0.021

Table 6. Estimated models after simplification

	Models
Model 1	$\hat{Y} = 2.29083 + 0.1533802X_{24}$
Model 2	$\hat{Y} = 2.92797 + 0.094988X_{24} + 0.061959X_{12}$
Model 3	$\hat{Y} = 2.507393 + 0.075823X_{24} + 0.117352X_{12} - 0.0000248154X_6^2$
Model 4	$\hat{Y} = 2.448779 + 0.078338X_{24} + 0.118904X_{12} - 0.000037223X_6^2 + 0.045611X_a$
Model 5	$\hat{Y} = 2.068975 + 0.083249X_{24} + 0.077479X_{12} - 0.0000682X_6^2 + 0.039561X_a + 0.103347X_6$

With this study, it was proofed that estimation collapse of ME by gas production for previous studies was caused due to apply of linear regression with ignoring the distribution of data. In this study, analysis was performed by regarding the distribution of original data. Data showed long tailed skewed distributions. This skewness was normalized with use of mean and variance of original distribution of data. To obtain user friendly models, obtained equations were simplified to leave the explanatory variables alone. Thus, reliable models to estimate the ME by using gas production were obtained. With this study, ME could be estimated

by only using gas production value that not achieved in previous studies which used such as NDF, ADF, crude protein and crude fat additionally in models (Mirzaeiaghsaghali et al., 2011; Geerkens et al., 2013; Ali et al., 2015).

## 4. Conclusion

It was assumed that general distribution of gas production data are long tailed ones such as Wakeby and Generalized Extreme Value which were determined in this study. The variance of previous studies on gas

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production allowed to declare that distribution of gas production values could be same with the distribution of data obtained from this study. Rising similar long tailed distributions on future studies for gas production will be expected.

Results suggested to the researchers working on gas production to build models up according to shape of the data. The mentioned models can be used to estimate ME values from gas production for roughage.

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