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In situ degradability of dry matter from four forage protein plants: *Erythrina variegata, Leucaena leucocephala, Moringa oleifera* and *Neonotonia wightii*

Degradabilidad *in situ* de la materia seca de cuatro plantas proteicas forrajeras: *Erythrina variegata*, *Leucaena leucocephala*, *Moringa oleifera* y *Neonotonia wightii*

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To determine the *in situ* degradability of dry matter of: *Erythrina variegata, Leucaena leucocephala, Moringa oleifera* and *Neonotonia wightii* (Glycine), two cannulated male sheep of Pelibuey breed, weighing 36 kg, were used. Incubation times of 0, 8, 16, 24, 36, 48 and 72 hours were used and the rumen bag method was applied. The trend in ruminal degradability was increasing in the order: Glycine, Leucaena, Erythrina and Moringa. For Moringa, a higher value of ruminal degradability was obtained at 72 hours, greater than 80 %, as well as effective degradability (59.35 %). It is concluded that, although the plants are promising for ruminant feeding, Moringa showed superior indicators in the parameters of ruminal degradation and *in situ* degradability of dry matter. Ruminal degradability studies are required that relate cutting frequency and secondary metabolite effects.

Key words: *chemical composition, legumes, nutritional value, ruminal degradability, trees*

Para determinar la degradabilidad *in situ* de la materia seca de: *Erythrina variegata, Leucaena leucocephala, Moringa oleifera* y *Neonotonia Wightii* (Glycine) se utilizaron dos ovinos machos canulados de la raza Pelibuey, con peso de 36 kg. Se utilizaron tiempos de incubación de 0, 8, 16, 24, 36, 48 y 72 horas y se aplicó el método de la bolsa en rumen. La tendencia de degradabilidad ruminal fue en incremento en el orden: Glycine, Leucaena, Erythrina y Moringa. Para Moringa, se obtuvo mayor valor de degradabilidad ruminal a las 72 horas, superior a 80 %, así como de degradabilidad efectiva (59.35 %). Se concluye que, aunque las plantas son promisorias para la alimentación de rumiantes, la Moringa presentó indicadores superiores en los parámetros de la degradación ruminal y degradabilidad *in situ* de la materia seca. Se requieren estudios de degradabilidad ruminal que relacionen la frecuencia de corte y efectos de metabolitos secundarios.

Palabras clave: *arbóreas, composición química, degradabilidad ruminal, leguminosas, valor nutritivo*

The biomass of trees and legumes plants has played an important role in ruminants feeding in recent years. Legumes forage plants have proven to be a favorable option to complement ruminant feeding. However, there are other plants with potential for foliage production, which have nutritional value comparable to legumes, such as *Moringa oleifera* (Gutiérrez *et al.* 2015). Other factors, such as environmental factors, influence the bromatological composition and nutritional value of plants, such as soil and climate conditions and different varieties, although there is still a lack of information to determine the nutritional value of different species.

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Studies of ruminal degradability infer the quality of nutrients in food. Currently, the *in sacco* method has been the most effective method to study food degradation within the rumen (Elizondo-Salazar and Monge-Rojas 2020 and Meza-Bone *et al.* 2022). The nylon bag technique for measuring the ruminal degradability of food samples remains highly useful in evaluating plant species with potential for ruminant feeding. The objective of this study was to determine the *in situ* ruminal degradability of dry matter (DM) from the plants *Erythrina variegata* (Erythrina), *Leucaena leucocephala* (Leucaena), *Moringa oleifera* (Moringa) and *Neonotonia Wightii* (Glycine).

The study was conducted at the Dr. Juan Tomás Roig Experimental Station of the Bioplant Center, belonging to Máximo Gómez Báez University of Ciego de Ávila.

Collection and processing of samples: The different foliage samples were collected in areas of Ciego de Ávila municipality, in livestock farms and at the University of Ciego de Ávila.

Composite samples of foliage from four protein plants were random collected: Erythrina, Leucaena, Moringa and Glycine. In each case, leaf-petiole samples were taken. They were dried at 65°C in a forced-air oven for 48 h.

The milling of all samples was performed to obtain particle sizes between 2.5 and 3 mm for ruminal degradability analyses and between 0.8-1 mm for chemical DM analyses. In all cases, a hammer mill with its corresponding sieves was used and the processed material was properly preserved until use.

Animal management and diets: Samples from two male Pelibuey sheep, with live weight of 36 kg and cannulated in the dorsal rumen sac were taken. The animals grazed in an area dominated by the creeping legume *Teramnus labialis* and had free access to water and common salt.

Procedure for separating soluble and insoluble fractions of foliage: Between 1-2 g of DM sample was placed in a beaker with 150 mL of distilled water at room temperature (20-25 °C) for 105 min, with intermittent stirring. The solution was filtered through a Whatman No. 1 filter paper (15 cm diameter) and the residue was washed with distilled water until approximately 500 mL of the filtrate was collected. The filter paper, containing the insoluble residues, was placed in an oven at 60 °C for 48 h. The paper with the dry residue was quickly transferred to a previously weighed plastic bag and was hermetically sealed. The bag was weighed after one hour and washing losses were calculated by weight difference.

In situ ruminal degradability. The determination of the DM ruminal degradability of the evaluated plant materials was carried out according to the nylon bag procedure or *in situ,* described by Mehrez and Ørskov (1977). A total of 5 g of sample of each collected plant material (Erythrina, Leucaena, Moringa and Glycine) were weighed in duplicate for each incubation time and animal. For this purpose, Ankom® Dacron/polyester nylon bags (ANKOM Technology) were used, with a pore size of 50±10 µm and internal dimensions of 10 x 20 cm, previously weighed and properly identified.

The bags were closed and immersed for 30 seconds in clean water, before their incubation in the rumen, in order to prehydrate them and generate the correction factor for particle escape at 0 h. They were introduced sequentially into the ventral compartment of the rumen of the animals, cannulated at 0, 8, 16, 24, 36, 48 and 72 h, and then they were simultaneously removed. For this purpose, a polyester bag attached to a 50.0 cm long nylon rope was used.

Then, each bag was manually washed with running water until the water ran clear. They were placed on aluminum trays and dried for 48 h at a temperature of 60 °C in a forced-ventilation oven. Later, they were transferred to a desiccator for 30 min and weighed on a precision balance to determine the DM disappearance. The difference between the initial weight of the sample placed in the nylon bags and the weight of the residue after ruminal incubation was used to determine the DM degraded in the rumen.

The DM was determined according to AOAC (1995). Residual DM contents were determined in triplicate by drying 2 g of sample at 105 °C for 24 h.

Data processing and statistical analysis: To determine the degradative characteristics, the exponential model proposed by Ørskov and Mc Donald (1979) was used. It was assumed that the DM degradation curve (DMD) over time follows a first-order kinetic process described as follows:

$$P = A \quad for \quad t_0 = 0$$

$$P = a + b\left(1 - e^{-ct}\right) \; t > t_0$$

where:

P: Ruminal degradation. Is the ruminal degradation of the indicator evaluated over the time (t) it remains in the rumen

a: Intersection of the degradation curve at time zero. Represents the rapidly degrading component

b: Potential degradability of the component at time t

e: Base of natural logarithms (2.71828)

c: Constant rate of degradation of fraction (b)

a + b: Total degradability of the component

t: Incubation time

A: Rapidly soluble fraction. Obtained by incubating the sample in a water bath at 39 °C for 30 minutes

To determine the effective ruminal degradability (ED), the McDonald model (1981) was used.

$$ED = A + \left(\frac{B \; c}{c + k}\right)$$

where:

ED: Effective ruminal degradability *in situ*

k: Fractional ruminal passage rate. k = 0.02 is assumed

B: Insoluble but potentially degradable fraction. *B=(a+b) -A* (Ørskov 2002)

c: Degradation rate of the fraction

For the mathematical estimation of the DM degradation kinetics of each of the materials, the NEWAY EXCEL program (Chen 2000) was used.

The parameters of ruminal kinetics and DM degradability of the plant materials evaluated (table 1) show that the effective degradability of DM maintained an increasing trend in the following order: Glycine, Leucaena, Erythrina and Moringa. The creeping legume Glycine had values below 50 %, while the rest of the plant materials were between 53.81 and 59.35 %, with the highest value for Moringa.

Table 1. Parameters of ruminal kinetics and effective degradability of DM from plant materials of protein plants

Parameters	Moringa	Erythrina	Leucaena	Glycine
A (%)	37.95	35.97	34.8	30.10
B (%)	52.55	52.53	51.78	45.15
A+B (%)	90.50	88.50	86.58	75.25
c (Fraction h^{-1})	0.081	0.075	0.072	0.063
R^2	0.99	0.97	0.99	0.98
ED (%) k: 0.02	59.35	56.00	53.81	45.35

A: soluble fraction, B: insoluble but potentially degradable fraction, A+B: potential degradability, c: degradation rate of fraction B, R^2: coefficient of determination belonging to the model, ED: effective ruminal degradability.

The model used in the analysis showed high coefficients of determination (R^2) for each of the plant materials. The values of the soluble fraction (A) also showed a tendency to increase in the same order as the mentioned plant materials. The potentially degradable fraction (B) presented similar values in Moringa, Erythrina and Leucaena, while in Glycine it was less than 50% of the DM. Likewise, the potential degradability (A+B) showed the same trend in the plant materials as the soluble fraction (A). Moringa highlighted with 90.5% potential degradability and Glycine with the lowest value.

The evolution of the disappearance of DM after *in situ* ruminal incubation (figure 1) showed an increase as the incubation time increased for plant materials. Moringa showed higher ruminal degradability values than the rest of the plants at each hour of evaluation. The trend of ruminal degradability of plant materials was increasing in the following order: Glycine, Leucaena, Erythrina and Moringa. For the latter plant, a ruminal degradability of more than 80 % was obtained at 72 h, while Glycine showed a value less than 60 %.

Degradation kinetics showed an increase in the disappearance of DM with the incubation time in the rumen. Likewise, the fit of the *in situ* ruminal degradability data to the proposed model, $P = a + b (1 - e^{-ct})$ showed R^2 equal to or higher than 97.0 for the studied plants. This shows a correct fit of the degradation values to the model (table 1). The above explains the degradative performance of these protein plant materials

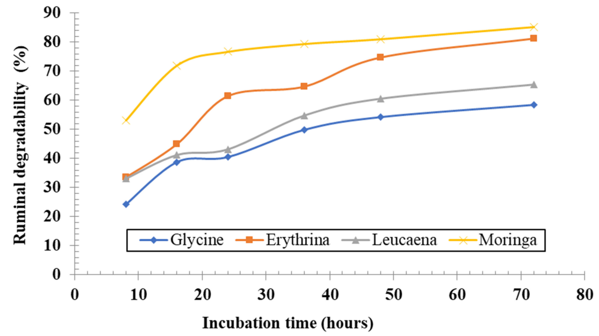

Figure 1. *In situ* ruminal degradability of the DM of protein plants

and shows that the structural compounds of the cell wall did not prevent the action of ruminal cellulolytic microorganisms (Gutiérrez *et al.* 2015).

La O *et al.* (2018) with *Gliricidia sepium* forage, from a saline and high drought ecosystem, found values of effective ruminal degradability of DM between 63.1 and 67.6 % for different ruminal turnover constants. Likewise, the values of the soluble fraction A) 40.7 % and insoluble fraction B) 36.76 % differed from those found in the evaluated plants (table 1). However, the A+B fraction of Moringa, Erythrina and Leucaena was superior to *Gliricidia sepium*, according to La O *et al.* (2018). Apparently, these differences can be attributed to the different proportions of soluble carbohydrates and the bromatological composition, as well as other factors related to edaphoclimatic conditions.

Valenciaga *et al.* (2018) found different results in the degradability parameters of plant materials of *Tithonia diversifolia*, with lower values in the "a" fraction and higher values in the "b" fraction, compared with the evaluated plants (table 1). The ruminal degradability results of the protein plants in this study may have been related to their chemical composition (soluble carbohydrate content), which favored higher degradability of the Moringa dry matter at 72 h. The ruminal fermentation of these plants could promote greater availability of compounds such as ammonia, amino acids, peptides and branched short-chain fatty acids, which favors the synchronization of nitrogen and energy for the microorganisms of the rumen (Valenciaga *et al.* 2018). Gutiérrez *et al.* (2024) confirm the benefits in ruminal degradability when mixing ryegrass (*Lolium perenne*) and two species of clovers (*Trifolium repens* and *T. pretense*). These authors found that the mixture with 70 % ryegrass and 30 % clover had a potential degradability (AB) between 90 and 96 %, results similar to the potential degradability of Moringa in this study.

The results of effective DM degradability (table 1) differ from most tropical grasses, which have values lower than 40 % (Ascencio-Rojas *et al.* 2019). In this sense, better environmental conditions and ruminal degradability of forages can be achieved when shrub plants with higher protein levels than grass forages are included (Meza-Bone *et al.* 2022). Other authors determined higher DM degradability when the protein level was increased with the incorporation of legume fruits (Sosa-Pérez *et al.* 2023).

The obtained results are related to the chemical composition of the studied plants. The protein content can be considered to be in a higher range, in relation to forage grasses used for ruminants feeding. Furthermore, with high levels of soluble carbohydrates, which indicates a possible advantage of its use in ruminants, since with the amount of non-structural carbohydrates, a more efficient use of the degradable protein in the rumen is expected (Valenciaga *et al.* 2018).

The results of the ruminal degradation kinetics (figure 1) agree with other studies in non-legumes plants, such as those of Ascencio-Rojas *et al*. (2019), who obtained 77 % degradation for *Moringa oleifera*, Choque-Durand *et al.* (2018) found 70 % for *Erythrina sp*. Gutiérrez *et al.* (2015) obtained *in situ* DM degradability of 71.26 % in Moringa samples in a mixture of *Cenchrus purpureus* with Moringa (40:60), degradability of 69.79 % and 67.29 % degradability with 40 % Moringa and 60 % *Cenchrus purpureus*.

It is concluded that Moringa showed superior indicators in the parameters of ruminal degradation, such as in the *in situ* degradability of DM, followed in descending order by Erythrina, Leucaena and Glycine. However, they show promise for use in ruminant feeding. Studies on ruminal degradability are needed to determine the relation between cutting frequency and the effects of secondary metabolites.

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