

An Alternative Robust Model for *in situ* Degradation Studies “Korkmaz-Uckardes”

Research Article

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ABSTRACT

The first purpose of this study is to present an alternative robust model in order to describe ruminal degradation kinetics of forages and to minimize the fitting problems. For this purpose, the Korkmaz-Uckardes (KU) model, which has a logarithmic structure, was developed. The second purpose of this study is to estimate, by using the Korkmaz-Uckardes (KU) model, the parameters t_p (the time to produce p% of partial dry matter disappearance in rumen) and Rt_p (the rate of the partial dry matter disappearance in rumen at the time t_p). These parameters will provide more useful data to compare feedstuffs in *in situ* degradation studies. The third purpose is to evaluate the performance of the Korkmaz-Uckardes (KU) model. Statistical criteria used to evaluate the performance of a model were analysis of residuals (Runs and Durbin Watson test) and goodness-of-fit test (residual mean square, coefficient of determination and accuracy factor). The analysis of residuals of the Korkmaz-Uckardes (KU) model was found to be non significant ($P>0.05$). The residuals are normally distributed. According to the goodness-of-fit test, the Korkmaz-Uckardes (KU) model applied to all data showed a very good fit (residual mean square (RMS)=15.854, $R^2=0.9853$ and accuracy factor (AF)=1.0387). According to the Pearson's correlation analysis, a significant relationship ($r^2=0.969$) was found between observed and predicted values ($P<0.001$). The results indicate that the Korkmaz-Uckardes (KU) model can be used as an alternative model for describing and interpreting *in situ* dry matter degradation from natural feedstuffs.

KEY WORDS *in situ* degradation, Korkmaz-Uckardes model, mathematical model.

INTRODUCTION

Mathematical models have been used in animal feed studies since 1939 (Axelsson, 1939). Especially, the occurrence of *in vivo*, *in vitro* and *in situ* techniques between the years 1960 and 1980 increased the demand of mathematical models. In the past 50 years, mathematical models have been commonly used to describe and interpret *in situ* digestion kinetics.

During the last few decades, many researchers have developed a few mathematical models, some researchers, such as Blümmel and Orskov (1993), (France *et al.* 1993; France

et al. 2000; France *et al.* 2005), Groot *et al.* (1996), Schofield *et al.* (1994) and Dhanoa *et al.* (2000) used the models in different discipline in animal feed studies. However, some researchers such as Wang *et al.* (2011) and Sahin *et al.* (2011) increased the effectiveness of the model by modifying the models used. The mathematical model must contain parameters with biological meaning. Otherwise, it is difficult to estimate the initial values of parameters (Zwitering *et al.* 1990). A poor selection of the initial value can lead to wrong solutions or never converge to a solution (Motulsky and Ransnas, 1987). Therefore, this situation requires restarting by different initial values again and

again in some models (Lopez *et al.* 1999; Lopez *et al.* 2004; Calabro *et al.* 2005).

The first objective of this study is to present an alternative robust model in order to describe ruminal degradation kinetics of forages and to minimize the fitting problems. The second objective is to estimate new parameters such as t_p and Rt_p by using the KU model. These parameters will provide more useful data to compare feedstuffs in *in situ* degradation studies. The third objective was to evaluate the performance of the KU model. For these purposes, the goodness-of-fit and error analysis are also included in the study.

MATERIALS AND METHODS

Two commercially available and widely used forages consisting of Gleditsia Triacanthos and Alfalfa hay (*Medicago sativa*) were used in this experiment. Three ruminally fistulated sheep (two years old and with body weight of average 60 kg), maintained on a 900 g good quality Alfalfa hay and 300 g concentrate diet according to their requirements were used.

The *in situ* dry matter (DM) degradation analysis was carried out according to the procedure described by Mehrez and Orskov (1977). 5-gram Gleditsia Triacanthos samples dried and milled through a 1-mm sieve were weighed into nylon bags and incubated in three rumen fistulated sheep for 3, 6, 12, 24, 48, 72 and 96 h.

The sheep were fed twice a day with forage Alfalfa hay and concentrate diet. On removal the nylon bags were thoroughly washed with cold running water, until no further coloured liquid could be extruded, and dried at 60 °C for 48 h. The DM degradation losses (%) for each incubation time were determined.

The Korkmaz-Uckardes (KU) model

In situ degradability shows a logarithmic increase. Therefore, it needs to use the models which have logarithmic structures.

The Orskov and exponential models have logarithmic structures and are the most common models used. However, some researchers used models which have sigmoidal structures, such as the Logistic and Gompertz models (Lopez *et al.* 1999; Wang *et al.* 2011). The main reason for this is that the amount of bacteria and fungus increases depending on the forage in rumen. Lopez *et al.* (1999) reported that sigmoidal models have a fitting problem. Therefore, in this study, it is intended to use an alternative model which has a logarithmic structure and also minimizes the fitting problem. For this purpose, we directly focused on a model which includes the logarithmic model and then we proposed the empirical model below:

$$y = a - b_1 \ln(\exp(-ct) + 1) \quad (1)$$

Where:

t: time.

y: the value at t.

a: the maximum value.

b_1 : the shape parameter.

c: the rate parameter.

In the equation (1), when t tends to infinity, y approaches a, which is the maximum value. However, the equation (1) does not contain parameters with biological meaning. Therefore, this model should be converted to another model which has biological parameters. The following transformations are gradually given. Firstly, we searched for the parameter b which gives the initial value (t=0) and then the following equation was obtained:

$$b = a - b_1 \ln(2) \quad (2)$$

After that the following equation was obtained.

$$b_1 = (a - b) / \ln(2) \quad (3)$$

Secondly, we obtained the following equation, which is the general equation of the Korkmaz-Uckardes model.

$$y = a - ((a - b) / \ln(2)) \times \ln(\exp(-ct) + 1) \quad (4)$$

Where:

y: the DM disappearance in rumen at the time t.

a: the maximum DM disappearance in rumen.

b: the DM disappearance in rumen at initial time.

c: the constant rate of degradation of (a-b) (%/h).

a-b: the potentially degradable fraction.

The first derivative of the equation (4), the absolute rate of degradability of the KU model, can be expressed as:

$$y' = (a - b)c \exp(-ct) / (\ln(2) \times (\exp(-ct) + 1)) \quad (5)$$

When t equals zero, the first derivative of the model gives the maximum rate:

$$y'(0) = (a - b) \times c / (2 \times \ln(2)) \quad (6)$$

Some researchers prefer the France model because it determines t_p (France *et al.* 1993; Theodorou *et al.* 1994; Lopez *et al.* 1999; Kamalak *et al.* 2004; Kamalak *et al.* 2005; Sallam *et al.* 2007). Recently, some researchers such as France *et al.* (2005) and Sahin *et al.* (2011) have showed the attainment of these parameters in different models.

The attainments of these important parameters in this study are as follows:

t_p can be found using the equation below.

$$y_0 + (y_\infty - y_0) \times p / 100 = y(t_p) \quad (7)$$

Where:

y_0 : the initial value.

y_∞ : the asymptotic value at the infinity.

p : a percentage value.

t_p : the time on p .

$y(t_p)$: the value at the time t_p .

The values of y_0 and y_∞ are b and a , respectively. If the KU model is rewritten in the equation (7), the following equation is obtained:

$$b + (a-b) \times p / 100 = a - ((a-b)/\ln(2)) \times \ln(\exp(-ct_p)+1) \quad (8)$$

If the equation (8) is solved for t_p , then the following general formula is obtained.

$$t_p = -\ln(\exp(-0.00693p+0.693)-1) / c \quad (9)$$

So, the time of the desired p % values can be found by using the equation (9).

For example, by using the equation (9), the time of the desired 25% values (t_{25}) can be found as:

$$t_{25} = -\ln(\exp(-0.00693 \times (25/100) + 0.693) - 1) / c$$

The general formula of Rt_p can be found by taking the first derivative of the KU model (4). So, the general formula of Rt_p can be expressed as:

$$Rt_p = \partial y / \partial t_p = 1.443 \times (a-b) \times c \exp(-ct_p) / (\exp(-ct_p)+1) \quad (10)$$

By using the equation (10), the values of Rt_0 , Rt_{25} , Rt_{50} , Rt_{75} and Rt_{95} can also be found easily.

Model fitting

The degradation data of DM were fitted to the KU model using the Levenberg-Marquardt algorithm and the NLIN procedure of SAS package (SAS, 1999). A sample set of 12 *in situ* 96 h degradability curves was used in this study. The initial values were defined for each parameter and for each data set. The initial values of all parameters were chosen as unity. However, changing the initial values of parameters has not caused any different effect on the residual mean

square (RMS). This situation has only caused a slight increase in the number of iteration. The KU model never caused fitting problems. In all cases, the RSS values and the parameters for the KU model were the same.

Statistical evaluation

The goodness-of-fit of model was evaluated by using residual mean square (RMS), the coefficient of determination (R^2) and accuracy factor (AF), where RMS is defined as the residual sum of square divided by its degree of freedom. For RMS, the following equation was used

$$\begin{aligned} \text{RSS} &= \sum (y_i - \hat{y}_i)^2 \\ \text{RMS} &= \text{RSS} / (n-p) \\ R^2 &= 1 - \text{RMS} / (S_y^2) \end{aligned}$$

Where:

RSS: the residual sum of square.

n : the number of data points.

p : the number of parameters of the model.

(S_y^2) : the total variance of the y -variable (Bibby, 1977).

AF was used to evaluate the performance of the model in terms of the average deviation between predicted and observed values (Lopez *et al.* 2004). AF was calculated as:

$$\text{AF} = \exp[\sqrt{\sum (\ln y - \ln \hat{y})} / n]$$

Regression of observed versus predicted degradation values is a common approach to evaluate model's behaviour. The significance of the regression parameters was statistically analysed using hypothesis test for slope 1 and intercept 0, according to Pineiro *et al.* (2008). Nevertheless, Pearson's correlation coefficients were used for the relationship between predicted and observed values (SAS, 1999).

Examination of Residuals

Analysis of residuals is important to decide on the suitability of a model. There are two important criteria for the validity of the fitted model. These criteria are normal distribution and independent residuals. Runs and Durbin Watson (DW) tests were used for independence of residuals. For the normality of residuals, the Runs test, described by Motulsky and Ransnas (1987) and DW test, described by Draper and Smith (1981), were used.

RESULTS AND DISCUSSION

The initial value for each data and each parameter was defined as 1 and iteration results were given in Table 1. The number of iterations of the model ranged from 12 to 25.

Table 1 Summary of R^2 , accuracy factor (AF), durbin watson (DW) values and runs test after fitting model (n=12)

Korkmaz –Uckardes model		
Number of iteration	Minimal	Maximal
Average	5.08	16.75
Min	2	12
Max	11	25
RMS		
Average	15.854	
Min	1.057	
Max	58.204	
R^2		
Average	0.9853	
Min	0.9680	
Max	0.9958	
AF		
Average	1.0387	
Min	1.0117	
Max	1.0914	
Distribution of curves according to DW values (total curve=12)		
Significant	0	
Non significant	12	
Distribution of curves according to the number of runs of sign (total curve=12)		
4	2 ^{ns}	
5	6 ^{ns}	
6	4 ^{ns}	

AF: accuracy factor and DW: durbin watson.

NS: nonsignificant and RMS: residual mean square.

 R^2 : coefficient of determination.

The average number of iteration was 16.75. To change the initial values, the values of RMS and parameter were not changed. The KU model provided a good fit with the Levenberg-Marquardt algorithm. When the possible initial values for each parameter were selected, the iteration number ranged from 2 to 11. The average number of iteration was 5.08. The proportion of the variation explained was very high. The goodness-of-fit tests were given in Table 1. The average value of RMS of the KU model was 15.854. R^2 values of the model ranged from 0.9680 to 0.9958. R^2 values were close to unity in most cases. The average value of R^2 of the KU model was 0.9858. A similar trend was observed when AF was used (Table 1). AF values of the model ranged from 1.0117 to 1.0914 (Table 1). AF values were close to unity in most cases. The average value of AF of the KU model was 1.0387. According to these results, the KU model has shown a perfect fit to *in situ* data. For serial correlation or autocorrelation of the errors, durbin watson (DW) statistics was used. DW statistics was used to determine whether the errors are scattered randomly around the zero line. DW values obtained are given in Table 1. The distribution of number of runs of sign was shown in Table 1. The distribution of the 12 curves for each fitted models was illustrated by dividing them into three categories, which were the number of curves with 4, 5 and 6 runs of

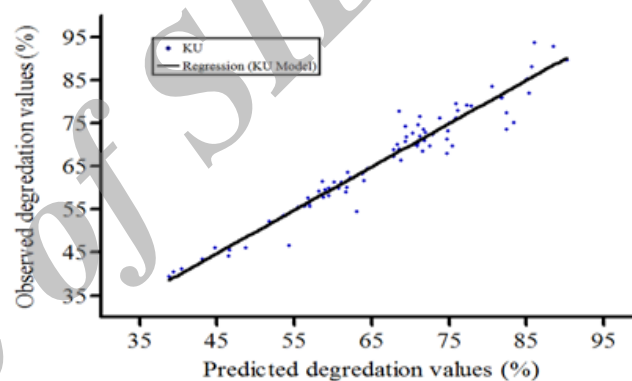
sign, respectively (Table 1). A small number of runs of sign were obtained when the residuals were not randomly distributed. So residuals of the same sign tend to cluster on some parts of curve. Such clustering indicates that the data points differ systematically from the predictions of the curve. The result of observed versus predicted regression *in situ* degradation was shown in Table 2 and Figure 1.

Table 2 The result of observed versus predicted regression *in situ* degradation, and the result of correlation after fitting model

Korkmaz –Uckardes model	
Values of hypothesis test for the parameters of observed (in the y-axis) versus predicted regression (in the x-axis) <i>in situ</i> degradation, and correlation	
Intercept	-0.337 ^{ns}
Slope	1.003 ^{***}
R^2	0.969 ^{***}

NS: nonsignificant.

*** P<0.001.

 R^2 : coefficient of determination.**Figure 1** Scatter plot of observed versus predicted regression. The observed and predicted *in situ* degradation values are at the each degradation time

As a result of hypothesis test, for the parameters of regression, the values of intercept and slope were considered as 0 and 1, respectively. According to the Pearson's correlation analysis, a significant relationship ($r^2=0.969$) between observed and predicted values ($P<0.001$) was found.

The digestion kinetics results of the KU model for *Gleditsia Triacanthos* were shown in Figure 2. Time to produce p % of total degradation of the specific time periods, being an example to all of the models, was taken into the consideration. The production of any other percent of total degradation could also be found by putting a desired time instead of 25, 50, 75 and 95% in Table 2. Similarly, the same procedures could also be done for Degradation Rate. The Degradation Rates of the model, Rt_0 , Rt_{50} , Rt_{75} and Rt_{95} were given in Table 3. Lopez *et al.* (1999); Lopez *et al.* (2004), Calabro *et al.* (2005) and France *et al.* (2005) reported that they repeated the analysis many times for selecting the most appropriate initial values and obtaining the lowest RSS value.

In particular, for some inconsistent models with very large data set, this process becomes time-consuming.

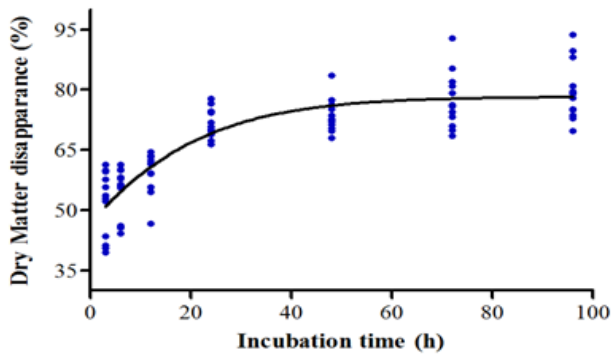


Figure 2 The solid curve was determined as the KU model:

$$y = a - \frac{(a-b)}{\ln(2)} \times \ln(\exp(-ct)+1)$$

The best fit values were determined by $a = 78.24 \pm 1.33$, $b = 46.83 \pm 2.09$ and $c = 0.062 \pm 0.01$

Motulsky and Ransnas (1987) expressed that a poor or wrong selection of the initial values could cause some undesired consequences such as increase in the number of iteration which does not minimize RSS, never converging to a solution or converging to a wrong solution. Although the initial value was entered as “1” for the value of each parameter of the KU model, the mentioned problems above were not seen in our study. In addition, the number of iterations did not increase too much. Moreover, we could not find any problem in fitting and the values of RSS and the parameter were not changed.

Table 3 The time to produce (t_p) 25, 50, 75 and 95% of total degradation of the model and the rate of the partial DM disappearance (Rt_p) for t_0 , t_{25} , t_{50} , t_{75} and t_{95} (Mean \pm SE)

Korkmaz-Uckardes model				
-	t_{25}	t_{50}	t_{75}	t_{95}
Rt_0	6.00 \pm 0.32	13.80 \pm 0.75	26.07 \pm 1.42	52.38 \pm 2.86
Rt_{25}	1.20 \pm 0.18	0.86 \pm 0.13	0.47 \pm 0.07	0.10 \pm 0.01

DM: dry matter; t_{25} : time to produce 25% of partial DM disappearance; t_{50} : time to produce 50% of partial DM disappearance; t_{75} : time to produce 75% of partial DM disappearance; t_{95} : time to produce 95% of partial DM disappearance; Rt_0 : the rate of the partial DM disappearance for t_0 ; Rt_{25} : the rate of the partial DM disappearance for t_{25} ; Rt_{50} : the rate of the partial DM disappearance for t_{50} ; Rt_{75} : the rate of the partial DM disappearance for t_{75} and Rt_{95} : the rate of the partial DM disappearance for t_{95} .

Motulsky and Ransnas (1987), Lopez *et al.* (1999) and Korkmaz *et al.* (2011) expressed that there is not a single critical value to assess fitting performance and goodness-of-fit of the model. To express very good performance and goodness-of-fit of the model, several different ways should be evaluated. The main statistical tests used for the models are goodness-of-fit and residual analysis. The criteria of R^2 and AF were used to evaluate the performance and goodness-of-fit of the model.

Lopez *et al.* (1999) explained that if R^2 and AF values are very close to unity, then it indicates that the model

shows a very good fit to data set. Moreover, Branyi *et al.* (1999) expressed that if AF value is close to unity, the predicted and the observed values indicate a perfect agreement. R^2 and AF values of the KU model were very close to unity. According to these results, the KU model has shown a perfect fit to *in situ* data.

Draper and Smith (1981) reported that if the DW value is significant, the errors are not scattered randomly around the zero line and the model did not fit well to the data set. In Table 1, DW values of the KU model are non significant ($P > 0.05$) and the errors are scattered randomly around the zero line. Wang *et al.* (2011) found that DW values were significant in a few times in the used sigmoidal models, the Exponential, the Gompertz, the Logistic and the generalized Mitscherling and Michaelis-Menten models, and the Logistic model modified by them, in their studies. In a similar way, Lopez *et al.* (2004) found that DW values in their study were significant in a few times.

Motulsky and Ransnas (1987) reported that according to analysis of residuals, too few numbers of runs of sign did not show a random distribution of errors. Very few runs indicate the cluster in the same marked errors in some parts of the curve. The numbers of runs of sign of the KU model were given in Table 1. At the same time, according to Draper and Smith (1981), the error sequences of the KU model was non-significant ($P > 0.05$). Lopez *et al.* (2004) found that the runs values were significant in a few times in the sigmoidal models used in their studies.

Pineiro *et al.* (2008) and Wang *et al.* (2011) reported that there was a significant positive correlation between observed and predicted values of the models and the model fit well to the data set. There was also a significant positive correlation between observed and predicted degradation values of the KU model ($P < 0.01$).

The significance of the regression parameters was statistically analysed to test the hypothesis of “intercept= 0” and “slope= 1” according to Pineiro *et al.* (2008). Briefly, perfect agreement between observations and predictions was represented by intercept and slope of 0 and 1, respectively. The results of observed versus predicted regression of DM degradation were given in Table 2 and Figure 1. According to these results, the intercept and slope of the KU model was found to be 0 and 1, respectively. Moreover, there was a significant positive correlation between observed and predicted *in situ* degradation values of the KU model ($P < 0.001$). Wang *et al.* (2011) observed that the goodness-of-fit of some sigmoidal models do not agree with these criteria. This can be related to sigmoidal structure of the models.

The digestion kinetics results of the KU model for *Gleditsia Triacanthos* were shown in Figure 2. Kamalak *et al.* (2012) and Parissi *et al.* (2005) have determined the

digestion kinetics for *Gleditsia Triacanthos* by using Orskov model in their studies. The value of the maximum degradability of the KU model is similar to the result reported by Kamalak *et al.* (2012). The results of the other parameters are not similar because Kamalak *et al.* (2012) reported that they used polyethylene glycol in their studies. They reported that this substance has changed the digestion kinetics of the forage. The values of constant rate of degradation of (a-b) and the potentially degradable fraction (c), reported by Parissi *et al.* (2005), are similar to the results of the KU model. In our study, time to produce 25, 50, 75 and 95% of total degradation of model were slightly higher than time to produce 25, 50, 75 and 95% of total degradation of the exponential model used by Sahin *et al.* (2011). The value of t_{50} in the KU model is compatible with the value reported by Wang *et al.* (2011). However, the studies of these researchers did not include the total degradation times for the general p %, it includes total degradation times for only 50 %. Our estimate for the degradation rate Rt_{50} was slightly higher than the value reported by Wang *et al.* (2011). Also, the study of Wang *et al.* (2011) did not include other digestion rates.

As a result, in addition to the parameters of a model (a, b, c and...) using the KU model, the estimations of " t_p " and " Rt_p " provided more useful data to compare feedstuffs in terms of both *in situ* degradation and *in vitro* fermentation studies. A few researches reported that there was a significant high positive correlation between *in situ* and *in vitro* (Kamalak *et al.* 2005; Canbola *et al.* 2005; Bueno *et al.* 2010). Since the results of *in situ* and *in vitro* studies are compatible with each other, the availability of *in vitro* studies of the model could be investigated.

CONCLUSION

In this study, in addition to the models widely used in *in situ* studies, a new alternative model has been developed in order to minimize the fitting problems. The results indicate that there is no fitting problem and the KU model shows a perfect fit to the data set. Besides, the attainment of some important parameters not included in the model was given. These parameters provided more useful data to compare feedstuffs in terms of *in situ* degradation. In summary, our study indicates that KU model could be used as an alternative model for describing and interpreting *in situ* dry matter degradation from natural feedstuffs. However, different *in situ* measurement studies in wider ranges of feedstuffs could be done to investigate the performance of the KU model. In the future, in *in situ* and *in vitro* studies, the agreement with other algorithms of the KU model can be investigated and also the performance of the KU model could be compared with the performance of other models.

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