

## Comparison of Different Nonlinear Models for Degradability In Situ Dry Matter of Maize Harvested at Various Maturity Stages

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### Research Article

#### Article History:

Received: 06.08.2021

Accepted: 13.10.2021

Published online: 08.03.2022

#### Keywords:

Data Analysis

Degradability

Maize silage

Nonlinear model

The goodness of fit

### ABSTRACT

The present research was managed to propose alternative nonlinear models to describe ruminal degradation kinetics from data get by the incubation in the rumen using the polyester bag technique. For this purpose, data were used from maize silage harvested at three different maturity stages: medium flowering, milk line, and hard dough. Orskov model was compared to nonlinear models: Monomolecular, Mitscherling, Logistics, and Verhulst models. These models were calculated according to  $R_{adj}^2$ , MSE, MAE, MAPE, and BIC which are goodness of fit criterias, and the Logistics and Verhulst models identified the best model in all of the models. Monomolecular and Mitscherling models indicated similar results with Orskov model. As a result, apart from the Orskov Model, it has been shown that Monomolecular, Mitscherling, Logistics, and Verhulst nonlinear models can be used as well for maize silage obtained in different maturity stages using nylon bag technique in animal feeding studies. Additionally, the Logistics and Verhulst models are capable of calculating the time at inflection point and digestion at inflection point are important advantages specific to these two models.

## Farklı Olgunluk Aşamalarında Hasat Edilen Mısırın, in Situ Kuru Madde Parçalanabilirliği İçin Farklı Doğrusal Olmayan Modellerin Karşılaştırılması

### Araştırma Makalesi

#### Makale Tarihi:

Geliş tarihi: 06.08. 2021

Kabul tarihi: 13.10.2021

Online Yayınlanma: 08.03.2022

#### Anahtar Kelimeler:

Veri Analizi

Sindirilebilirlik

Mısır Silajı

Doğrusal olmayan modeller

Uyum iyiliği kriterleri

### ÖZ

Polyester torba tekniği kullanılarak yapılan bu çalışmada, mısır silajının rumendeki inkübasyonundan elde edilen veriler kullanılarak silajların rumende parçalanabilirlik kinetiğininin doğrusal olmayan alternatif modeller önermek için yürütüldü. Bu çalışmada, mısırın orta çiçeklenme, süt olum ve sert hamur olum olmak üzere üç farklı olgunluk döneminde hasat edilmesiyle elde edilen silajların verileri kullanılmıştır. Orskov modeli, Monomolecular, Mitscherling, Logistics ve Verhulst modellerinin yer aldığı doğrusal olmayan modellerle karşılaştırıldı. Bu modeller  $R_{adj}^2$ , MSE, MAE, MAPE ve BIC uyum kriterlerine göre karşılaştırmış ve Logistics ve Verhulst modelleri tüm modellerde en iyi model olarak bulunmuştur. Monomoleküler ve Mitscherling modelleri, Orskov modeli ile benzer sonuçlar vermiştir. Sonuç olarak, hayvan besleme çalışmalarında polyester torba tekniği ile farklı olgunluk aşamalarında elde edilen mısır silajı için Orskov Modeli dışında Monomolecular, Mitscherling, Logistics ve Verhulst nonlinear modellerinin kullanılabileceği gösterilmiştir. Logistics ve Verhulst modellerinin en iyi modelleri olarak seçilmelerinin yanı sıra dönüm noktası zamanını ve değerini hesaplayabilmesi bu iki modele özgü önemli bir avantajdır.

**To Cite:** Hızlı H., Kılıçalp N. Comparison of Different Nonlinear Models For Degradability In Situ Dry Matter of Maize Harvested at Various Maturity Stages. Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi 2022; 5(1):12-21.

## Introduction

Mathematical models are important because biological interpretations can be made on the parameters they contain (Ratkowsky, 1989; Seber and Wild, 1989). The first mathematical model in animal nutrition studies was used on the dry matter digestibility of feeds (Axelsson, 1939). In the following years, mathematical models gained importance in explaining the values obtained from in situ and in vitro methods used in determining the quality of nutritional feed composition. Ørskov and Donald (1979) used the first method developed by modifying the exponential equation to predict the digestibility of the feed proteins in the rumen. One of the most commonly used feed evaluation methods in ruminant feeding is in situ technique (Ørskov et al., 1980). Lo'pez et al., (1999) used fragmentation curves to determine the degradation kinetics of each feed substrate in the rumen. This method (in sacco) is an important method for determination of degradability of roughage and concentrated feeds in the rumen, and to estimate feed consumption and daily live weight gain of animals. But, there are other many methodologies measuring the affect of degradability of feed samples (Nocek, 1988; Huntington and Givens, 1995). Beuvink and Kogut (1993) conducted a study to estimate the digestive parameters of different meadow silages with different models. Chilibroste et al., (1998) compared the in situ with the in vitro technique using the Groot model. France et al., (1998) used Mitscherlich, Generalized Michaelis Menten and Gompertz and Logistic Growth models to define gas production profiles. Macheboeuf and Milgen (1998) compared the models according to the standard errors (MSE) of the models using five models to estimate the organic matter digestibility of feed in horses. Olaisen et al., (2003) used the in situ method to estimate the concentrations of dry matter and protein digestibility and applied the Von Bertalanffy model to the values they obtained. France et al., (2005) interpreted gas production parameters using Gompertz, sigmoidal, Morgan and France models. Tedeschi et al., (2005) emphasized the importance of using mathematical models in accordance with the experimental data of the in situ technique obtained from animal feeding studies in the information obtained about feeds. Nasri et al., (2006) studied the dry matter and crude protein digestibility of soybeans using different mathematical models and calculated which model is better with various criteria of goodness of fit. Hackmann et al., (2008) measured Relative Feed Value (RFV), related to in situ degradation parameters of grass and legume forages, using DM, CP, and NDF degradation data with six alternative nonlinear models by using some goodness of fit criteria such as residual sums of squares (SSRES), residual mean square (MSRES), Akaike's information criterion (AIC) values. Korkmaz and Üçkardeş (2014) presented an alternative model called Korkmaz-Üçkardeş, which has a logarithmic structure to describe the ruminal degradation kinetics of feeds. Üçkardeş and Efe (2014) investigated the use of some different mathematical models at four different legume forage crops, and the goodness of fit of these models to in vitro gas production data using goodness of fit criteria such as  $R_{adj}^2$ , MSE, AF and BIC.

The purpose of this study was to compare the suitability of defining rumen degradation kinetics from maize silage in five nonlinear models named Orskov, Monomolecular, Mitscherlich, Logistic and

Verhulst and to show their usability. The usability of these models were tested to different goodness of fit criterias such as Adjusted Determination of Coefficient ( $R_{adj}^2$ ), Mean Square Error (MSE), Mean Absolute Error (MAE), Mean Absolute Percentage Error (MAPE) and Bayes Information Criterion (BIC). For statistical analyses, one-way analysis of variance and then Duncan multiple comparison test were applied. Thus, this study offers researchers working on different forage crops that the option of using different models can be used other than using the Orskov model.

## **Materials and Method**

### **Material**

#### **Animal material and in situ incubations**

Nylon bag technique was used to determine the degradability of silage samples in the rumen. For this purpose, a rumen fistula (3,5 cm inner diameter) was replaced to the rumens of approximately 500±0,4 kg live weight, three-year old, three Hostein heifers (Ørskov and Donald, 1979). The animals were weighed and placed in individual compartments before digestion trial started and adapted to the trial conditions for three weeks. Feeds given to heifers; 125% of maintenance level and roughage: concentrate feed rate was prepared at the ratio of 60:40. The heifers were fed twice in a day as recommended (NRC, 2007), at 8,30 in the morning and 16,30 in the evening respectively, with a specific feed ration consisting of alfalfa hay and triticale hay mixture (60:40) as roughage, barley as a concentrate feed, and additional mixture of vitamins and minerals. Clean water always was available in front of the heifers. In situ dry matter degradability for each incubation period was calculated by the equations proposed in Table 1.

#### **In Situ Procedure**

Silage samples were dried at 65 °C for 48 hours and then grinded through laboratory hammer mill with a 2,5 mm screen. The 5-gram samples were filled in polyester bags (5x10 cm size and 40-50 microns pore diameter) and then put into the rumen for incubations of 0, 12, 24, 48, 72 and 96 hours to determine DM degradability. The degradation of DM was recorded at each incubation period for each harvesting stage of silages.

### **Method**

Many researchers indicate that the degradation of feeds in the rumen is a function of the length of the feed remaining in the rumen (France et al., 1998; Dhanoa et al., 2007). Table 1 shows Orskov, Monomolecular, Mitscherling, Logistics and Verhulst nonlinear model equations explaining the ruminal degradation kinetics from the data obtained by incubation in the rumen using the polyester bag technique (in situ). In equations (Y), represents digestion at t time, t is incubation time or digestion time, A is parameter total digestion amount, B is the curve asymptote at infinite time, and k rate of degradation of fraction. Time at inflection point (TIP) and Digestion at Inflection Point (DIP) indicate

where the digestion rate is highest. After these points the digestion rate decreases gradually. The ability to calculate TIP and DIP in Logistics and Verhulst models is an important advantage specific to these two models.

**Table 1.** Model expressions, parameters, inflection points of the non linear equations

Models	Equations	TIP (X-axis)	DIP (Y-axis)
Orskov	$Y = A+B(1 - e^{-kt})$	-	-
Monomolecular	$Y=A-Be^{-kt}$	-	-
Mitscherling	$Y=A(1-Be^{-kt})$	-	-
Lojistic	$Y = A/(1+e^{B - kt})$	$\ln(B)/k$	$A/2$
Verhulst	$Y = A/(1-Be^{-kt})$	$-\ln(-1/B)/k$	$A/2$

A, B, k are model parameters; TIP:Time at Inflection Point; DIP:Digestion at Inflection Point; Y: The degradation rate of dry matter. k: rate of degradation of fraction.

Parameter estimates in the models were made with the Levenberg-Marquardt iteration method by using the NLR procedure in the SPSS program (IBM SPSS, 2011). Models were evaluated by performing ANOVA and Duncan's multiple comparison test on the statistical goodness of fit measurements (MSE, MAE, MAPE and BIC) (Waller and Duncan, 1972; Nasri et al., 2006; Chai and Draxler, 2014; Pham, 2019). When comparing models, MSE MAE, MAPE and BIC, those with the smallest value were rated as the best (Kaps and Lamberson, 2004; Pham, 2019). Also, Piłatowska (2011) reported that using  $R_{adj}^2$  in nonlinear models is more appropriate than using the coefficient of determination ( $R^2$ ).

## Results and Discussion

Table 2 shows the mean and standard error of the goodness of fit criterias,  $R_{adj}^2$ , MSE, MAE, MAPE, BIC, calculated using the digestibility values of maize silage, harvested at MF, ML and HD. Orskov, Monomolecular, Mitscherling, Lojistic and Verhulst models compared with each other using the goodness of fit criterias via Duncan's multiple comparison tests. The  $R_{adj}^2$  value was found the highest harvested maturity stages. In the MF and ML 98%, secondly the HD followed 94% in all models. The results suggested that all models can be used for all harvested maturity stages periods. In those stages, in terms of MSE, MAE, MAPE and BIC criteria values, Logistics and Verhulst models were found both small and similar while Orskov, Monomolecular and Mitscherling models were both highest and similar.

**Table 2.** Goodness of fit criteria analysis of variance and Duncun test results of nonlinear models of maize silage harvested at three different maturity stages (Mean  $\pm$  SE)

Models and Harvest Stage	Goodness of Fit				
	$R_{adj}^2$	MSE	MAE	MAPE	BIC
<b>MF</b>					
Orskov	0,98 $\pm$ 0,05	316,14 $\pm$ 115,28 <sup>b</sup>	13,80 $\pm$ 3,00 <sup>b</sup>	2,38 $\pm$ 0,47 <sup>b</sup>	3,56 $\pm$ 078 <sup>b</sup>
Monomolecular	0,98 $\pm$ 0,05	316,14 $\pm$ 115,28 <sup>b</sup>	13,80 $\pm$ 3,00 <sup>b</sup>	2,38 $\pm$ 0,47 <sup>b</sup>	3,56 $\pm$ 078 <sup>b</sup>
Mitscherling	0,98 $\pm$ 0,05	316,14 $\pm$ 115,28 <sup>b</sup>	13,80 $\pm$ 3,00 <sup>b</sup>	2,38 $\pm$ 0,47 <sup>b</sup>	3,56 $\pm$ 078 <sup>b</sup>
Lojistic	0,98 $\pm$ 0,05	227,20 $\pm$ 79,94 <sup>a</sup>	12,46 $\pm$ 2,27 <sup>a</sup>	2,14 $\pm$ 0,33 <sup>a</sup>	3,22 $\pm$ 0,59 <sup>a</sup>
Verhulst	0,98 $\pm$ 0,05	227,20 $\pm$ 79,94 <sup>a</sup>	12,46 $\pm$ 2,27 <sup>a</sup>	2,14 $\pm$ 0,33 <sup>a</sup>	3,22 $\pm$ 0,59 <sup>a</sup>
P value	NS	***	**	*	*
<b>ML</b>					
Orskov	0,98 $\pm$ 0,05	471,63 $\pm$ 132,64 <sup>b</sup>	18,74 $\pm$ 2,93 <sup>b</sup>	3,51 $\pm$ 046 <sup>b</sup>	4,84 $\pm$ 0,76 <sup>b</sup>
Monomolecular	0,98 $\pm$ 0,05	471,63 $\pm$ 132,64 <sup>b</sup>	18,74 $\pm$ 2,93 <sup>b</sup>	3,51 $\pm$ 046 <sup>b</sup>	4,84 $\pm$ 0,76 <sup>b</sup>
Mitscherling	0,98 $\pm$ 0,05	471,63 $\pm$ 132,64 <sup>b</sup>	18,74 $\pm$ 2,93 <sup>b</sup>	3,51 $\pm$ 046 <sup>b</sup>	4,84 $\pm$ 0,76 <sup>b</sup>
Lojistic	0,98 $\pm$ 0,05	439,62 $\pm$ 109,38 <sup>a</sup>	18,13 $\pm$ 2,82 <sup>a</sup>	3,39 $\pm$ 053 <sup>a</sup>	4,68 $\pm$ 0,73 <sup>a</sup>
Verhulst	0,98 $\pm$ 0,05	439,62 $\pm$ 109,38 <sup>a</sup>	18,13 $\pm$ 2,82 <sup>a</sup>	3,39 $\pm$ 053 <sup>a</sup>	4,68 $\pm$ 0,73 <sup>a</sup>
P value	NS	***	*	*	*
<b>HD</b>					
Orskov	0,94 $\pm$ 0,08	1410,38 $\pm$ 371,59 <sup>b</sup>	31,10 $\pm$ 5,63 <sup>b</sup>	6,12 $\pm$ 1,01 <sup>b</sup>	8,03 $\pm$ 1,45 <sup>b</sup>
Monomolecular	0,94 $\pm$ 0,08	1410,38 $\pm$ 371,59 <sup>b</sup>	31,10 $\pm$ 5,63 <sup>b</sup>	6,12 $\pm$ 1,01 <sup>b</sup>	8,03 $\pm$ 1,45 <sup>b</sup>
Mitscherling	0,94 $\pm$ 0,08	1410,38 $\pm$ 371,59 <sup>b</sup>	31,10 $\pm$ 5,63 <sup>b</sup>	6,12 $\pm$ 1,01 <sup>b</sup>	8,03 $\pm$ 1,45 <sup>b</sup>
Lojistic	0,94 $\pm$ 0,08	1385,48 $\pm$ 403,02 <sup>a</sup>	30,00 $\pm$ 5,89 <sup>a</sup>	5,81 $\pm$ 1,12 <sup>a</sup>	7,75 $\pm$ 1,52 <sup>a</sup>
Verhulst	0,94 $\pm$ 0,08	1385,48 $\pm$ 403,02 <sup>a</sup>	30,00 $\pm$ 5,89 <sup>a</sup>	5,81 $\pm$ 1,12 <sup>a</sup>	7,75 $\pm$ 1,52 <sup>a</sup>
P value	NS	**	**	*	*

The meaning of a,b letters should be explained Model Comparison was made within the column and in their own Harvest Stages. a was determined as the 1st group, b as the 2nd group. NS: Non significant; \*: P< 0,05; \*\*: P< 0,01; \*\*\*: P< 0,001; MF: Medium flowering; ML: Milk line; HD: Hard dough.

The mean values of MSE, MAE, MAPE and BIC of the Logistics and Verhuls models in the MF, ML and HD harvest periods were 227,20, 12,46, 2,14 and 3,22; 439,62, 18,13, 3,39 and 4,68; 1385,48, 30,00, 5,81 and 7,75, respectively. Also, avarage values of MSE, MAE, MAPE and BIC at the MF, ML and HD periods were obtained by using Orskov, Monomolecular and Mitscherling models as 316,14, 13,80 and 2,38; 3,56, 471,63, 18,74 and 3,51; 1410,3, 31,10, 6,12 and 8,03, respectively. Each harvest period was compared according to Duncan's multiple comparison test in terms of goodness of fit criteria between the models. Accordingly, the difference between  $R_{adj}^2$  values of all models used for silage maize at different harvest periods was not significant (P>0.05). Likewise, Logistic and Verhulst models according to Goodness of fit all criteria were found significant (P<0.05) in all harvest periods.

Logistic and Verhulst models were observed to be better than Orskov, Monomolecular and Mitscherling models. Üçkardeş and Efe (2014) reported that, The Verhulst model's MSE values and also in some cases the Logistic model's MSE values have had higher values than the Orskov, Verhulst, Janoscheck, Weibull, Bridges, Mitscherling, Monomolecular and Von Bertalanffy models, but they found that those models's MSE values were same to each other. Lopez et al., (1999) and Üçkardeş and Efe (2014) reported that the values of MSE and  $R_{adj}^2$  did not provide a well data set for the comparison among goodness of fit criterias. In addition, Üçkardeş and Efe (2014) reported that Verhulst and Logistic models showed higher performance than other models in terms of goodness of fit in the comparison made with BIC values. Wang et al., (2011) emphasized that using models with low performance in terms of goodness of fit criteria may be misleading the conclusion. According to France et al., (2005) and Calabro et al., (2005), dependence on a few models should be avoided since different degradation curves can be obtained in the case of using different species of ruminants or the amount of organic matter forage material and also, in some situations flexible models may perform better than Orskov model.

### **Comparison of Degradation Parameters**

The nonlinear models used in this study were investigated and analysed comparatively considering the feed degradation processes in the rumen to produce an alternative solution in this topic according to the literature. Table 3 shows the model parameters of degradation of the maize silaj harvested at MF, ML and HD periods calculated by using Orskov, Monomolecular, Mitscherling, Logistic, and Verhulst models.

The highest average value of parameter A (759,80 in the period of MF; 754,99 in the period of ML; 753,15 in the period of HD) were estimated by using the Monomolecular and Mitscherling models. The parameter A calculated with the Monomolecular and Mitscherling models were higher than that of Orskov, Logistic and Verhulst models (Table 3). The highest mean B parameter values (590,07 for in MF period, 618,60 for in ML period and 608,77 for in HD period) were estimated by using the Orskov, Monomolecular models, but values were different in the results of other models; Mitscherling, Logistic and Verhulst (Table 3). The k parameter's means was found the highest in Logistics model for MF, ML and HD periods, similar in Orskov, Monomolecular and Mitscherling models and, it was negative only in Verhulst model. When the mean and standard errors of the parameter values of the models are examined, Parameter A has been observed to be high in most models but Orskov's model (Table 3).

**Table 3.** Degradation parameters of dry matter and Correlation, TIP, DIP of the models

Harvest Stage and Models	Degradation Parameters (g/kg Mean ± SE)			Correlation			TIP	DIP
	A	B	k	r <sub>AB</sub>	r <sub>AK</sub>	r <sub>BK</sub>		
<b>MF</b>								
Orskov	169,72±33,95	590,07±25,41	0,03±0,004	-0,84	-,882	,527		
Monomolecular	759,80±18,48	590,07±25,41	0,03±0,004	-0,18	-,895	,527		
Mitscherling	759,80±18,48	0,77±0,04	0,03±0,004	-0,60	-,895	,838		
Lojistic	737,35±10,52	0,66±0,77	0,05±0,005	-0,33	-,751	,787	-8,31	368,67
Verhulst	737,35±10,52	-1,95±0,15	-0,05±0,005	0,33	,751	,787	-2,32	368,67
<b>ML</b>								
Orskov	136,40±37,96	618,60±27,18	0,02±0,005	-0,70	-,874	,296		
Monomolecular	754,99±27,28	618,60±27,18	0,02±0,005	0,03	-,921	,296		
Mitscherling	754,99±27,28	0,82±0,04	0,02±0,005	-0,62	-,921	,778		
Lojistic	722,80±16,11	0,82±0,10	0,05±0,006	-0,31	-,774	,757	-3,97	361,4
Verhulst	722,80±16,11	-2,27±0,23	-0,05±0,006	0,31	,774	,757	-2,17	361,4
<b>HD</b>								
Orskov	144,38±59,20	608,77±45,43	0,02±0,008	-0,31	-,864	-,173		
Monomolecular	753,15±62,38	608,77±45,43	0,02±0,008	0,43	-,946	-,173		
Mitscherling	753,15±62,38	0,81±0,06	0,02±0,008	-0,60	-,946	,704		
Lojistic	706,95±33,23	0,83±0,17	0,04±0,009	-0,28	-,811	,704	-3,72	353,47
Verhulst	706,95±33,23	-2,31±0,39	-0,04±0,009	0,28	,811	,704	-2,15	353,47

MF: Medium flowering; ML: Milk line; HD: Hard dough, Sig.=significant TIP : Time at inflection point on X-axis; DIP: Degradation at Inflection Point on Y-axis; k: rate of degradation of fraction.

The parameter B and k (degradation rate) was estimated similarly in Orskov and Monomolecular Models in all models. It is seen that alternative models compared to Orskov model can also be used in feeding studies. Correlation between parameters A and B was negatively highest in the Orskov model, correlation between A and k was negatively highest in Monomolecular and Mitscherling, and correlation between B and k was positively and equally highest in the Verhulst and Mitscherling Models. Logistic and Verhulst Models have a turning point time and value. TIP values in the Logistic model for MF, ML and HD periods were found as -8,31, -3,97 and -3,72, respectively, and -2,32, -2,17 and -2,15 in the Verhulst model. Remarkably, those negative results states that degradation of the feed starts before placing in the rumen. And also, the value in the Logistics model shows degradation starting time (TIP) earlier than Verhulst model according to comparison of values in both models. Also, according to TIP values shown on Table 3, Logistic's model reflects better performance than Verhulst model's performance. DIP values were similar for MF, ML and HD periods in both models, 368,67, 361,40 and 353,47, respectively. According to the DIP and TIP results, the best digestion in the shortest time was at the term of MF, ML, and HD, respectively. This results cause a striking inference which is

supported by Gürsoy and Macit (2020) that; as the time goes up after harvesting, degradation speed of plant materials slows down because of the level of their water content, and their digestion capacity decreases.

## **Conclusion**

The maize silage, harvested at three different maturity stages, MF, ML and HD of ruminal degradation kinetics, from data obtained by the incubation in the rumen using polyester bag technique (in situ) showed significantly difference among  $R_{adj}^2$ , MSE, MAE, MAPE and BIC criteria values. Logistics and Verhulst models were found the best models in this study. Further, Logistic model results gave even better results than Verhulst model did. Additionally, the ability to calculate the turning point time and value is an important advantage specific to these two models. Monomolecular and Mitscherling models gave similar results with Orskov model. As a result, it has been shown that Monomolecular, Mitscherling, Logistics and Verhulst nonlinear models apart from the Orskov Model can be used for maize silage obtained in 3 different maturity stages obtained by nylon bag technique in animal feeding studies.

## **Conflict of Interest**

The authors declare that there is no conflict of interest.

## **Authors' contributions**

HH contributed to the project idea, design and writing of the study. NK was responsible for the field trials and laboratory analysis, and the article was reviewed by all authors.

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