**In situ** ruminal degradation of sallow tree leaves using different mathematical models

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**ABSTRACT**

**Objective.** Sallow leaves are the leaves of sallow trees that fall in the fall, and their disposal represents an environmental problem since they are potential pollutants. One of their most promising alternatives uses feedstuffs in ruminant diets. **Materials and methods.** Four mathematical models were used to describe the ruminal disappearance of dry matter (DM) and crude protein (CP) of sallow leaves: non lagged exponential (Model I); lagged exponential (Model II); Gompertz (Model III), and generalized Mitscherlich (Model IV). **Results.** Results of DM and CP degradability characteristics showed that all the models fitted well ($r^2>0.98$) to the disappearance data. There were minor differences between the models in terms of statistical evaluations. However, the models differed in the estimated parameters depending on the model’s nature and structure and the parameters included. **Conclusions.** Given that some models estimated negative values for the studied parameters, they were not biologically acceptable. For this reason, the only model I can be used for estimating the degradability of DM and CP of sallow leaves. In terms of effective degradability (ED) of DM, model III was not used in the calculations because of calculating negative values for part A. Sallow leaves constitute the largest possible proportion of the ruminal degradability fractions, and effective degradability can be used as a forage source in ruminant feeds.

**Keywords:** Degradability; In sacco technique; mathematical models; sallow leaves (Source: USDA).

**RESUMEN**

**Objetivo.** Las hojas cetrinas son las hojas de los árboles cetrinos que caen en el otoño y su eliminación representa un problema ambiental ya que son contaminantes potenciales. Uno de sus usos alternativos más prometedores es como pienso en dietas para rumiantes. **Materiales y Métodos.** Se utilizaron cuatro modelos matemáticos para describir la desaparición ruminal de materia seca (MS) y proteína cruda (PC) de hojas cetrinas: exponencial no rezagado (Modelo I); exponencial rezagado (Modelo II); Gompertz (Modelo III) y Mitscherlich generalizado (Modelo IV). **Resultados.** Los resultados de las características de degradabilidad de DM y CP mostraron que todos los modelos se ajustaban bien ($r^2>0.98$) a los datos de desaparición y había pequeñas diferencias entre los modelos en términos de...
INTRODUCTION

The usability and cost of commercial feedstuff are major problems for ruminant farms. Accordingly, it is necessary to replace cost-effective alternative ingredients with high nutritional and protein value (1,2). Agricultural by-products such as sesame meal can have a major impact on reducing production costs (3,4). Plants leave such as Sallow can be used as a qualified and cheap protein source (5) for ruminants. Tree leaves such as Sallow (Salix Alba), available in the country, can be used as a low-priced feed source. Still, they need to have enough information about the nutrients and the availability of these substances.

Ruminal fermentation particularity of feedstuffs can be studied using in vivo, in situ, and in vitro techniques. Dacron polyester or in sacco technique has been used widely for estimating ruminal nutrient degradation because it is a relatively simple, low-cost method compared with other methods. The Nylon-bag technique provides a useful means to estimate rates of disappearance and potential degradability of feedstuffs and feed constituents (6).

It is well-established that feed contents play the most important role in the animal products and longevity of organisms and environmental concerns. Various methods have so far been suggested to estimate the proportion of good and bad contents of feeds. But if digestible feeds are identified, and their degradation curves are plotted, it will provide more useful information for judging feeds. Ideally, a function is required to model both a range of shapes with no inflection point and a range of sigmoidal conditions in which the inflection point is variable. Food security is of global concern thus more attention shall be given to the identification of alternative sources. To combat and or to marginally prevent the issue, human edible grains can be excluded from the livestock feed. These grains can be substituted by nutrient rich process discards and or by-products such as tree leaves. The present study aimed to figure out the in situ digestion parameters of sallow leaves (Salix Alba) as an alternative fibrous feedstuff, not commonly used in animal diets, using different mathematical models to identify the best model fitting the data.

MATERIALS AND METHODS

Sample collection. Sallow leaves were harvested in mid-August from the city of Bonab in the East-Azarbaijan of Iran. Afterward, the dried leaves were milled in a Willy mill through a 2 mm Screen sieve for chemical analysis and in situ degradability assays.

In situ degradation. Two yearlings ruminally cannulated wethers (35±1.8kg) were used for in situ degradability experiment. The chemical composition and nylon bag data were given in the previous article (5). Since the mathematical models and disappearance curves of the ruminal degradation have been studied in this study, I will explain these methods.

Mathematical models. Digestion models, due to their dependence on the nature of the feed, consist of sequential equations, a linear distribution, and a curve of differential and integral equations that should be used to show the type of digestion process (7,8).

Models I and II are first-order kinetics models without and with a lag phase, respectively. Model III is Gompertz curve. Model IV is Generalised Mitscherlich, the model I (results in the model I for d=0), with a square root time dependence component (9,10,11). The models that we used included:

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Palabras clave: Degradabilidad; técnica in sacco; modelos matemáticos; hojas cetrinas (Fuente: USDA).
First-order kinetics model without lag phase
\[ P = a + b \left(1 - e^{-ct}\right) \]

First-order kinetics model with lag phase
\[ P = a + b \left(1 - e^{-c(t+L)}\right) \]

Gompertz model
\[ P = a + b \left(K - \frac{K^{exp(-ct)}}{K - 1}\right) \]

Generalised Mitscherlich model
\[ P = a + b \left(1 - e^{-c(t-L)-d(\sqrt{t}-\sqrt{L})}\right) \]

where, \( Y \) = the quantity of either DM or CP, \( a, b, c, d \) and \( k \) = parameters to be estimated, and \( t = \) time.

The DM and CP degradability data were fitted to each model by nonlinear regression using the Levenberg-Marquardt procedure of MATLAB (2019a). Nowadays, optimization methods are widely used in various sciences (12,13); for this purpose, I used the MATLAB curve fitting toolbox and the numerical algorithm based on the Levenberg-Marquardt method. The models were identified through the editor toolstrip, and the starting points and ranges required for the models were defined. I used the goodness of fit measure function to measure the fit curves’ error values in the studied models. The mathematical model fitting stopped when changing in residuals less than tolerance.

**Effective degradability.** ED was calculated according to Ørskov et al (14) equation:

\[ ED = a + \frac{bc}{(c+k)} \]

where ‘a’, ‘b’ and ‘c’ are the constants as described earlier in the different mathematical models above and ‘k’ is the rumen fractional outflow rate (0.02/h, 0.03/h, 0.04/h, 0.05/h, or 0.06/h).

**RESULTS**

**Statistical Models Output.** The different digestible models on the DM and CP degradability of sallow leaves were presented in Tables 1 and 2. Comparing various fitted models for DM degradability of sallow leaves based on the coefficient of determination (\( r^2 \)) and Adjusted (\( r^2 \)) showed that models I and IV fitted the best. In our study, model IV showed the highest goodness parameters to selecting. The process of fitting continued until a change in residuals less than tolerance and then fitting stopped. According to the fitted model (model IV), there was a 3.156-hour lag time in degradability; it can be said that the degradability of sallow leaves takes 3.156 h to be started, and this may be due to the formation of secondary parts of the cell wall in the leaves, leading to an increase in structural compounds and enhanced lignin content which hinders the launch of microbial degradation.

**Table 1.** Estimated DM degradability parameters of sallow leaves using different mathematical models with MATLAB.

| Parameter | a | b | c | L | d | k | SSE | R-Square | Adj R-Square | Iter |
|-----------|---|---|---|---|---|---|-----|----------|-------------|------|
| Model I\(^3\) | 19.48 | 52.29 | 0.0529 | - | - | - | 41.426 | 0.9892 | 0.9869 | 5 |
| Model II | 20.86 | 50.92 | 0.0529 | 0.0267 | - | - | 41.426 | 0.9892 | 0.9852 | 5 |
| Model III | -196.1 | 51.63 | 0.0578 | - | - | 0.7732 | 46.014 | 0.9881 | 0.9836 | 109 |
| Model IV | 27.94 | 32.75 | 0.0632 | 3.156 | 0.0465 | - | 34.412 | 0.9911 | 0.9860 | 8 |

\( a = \) rapidly soluble fraction (%); \( b = \) slowly degradable fraction (%); \( c = \) degradation rate constant (%/h) of fraction ‘b’; \( L = \) lag time (h); \( d = \) is the parameter pertaining to the variable fractional rate of degradation; \( k = \) slope, or degradation rate coefficient (h\(^{-1}\));

SSE = Sum of Squares Due to Error; R-Square= the square of the correlation between the response values and the predicted response values; Adj R-Square= Degrees of Freedom Adjusted R-Square; Iter = iteration number of MATLAB.

Model I: First-order kinetics model without lag phase; Model II: First-order kinetics model with lag phase; Model III: Gompertz model; Model IV: Generalised Mitscherlich model.
Table 2. Estimated CP degradability parameters of sallow leaves using different mathematical models with MATLAB.

| Parameter | SSE  | R-Square | Adj R-Square | Iter |
|-----------|------|----------|--------------|------|
| a         | 36.47| 45.53    | 0.0459       | -    |
| b         | 25.95| 56.04    | 0.0506       | -    |
| c         | -142 | 44.90    | 0.0566       | -    |
| L         | 39.54| 41.44    | 1.516        | -    |
| d         | 38.098| 38.089  | 0.9867       | 0.9867| 0.9818| 0.9811| 109 |
| k         | 38.098| 38.089  | 0.9867       | 0.9867| 0.9818| 0.9811| 109 |
| Model I   | 5    | 5        | 5            |      |
| Model II  | 5    | 5        | 5            |      |
| Model III | 5    | 5        | 5            |      |
| Model IV  | 5    | 5        | 5            |      |

a = rapidly soluble fraction (%); b = slowly degradable fraction (%); c = degradation rate constant (%/h) of fraction ‘b’; L = lag time (h); d = the parameter pertaining to the variable fractional rate of degradation; k = slope, or degradation rate coefficient (h⁻¹);
SSE = Sum of Squares Due to Error; R-Square = the square of the correlation between the response values and the predicted response values; Adj R-Square = Degrees of Freedom Adjusted R-Square; Iter = iteration number of MATLAB.

Model I, First-order kinetics model without lag phase; Model II, First-order kinetics model with lag phase; Model III, Gompertz model; Model IV, Generalised Mitscherlich model.

Effective Degradability. Table 3 shows the sallow leaves’ main effect for effective degradability (ED) at the five rates of passage considered (0.02/h, 0.03/h, 0.04/h, 0.05/h, or 0.06/h). The ED declined as passage rates increased because if the passage rates increased, the rumen microorganisms would not have enough time to affect the feed. According to the results, model II showed a higher amount of dry matter effective degradability, but the amount of effective degradability for the crude protein in model IV was greater. This is due to the different behavior of different models for the degradability of dry matter and crude protein.

Table 3. Estimated effective degradability (ED) of dry matter and crude protein of sallow leaves using different mathematical models.

| DM | k=0.02 | k=0.03 | k=0.04 | k=0.05 | k=0.06 | k=0.02 | k=0.03 | k=0.04 | k=0.05 | k=0.06 |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Model I | 57.43 | 52.85 | 49.26 | 46.36 | 43.98 | 68.18 | 64.01 | 60.80 | 58.26 | 56.21 |
| Model II | 57.81 | 53.36 | 48.96 | 47.04 | 44.72 | 64.98 | 59.84 | 55.90 | 52.78 | 50.24 |
| Model III | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| Model IV  | 52.82 | 50.15 | 48.00 | 46.23 | 44.74 | 68.51 | 64.72 | 61.81 | 59.50 | 57.63 |

DM = effective ruminal degradability of dry matter; CP = effective ruminal degradability of crude protein; k = the rumen fractional passage rate.
Model I: First-order kinetics model without lag phase; Model II: First-order kinetics model with lag phase; Model III: Gompertz model; Model IV: Generalised Mitscherlich model.

DISCUSSION

Statistical Models Output. Comparing different models for estimating ruminal CP degradation parameters of sallow leaves revealed that models I and II reported by Ørskov and McDonald (11) reach convergence. In contrast, other models were not biologically acceptable because of estimated negative values (Table 3). Within the fitted models, the model I can choose is the best model, given the higher amount of Adjusted (r²).
If we are considering models I and II, we can see that the terms of the lag phase, significant changes in “a” and “b” fractions, because in the specified range, the slope of the curve were changed.

Yanez Ruiz et al (15) reported that the Olive lives DM rapidly soluble fraction (a) and a slowly degradable fraction (a) were 28.2 and 45.2 in goat respectively, and in sheep achieved parameters were 29.2 and 24.0 respectively that was similar to our data. Still, there results for ruminal degradability of CP for both animal varieties were lower than our achievements. Obtained data for DM degradability were higher than Elahi and Rouzbehah (16).
The results obtained in the present study for CP degradability parameters were in consist with Waghorn et al (17) reported data.

**Effective Degradability.** The ruminal biodegradability of CP affects the efficiency of nitrogen use for microbial protein synthesis. Starch fermentation rates can also affect ammonia consumption by altering the energy supply for microbial growth. It can be concluded that any of the evaluated models can be used to estimate DM’s degradability, while only models I and II can be used for estimating the degradability of CP of sallow leaves. However, considering the tested models’ similar performance, the biological characteristics of the models should be taken into account to implement the estimated parameters for practical use. Besides, sallow leaves may be used in ruminant rations as an alternative feed source to roughage. But, more in vivo studies together with in situ and in vitro researches are required to determine the actual nutritive value of tree leaves for ruminant animals.

In conclusions it can be concluded that only models I and IV can be used for estimating the degradability of DM and CP of Sallow leaves. However, considering similar performance of the tested models, the biological characteristics of the models should be taken into account in order to implement the estimated parameters for practical use. In addition, Sallow leaves may be used in ruminant rations as an alternative feed source to roughage. Nevertheless, more in-vivo along with in-situ and in-vitro studies are required to determine the actual nutritive value of Sallow leaves for ruminant animals.

**Conflict of interest**

The author declares that he has no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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