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INVESTIGATION ON THE USABILITY OF SOME MATHEMATICAL MODELS IN *IN VITRO* GAS PRODUCTION TECHNIQUES

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This study was derived from PhD thesis.

ABSTRACT

The aim of this study was to investigate the usage of some mathematical models in *in vitro* gas production techniques. With this aim, the Logistic, Orskov, Verhulst, Janoscheck, Weibull, Bridges, Mitscherling, Monomolecular and Von Bertalanffy models were used, respectively. The goodness of fit of these models to *in vitro* gas production data was examined by using various criteria, such as Mean Square Error (MSE), Adjusted coefficient of determination ($\overline{R}^2$), Accuracy factor (AF) and Bayesian Information Criterion (BIC). However, autocorrelation and the distribution of residuals were examined with the Durbin Watson and Shapiro Wilks tests, respectively. Although the Verhulst and Logistic models showed a lower goodness of fit according to the other models, these models were found to be suitable for *in vitro* gas production studies in the result of all the criteria and tests. As a result, it was determined to be suitable for the *in vitro* gas production technique of other models except for the Orskov model.

Key words: mathematical model; Orskov model; *in vitro*; animal nutrition

INTRODUCTION

Mathematical models have been used for a long time to determine the kinetics of digestion of forage. The first mathematical model in ruminant nutrition is the study to determine the dry matter digestibility of forage obtained by the *in vitro* method by Axelsson (1939) with the estimated regression equation. McMeekan (1943) added the standard errors of the regression equation and the correlation coefficient to this model. However, due to substantially high standard errors of the studies further work was carried out in those years to reduce the standard error (Kivimae, 1960). However, the demand for mathematical models increased in following years with the invention of *in vitro* and *in situ* techniques (Tilley *et al.*, 1960; Tilley and Terry,1963; Mcleoad and Minson,1969; Menke *et al.*, 1979). Recently, many mathematical modeling studies have been carried out to describe production data better (France *et al.*, 2005; Sahin *et al.*, 2011; Wang *et al.*, 2011). Forage digestion kinetics can be estimated more accurately by means of these models.

The aim of this study is to investigate the usability of some mathematical models in *in vitro* gas production techniques. For this purpose, nine models were discussed and tried to determine new models which are not scanned and reached in earlier *in vitro* studies except for the Orskov model. Furthermore, a new form of Logistic and Monomolecular models are discussed in this study. The models used in this study are Logistic, Verhulst, Janoschek, Weibull, Bridges, Mitscherling, Monomolecular and Von Bertalanffy, respectively.Various goodness of fit criteria were used to identify similarities and differences between these models.

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MATERIAL AND METHODS

The data set used in this study consists of dry matter digestibility values of each of three replications of four different legume forage crops (white clover - *Trifolium repens* L., red clover - *Trifolium pretense* L., common vetch - *Vicia sativa* L. and yellow sweet clover - *Melilotus officinalis* L.) obtained by using *in vitro* technique and taken at the hours 3, 6, 24, 48, 72 and 96 from Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Animal Science, and used with written permission of the researchers related.

In this study, the equation of Orskov model which is selected for control and of other models are given in Table 1 and also parameter meanings are given in Table 2.

Statistical Analyses

Determination of the goodness of fit

The goodness of fit of each model is evaluated by using Mean Square Error (MSE), Adjusted coefficient of determination ($\bar{R}^2$), Bayesian Information Criterion (BIC). Formulas of these criteria are:

Mean Square Error (MSE) = MSE $\frac{1}{n}\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2$

Adjusted coefficient of determination = $\bar{R}^2 = 1 - \dfrac{\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2/(n-k)}{\sum_{i=1}^{n}(Y_i - \bar{Y})^2/(n-1)}$

Accuracy Factor = $AF = e\left[\sqrt{\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2/n}\right]$

Bayesian Information Criterion = BCI = $nln\left(\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2/n\right) + kln(n)$

and these expressions refer to;

E : Exponential expression (2.7182),
$\hat{Y}_i$: Predictive value,
Y_i : Observed value,
$\bar{Y}$: Mean of observed values,
n : Sample size,
ln : Natural logarithm.

Examination of errors

Shapiro Wilk (SW) test is used to determine whether the errors in the models are distributed normally (West, 1999). Durbin Watson (DW) statistics was used to determine whether there is an autocorrelation among the errors in models (Lopez *et al.*, 2004).

All models are fitted by using SAS (8.0) package program NLIN command and Levenberg Marquardt algorithm (SAS, 1999). It is tested by one-way analysis of variance (One Way ANOVA) whether MSE, $\bar{R}^2$, AF and BIC criteria are statistically different from each other. Tukey's multiple comparison technique was used for the purpose of determining the difference between the criteria which is significant (Pearse and Hartley, 1966). The level of significance is taken as $P < 0.05$.

RESULTS AND DISCUSSION

The values of model parameters are given in Table 3. Similar results are received when Orskov model which is used for control, and parameter values of other models, are compared. As a result, it has been determined that the parameter values of the Orskov model are compatible with the parameter values of new models. The results of the DW test of the models are also given in Table 3. There has not been any autocorrelation in model errors according to the DW test ($P > 0.05$). The results of DW test has come out to be non-significant, which is in accordance with the findings of Lopez *et al.* (1999) and Uckardes *et al.* (2013), who reported that the model does not include the systematic error and therefore the model could be used.

The SW test has been used to determine whether errors in the models are normally distributed and the results are given in Table 4. As a result of this test, the errors in all models show a normal distribution ($P > 0.05$). There is no systematic deviation in the errors of models for both of the results of DW and SW tests;

Table 1: The models used in the study

Models	Equations
1. Logistics	$Y = a / (1 + e^{b-ct})$
2. Orskov	$Y = a + b (1 - e^{ct})$
3. Verhulst	$Y = a / (1 - be^{ct})$
4. Janoscheck	$Y = a - (a - b)e^{-ct^d}$
5. Weibull	$Y = a - be^{-ct^d}$
6. Bridges	$Y = a + b (1 - e^{-(ct^d)})$
7. Mitscherling	$Y = a (1 - be^{-ct})$
8. Monomolecular	$Y = a - be^{-ct}$
9. Von Bertalanffy	$Y = a - (a - b)e^{-ct}$

e: exponential

Table 2: The models used in the study and parameter expressions

Models	Parameter Expressions				
	Initially obtained amount of gas or the amount of digestion	Slowly obtained amount of gas or the amount of digestion	Speed of the amount of slowly obtained gas or digestion speed	Total production amount of gas or amount of digestion	Key characteristic of the curve / *Shape parameter*
1. Logistics	ln(a/d-1) (*)	a- ln(a/d-1)	c	a	b
2. Orskov	a	b	c	a+b (*)	-
3. Verhulst	a/(1-b) (*)	a- a/(1-b) (*)	c	a	b
4. Janoscheck	b	a-b	c	a	d
5. Weibull	a-b	b	c	a	d
6. Bridges	a	b	c	a+b (*)	d
7. Mitscherling	a(1-b) (*)	a- a(1-b) (*)	c	a	-
8. Monomolecular	a-b (*)	b	c	a	-
9. Von Bertalanffy	b	a-b	c	a	-

- : unavailable (*): it was obtained from the equation

thus these models are determined to be suitable for *in vitro* gas production studies.

The results of the goodness of fit criteria are given in Table 5. While the results of analysis of variance of BIC values of models of common vetch forage crops has come out to be non-significant ($P > 0.05$), the results of analysis of variance models of other forage crops have been found to be significant ($P < 0.05$; $P < 0.01$; $P < 0.001$). Statistically significant differences have been found between MSE and $\bar{R}^2$ values of the models which are used for four different legume forage crops ($P < 0.05$; $P < 0.01$). MSE values of the Verhulst model and also in some cases the Logistic models have been higher than the other models. However, the results of MSE values of other models are similar to each other. A similar situation is observed in the values of $\bar{R}^2$. Although $\bar{R}^2$ values are significant as a result of analysis of variance of yellow sweet clover forage crop, there has not been any difference as a result of the Tukey test. While the Verhulst model and also in some cases the Logistic model has given low results in terms of the values of $\bar{R}^2$, higher values of $\bar{R}^2$ are obtained from the other models. According to Lopez *et al*. (2004) and Korkmaz *et al*. (2011) are the values of MSE being high and values of $\bar{R}^2$ are being low; it shows that the model does not provide a good fit to the data set.

The results of AF are similar with that of $\bar{R}^2$ (Table 5). The results of the analysis of variance of yellow sweet clover forage crop are significant, but no differences are found as a result of the Tukey test. However, the AF results of the Logistic and Verhulst models of goodness of fit are lower as compared to other models. Lopez *et al.* (2004) reported that AF values which are low show that the model shows a good fit to the data set.

BIC criteria has a slightly different evaluation than other criteria. BIC has a more strict attitude toward the number of parameters than the other criteria (Alzahal *et al*., 2007). The result of the analysis of variance of common vetch forage crop is non-significant ($P>0.05$).

As a result, the Verhulst and Logistic models have showed lower performance than the other models in some cases in terms of the goodness of fit criteria. Therefore, it can be misleading to make an emphasis that these models should not be used. Wang *et al.* (2011) have reported that the models which have low performances increased goodness of fit by model modification.

Models may differ from each other in terms of behaviours (structure) and the number of parameters. A model might have a more flexible structure than another model or a model which is having less number of parameters, can fit more easily. In this study, comparing to other models, the Weibull, Janoscheck and Bridges models have the parameters which shape the curve and it is more difficult to make them fit. However, these models have a more flexible structure than other models. Although having more number of parameters seems like a disadvantage, this feature can be evaluated

Table 3: Parameter values of legume forage crops and Durbin Watson test results ($\bar{X} \pm S_{\bar{x}}$)

Models	Parameter				DW
	a	b	c	d	
Common vetch					
1. Logistics	367.27 ± 10.78	0.723 ± 0.029	0.093 ± 0.005	-	Ns
2. Orskov	90.64 ± 6.390	283.97 ± 5.259	0.054 ± 0.003	-	Ns
3. Verhulst	365.37 ± 8.348	-2.06 ± 0.033	0.094 ± 0.001	-	Ns
4. Janoscheck	376.23 ± 11.695	83.94 ± 6.439	0.064 ± 0.003	0.950 ± 0.001	Ns
5. Weibull	376.57 ± 11.726	294.067 ± 5.459	0.066 ± 0.003	0.940 ± 0.001	Ns
6. Bridges	85.35 ± 6.428	290.50 ± 5.373	0.062 ± 0.003	0.960 ± 0.001	Ns
7. Mitscherling	374.60 ± 11.529	0.759 ± 0.009	0.054 ± 0.003	-	Ns
8. Monomolecular	374.60 ± 11.529	283.97 ± 5.59	0.054 ± 0.003	-	Ns
9. Von Bertalanffy	374.60 ± 11.529	90.64 ± 6.387	0.054 ± 0.003	-	Ns
White Clover					
1. Logistics	374.20 ± 9.133	0.709 ± 0.040	0.081 ± 0.001	-	Ns
2. Orskov	97.84 ± 6.929	285.90 ± 4.325	0.046 ± 0.001	-	Ns
3. Verhulst	368.33 ± 7.890	-2.14 ± 0.071	0.095 ± 0.005	-	Ns
4. Janoscheck	385.87 ± 9.559	91.69 ± 6.958	0.054 ± 0.001	0.950 ± 0.001	Ns
5. Weibull	386.33 ± 9.586	295.97 ± 4.457	0.056 ± 0.001	0.940 ± 0.001	Ns
6. Bridges	93.99 ± 6.958	292.43 ± 4.391	0.053 ± 0.001	0.960 ± 0.001	Ns
7. Mitscherling	383.73 ± 9.494	0.746 ± 0.012	0.046 ± 0.001	-	Ns
8. Monomolecular	383.73 ± 9.464	285.90 ± 4.325	0.046 ± 0.001	-	Ns
9. Von Bertalanffy	383.73 ± 9.494	97.84 ± 6.929	0.046 ± 0.001	-	Ns
Red Clover					
1. Logistics	357.73 ± 17.246	0.866 ± 0.023	0.099 ± 0.003	-	Ns
2. Orskov	76.21 ± 5.303	289.37 ± 12.236	0.054 ± 0.002	-	Ns
3. Verhulst	358.37 ± 17.010	-2.318 ± 0.054	0.094 ± 0.003	-	Ns
4. Janoscheck	366.53 ± 18.273	69.52 ± 5.012	0.064 ± 0.002	0.950 ± 0.001	Ns
5. Weibull	367.53 ± 17.636	299.50 ± 12.689	0.066 ± 0.002	0.940 ± 0.001	Ns
6. Bridges	70.93 ± 5.073	295.93 ± 12.546	0.062 ± 0.002	0.960 ± 0.001	Ns
7. Mitscherling	365.57 ± 17.522	0.792 ± 0.005	0.054 ± 0.002	-	Ns
8. Monomolecular	365.57 ± 17.522	289.37 ± 12.236	0.054 ± 0.002	-	Ns
9. Von Bertalanffy	365.57 ± 17.522	76.21 ± 5.303	0.054 ± 0.002	-	Ns
Yellow Sweet Clover					
1. Logistics	348.00 ± 11.117	0.664 ± 0.044	0.096 ± 0.004	-	Ns
2. Orskov	90.69 ± 1.790	263.60 ± 12.200	0.057 ± 0.002	-	Ns
3. Verhulst	348.17 ± 12.479	-1.937 ± 0.039	0.095 ± 0.004	-	Ns
4. Janoscheck	355.70 ± 11.374	84.346 ± 2.007	0.067 ± 0.002	0.950 ± 0.001	Ns
5. Weibull	356.00 ± 11.374	273.067 ± 12.610	0.069 ± 0.002	0.940 ± 0.001	Ns
6. Bridges	85.68 ± 1.959	269.633 ± 12.433	0.065 ± 0.002	0.960 ± 0.001	Ns
7. Mitscherling	354.33 ± 11.375	0.743 ± 0.012	0.057 ± 0.002	-	Ns
8. Monomolecular	354.33 ± 11.375	263.60 ± 12.200	0.057 ± 0.002	-	Ns
9. Von Bertalanffy	354.33 ± 11.375	90.69 ± 1.790	0.057 ± 0.002	-	Ns

DW: Durbin Watson; Ns: $P>0.05$

Table 4: Shapiro Wilk W test results for errors in legume forage crops

Models	Common vetch		White clover		Red clover		Yellow sweet clover	
	W	SL	W	SL	W	SL	W	SL
1. Logistics	0.904	0.357	0.854	0.134	0.873	0.197	0.878	0.220
2. Orskov	0.858	0.147	0.894	0.297	0.927	0.524	0.884	0.243
3. Verhulst	0.911	0.400	0.871	0.912	0.872	0.193	0.892	0.287
4. Janoscheck	0.857	0.142	0.902	0.342	0.915	0.403	0.887	0.260
5. Weibull	0.857	0.141	0.901	0.338	0.933	0.574	0.887	0.257
6. Bridges	0.857	0.142	0.900	0.329	0.931	0.561	0.886	0.256
7. Mitscherling	0.862	0.159	0.895	0.301	0.927	0.524	0.891	0.279
8. Monomolecular	0.858	0.147	0.894	0.297	0.927	0.524	0.884	0.243
9. Von Bertalanffy	0.585	0.147	0.894	0.297	0.927	0.523	0.883	0.242

W>SL = P > 0.05; SL: Level of significance of difference

as an advantage. Zwitering *et al.* (1990) reported that a model which has less number of parameters should be preferred rather than the one which has more parameters in choosing the model. They stated that the reason why they proposed this preference was that the relationship between parameters may increase due to increase in number of parameters and therefore the model may fit with more difficulty. Moreover, these researchers reported that the model which has less parameters is simpler and therefore easier to use and because the less parameter solution is more stable since the parameters are less correlated.

However, Wang *et al.* (2011) reported that increased flexibility of the model and the number of parameters by adding a parameter shapes the curve in the logistic model. Schofield *et al.* (1994) turned Logistic and Gompertz models into a dual-phase structure by adding parameters to them and thus increase the effectiveness of the models. According to France *et al*. (2005) and Calabro *et al.* (2004), the reason of these studies is to show that dependence on only one or a few models should be avoided since different digestive curves can be obtained depending on the amount of organic matter forage material (quickly or slowly degradable) or in case of using different species of ruminants. Therefore, the models which have flexible structure, can outperform more in some cases as compared to the models which are more stable like Orskov model.

However, models can provide calculation of some parameters considered to be important for digestion, for example, active digestibility, the amount of gas production at a required time. Sahin *et al.* (2011) reported that they had obtained t_{25}, t_{50}, t_{75} and t_{95} times of exponential models. Besides, France *et al.* (2000) reported that they had obtained a general formula of t_p for the Generalized Michaelis-Menten, Generalized Mitscherlich and Logistic models. But these researchers emphasized that there is no analytic solution for these equations; thus they should be evaluated as numeric. These results show the slow but continuous increase in sigmoidal models. Uckardes (2013) noticed that the Mitscherlich model is modified by adding a new biologically meaningful parameter to describe the degradation kinetics and also developed new theoretical approaches to the modified Mitscherlich model regarding the description of *in situ* nylon bag and *in vitro* gas production techniques.

CONCLUSION

In conclusion, having a large number of models should not cause a confusion in deciding which model to use. That is because, reaction of the model will also be different depending on the feed materials used. While one model shows a very good fit to the data set in one study (due to the feed material), it may exhibit low performance in another study. Therefore, it might be necessary to select an appropriate model for the data set and having a large number of models referring to variety in a way rather than being a disadvantage.

As a result of this study, it is concluded that these models other than the Orskov model can be used to estimate *in vitro* gas production kinetics by using different forage crops.

Table 5: MSE, AF and BIC analysis of variance and tukey test results of nine different models of four different forage crops ($\bar{X} \pm S_{\bar{x}}$)

Models	Common vetch MSE	White clover MSE	Red clover MSE	Yellow sweet clover MSE
1. Logistics	475.5^{ab} ± 82.5	365.8^{b} ± 16.9	272.5^{bc} ± 64.3	481.0^{b} ± 48.9
2. Orskov	215.1^{ab} ± 52.2	146.3^{a} ± 12.3	91.9^{ab} ± 25.9	248.2^{a} ± 44.4
3. Verhulst	490.3^{b} ± 91.3	426.4^{b} ± 18.6	380.9^{c} ± 63.4	485.5^{b} ± 48.9
4. Janoscheck	187.2^{ab} ± 47.91	124.3^{a} ± 11.1	76.7^{a} ± 21.3	211.8^{a} ± 33.5
5. Weibull	181.8^{a} ± 47.1	120.2^{a} ± 10.8	73.9^{a} ± 20.4	208.5^{a} ± 35.1
6. Bridges	202.4^{ab} ± 58.3	128.6^{a} ± 11.3	79.6^{a} ± 22.3	215.1^{a} ± 31.8
7. Mitscherling	215.1^{ab} ± 52.2	146.3^{a} ± 12.3	91.9^{ab} ± 25.9	248.2^{a} ± 44.4
8. Monomolecular	215.1^{ab} ± 52.2	146.3^{a} ± 12.3	91.9^{ab} ± 25.9	248.2^{a} ± 44.4
9. Von Bertalanffy	215.1^{ab} ± 52.2	146.3^{a} ± 12.3	91.9^{ab} ± 25.9	248.2^{a} ± 44.4
Significance Level	**	***	***	***
Models	$\bar{R}^2$	$\bar{R}^2$	$\bar{R}^2$	$\bar{R}^2$
1. Logistics	0.9527^{b} ± 0.006	0.9646^{b} ± 0.002	0.9726^{b} ± 0.005	0.9417^{a} ± 0.011
2. Orskov	0.9787^{a} ± 0.004	0.9858^{a} ± 0.001	0.9911^{a} ± 0.002	0.9696^{a} ± 0.008
3. Verhulst	0.9512^{b} ± 0.006	0.9587^{b} ± 0.002	0.9694^{b} ± 0.007	0.9412^{a} ± 0.011
4. Janoscheck	0.9815^{a} ± 0.004	0.9880^{a} ± 0.001	0.9925^{a} ± 0.002	0.9741^{a} ± 0.006
5. Weibull	0.9821^{a} ± 0.004	0.9884^{a} ± 0.001	0.9928^{a} ± 0.002	0.9745^{a} ± 0.006
6. Bridges	0.9800^{a} ± 0.005	0.9876^{a} ± 0.001	0.9925^{a} ± 0.002	0.9738^{a} ± 0.006
7. Mitscherling	0.9787^{a} ± 0.004	0.9858^{a} ± 0.001	0.9911^{a} ± 0.002	0.9696^{a} ± 0.008
8. Monomolecular	0.9787^{a} ± 0.004	0.9858^{a} ± 0.001	0.9911^{a} ± 0.002	0.9696^{a} ± 0.008
9. Von Bertalanffy	0.9778^{a} ± 0.004	0.9858^{a} ± 0.001	0.9911^{a} ± 0.002	0.9696^{a} ± 0.008
Significance Level	***	***	***	*
Models	AF	AF	AF	AF
1. Logistics	1.083^{b} ± 0.0072	1.075^{b} ± 0.0096	1.066^{b} ± 0.0106	1.083^{a} ± 0.0125
2. Orskov	1.052^{a} ± 0.0075	1.047^{a} ± 0.0062	1.036^{a} ± 0.0081	1.055^{a} ± 0.0121
3. Verhulst	1.083^{b} ± 0.0020	1.071^{b} ± 0.0116	1.069^{b} ± 0.0110	1.083^{a} ± 0.0080
4. Janoscheck	1.048^{a} ± 0.0075	1.043^{a} ± 0.0057	1.033^{a} ± 0.0072	1.052^{a} ± 0.0115
5. Weibull	1.048^{a} ± 0.0078	1.043^{a} ± 0.0059	1.032^{a} ± 0.0072	1.051^{a} ± 0.0120
6. Bridges	1.049^{a} ± 0.0075	1.044^{a} ± 0.0062	1.033^{a} ± 0.0072	1.052^{a} ± 0.0120
7. Mitscherling	1.052^{a} ± 0.0075	1.047^{a} ± 0.0062	1.035^{a} ± 0.0076	1.058^{a} ± 0.0158
8. Monomolecular	1.052^{a} ± 0.0075	1.047^{a} ± 0.0062	1.036^{a} ± 0.0081	1.055^{a} ± 0.0121
9. Von Bertalanffy	1.052^{a} ± 0.0075	1.047^{a} ± 0.0062	1.036^{a} ± 0.0081	1.055^{a} ± 0.0121
Significance Level	***	***	***	*
Models	BIC	BIC	BIC	BIC
1. Logistics	44.87 ± 1.162	43.22^{b} ± 0.319	40.81^{a} ± 1.566	45.08^{a} ± 0.683
2. Orskov	39.14 ± 1.603	36.77^{a} ± 0.576	33.02^{a} ± 1.945	40.31^{a} ± 1.174
3. Verhulst	45.06 ± 1.239	44.30^{b} ± 0.307	41.71^{a} ± 1.590	45.15^{a} ± 0.679
4. Janoscheck	40.07 ± 1.688	37.57^{a} ± 0.606	33.72^{a} ± 1.919	41.19^{a} ± 1.044
5. Weibull	39.85 ± 1.708	37.33^{a} ± 0.612	33.46^{a} ± 1.906	41.06^{a} ± 1.109
6. Bridges	40.50 ± 1.890	37.81^{a} ± 0.599	33.97^{a} ± 1.928	41.32^{a} ± 0.979
7. Mitscherling	39.14 ± 1.603	36.77^{a} ± 0.576	33.02^{a} ± 1.945	40.31^{a} ± 1.174
8. Monomolecular	39.14 ± 1.603	36.77^{a} ± 0.576	33.02^{a} ± 1.945	40.31^{a} ± 1.174
9. Von Bertalanffy	39.14 ± 1.603	36.77^{a} ± 0.576	33.02^{a} ± 1.945	40.31^{a} ± 1.174
Significance Level	Ns	***	*	*

Ns: $P>0.05$; *:$P<0.05$; **$P<0.01$; ***: $P<0.001$; AF: Accuracy factor; MSE: Mean Square Error; BIC: Bayesian Information Criterion

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