

# Tropical Legumes Preserved in Silage and Hay Form and Its Nutritional Values in Laboratory Conditions

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### Abstract

This study aimed to evaluate the nutritional value of legumes leucaena and desmodium preserved in hay and silage form. We used a completely randomized design in a 2x2 factorial arrangement with two bulky conservation methods (hay and silage) and two legumes (leucaena and desmodium). For the bulky characterization was determined chemical composition and total digestible nutrients (TDN). For assessing the nutritional value, analysis of in vitro dry matter digestibility (IVDMD) and cell wall digestibility (IVCWD), and in situ effective degradability of dry matter (EDDM) and crude protein (EDCP) were performed. Data were submitted to ANOVA and Tukey test at 5% probability. Due to the characteristics of each specie leucaena presented a higher crude protein (CP) and TDN content compared to desmodium and lower fibrous fractions. The IVDMD presented interaction between the conservation form and forage (P<0.05), where leucaena hay was higher than the others treatments. The IVCWD also presented interaction (P<0.05), and the best values were obtained for leucaena silage. EDDM was superior in leucaena compared to desmodium (P<0.05). Regarding the method of preservation, the EDDM was higher in hay compared to silage. For the EDCP there was an interaction between conservation method and forage, where leucaena silage and hav were similar (P < 0.05), but higher than other treatments. Thus, leucaena has a nutritional value higher than desmodium and the conservation of legumes in the hay form presented a higher nutritional value to the conserved as silage.

Keywords: bulky, conserved forage, degradability, digestibility, protein



# 1. Introduction

Tropical legumes, due to their particularities, provide biological cover and nitrogen fixation in the soil, reducing erosion and weed infestation, as well as improving animal feeding due to its high protein content and higher digestibility when compared to grasses (Teixeira et al., 2010). Thus, legumes are alternatives for many areas with tropical pasture.

Among the so-called woody plants, leucaena (*Leucaena leucocephala*) highligths for adapting in tropical regions, presenting arboreal structure and producing large amounts of edible biomass (Possenti et al., 2008). In addition, it presents function for multiple forms of use, has high acceptability by the animals and excellent ability to regrow (Costa et al., 2011). However, some anti-nutritional factors present in leucaena can affect digestion in ruminants, such as tannin and mimosine (Makkar, 2003; Aganga and Tshwenyane, 2003).

According to Barreto et al. (2010) leucaena presents in its leaves around 10.15% of tannin. Despite this high tannin level, studies have shown that there is no negative effect of tannins on the production of microbial protein (Makkar, 2003). Possibly, the tannin present in these legumes is more harmful to fibrolytic bacteria than to proteolytic bacteria (Makkar, 2003). In vitro digestibility of leucaena obtained by Manella et al. (2002) were 57.2% and 61.5% for the period of water and drought, respectively, which can be considered high values despite high tannin levels. Another antinutritional factor present in leucaena is mimosine, a toxic substance that causes symptoms such as hair loss in ruminants not adapted to consuming it (Almeida et al., 2006), therefore, the use of leucaena in ruminants diets should not exceed 30% for unadapted animals (Aganga and Tshwenyane, 2003).

Desmodium is a legume whose genus has more than 600 species, among which *Desmodium ovalifolium*, highlights having excellent adaptation to different soils and climates and provides improvements in soil conditions (Barrios-Maestre et al., 2011).

However, its use in livestock has high tannin contents which affects animals intake and digestibility (Cruz, 1993). According to Abaunza et al. (1991) the tannin content found in *D. ovalifolium* CIAT-350 is around 17%.

In Brazil, a study evaluating several desmodium genotypes was performed in the state of Rondônia, and was observed that *D. ovalifolium* CIAT-350 was distinguished by its productivity in the rainy season, reaching a yield of 17.3 tons of dry matter (DM) per hectare (Costa and Oliveira, 1999). However, the genotype that was most outstanding by crude protein (CP) levels was *D. heterophilum* CIAT-349 with 15.2% CP. The *D. ovalifolium* CIAT-350 was in an intermediate position, with 12.0% CP. The IVDMD of these genotypes ranged from 50.3% for *D. heterophilum* CIAT-3782 to 38.7% for *D. ovalifolium* CIAT-350 (Costa and Oliveira, 1999). Macedo et al. (2010) obtained values of in vitro organic matter digestibility of 47.9% for *D. ovalifolium*.

Leucaena and desmodium are legume fodders that can be used to feed ruminants as pastures and in the form of conserved fodder, becoming a protein bank for feed shortage periods. In this context, the objective was to evaluate the nutritional value of leucaena and desmodium conserved as hay and silage.



### 2. Material and Methods

The legumes used in this experiment for the production of hay and silage were established at the Experimental Farm of Iguatemi of the State University of Maring á Paran á whose analysis of podozoic soil dark red was with the following conditions: pH in water 5.8; Al +++ 0.00; Ca +++ 2.1 and Mg 0.05; K +++ 0.12 cmoL/m<sup>3</sup>; P = meq/dm<sup>-3</sup>, at the implantation of these legumes. For the planting of leucaena (*Leucaena leucocephala*), seeds were scarified with water at 50 °C for 10 min. Then, planted with spacing between plants of 1.5 m, totaling five plants / m linear, in an area of 96 m<sup>2</sup>, divided into 5 sub-areas of 19.2 m<sup>2</sup>. Desmodium (*Desmodium ovalifolium*) CIAT-350 seeds were obtained scarified and were planted without inoculation with spacing 25 cm between plants, in an area of 20 m<sup>2</sup>, divided into 5 sub-areas of 4 m<sup>2</sup>.

The cutting of legumes occurred in late January. From leucaena were harvested the leaves and stems of plants and for desmodium cut was performed at 6 cm above the soil, before the beginning of the flowering phase. The materials were harvested from five uniform sub-areas for both legumes and separated into two fractions of each sub-area, one for hay making and one for silage making. The materials destined to hay production remained exposed to the sun for 18 hours on a tarp and were then turned over. After drying, these materials were packed in paper bags until the moment of laboratory analysis. These procedures, from harvesting and drying, were performed in each of the five sub-areas to obtain the replicates.

The silage materials (five replicates) were pre-wilted for a period of 4 h, then chopped ( $\pm 2$  cm). Subsequently, approximately 3 kg of the material was ensiled in PVC laboratory silos. At the time of ensiling, acetic acid was added in a ratio of 6 mL + 20 g of corn meal per kg of ensiled green matter.

The opening of silos occurred 30 days after ensilage. Immediately after opening the pH was determined according to Cherney and Cherney (2003). Ammonia nitrogen content [NH<sub>3</sub>-N (% of total N)] was determined according to AOAC (1980). Samples of silages and hay were oven dried at 55  $\,^{\circ}$ C for 72 h, divided into two parts, one part was milled in a knife mill with a 1 mm sieve for laboratory analysis and another part was milled in knife mill equipped with a 4 mm sieve screen to determine in situ degradability of DM and CP.

Laboratory analysis for silage and hay characterization including DM, ash, CP, ethereal extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were performed as described in Silva and Queiroz (2002). Total digestible nutrients (TDN) were calculated according to Weiss (1993).

In vitro dry matter digestibility (IVDMD) and in vitro cell wall digestibility (IVCWD) were performed using the technique described by Tilley and Terry (1963), adapted for the use of the Daisy II-Ankom® artificial rumen fermenter, proceeding weighing 0.5 g of sample from each replicate. Cows used as donors of ruminal fluid received total mixed ration (TMR) according to Table 1. This TMR was distributed twice a day, and at intervals the cows remained in a pasture predominating African star (*Cynodon nelemfuensis* Vanderyst).



Table 1. Ingredients and daily intake of natural matter and dry matter for cows used as donors of ruminal liquid for in vitro digestibility studies

Ingredients	Natural matter intake/cow/day (kg)	Dry matter intake/cow/day (kg)
Corn silage	16	4.80
Leucaena hay	1	0.85
Desmodium hay	1	0.86
Concentrated feed <sup>*</sup>	3	2.69
Total	21	9.20

\*Concentrated feed consisting of 78% corn, 20% soybean meal and 2% mineral salt.

For in situ degradability of DM and CP, samples from each of the five silos were mixed to form a composite sample. The nylon bag (10 x 17 cm) technique was used, with pores of  $53 \pm 10 \mu$ m. The samples were incubated in two non-lactating cows with rumen cannulas at the time intervals: 0, 6, 9, 24, 48, 72 and 96 h removing all the samples from the rumen at the same time. Cows were fed the TMR described in Table 1.

Percentages of degradation of DM and CP at each time were calculated by the proportion of materials remaining in the nylon bags after incubation in the rumen. The degradabilities of DM and CP were calculated using the equation described by Orskov and McDonald (1979).

$$P = a + b$$
 (1-e-ct).

Where:

P = amount degraded in time (h); a = intersection of the curve at time zero and can be interpreted with the rapidly soluble fraction; b = represents the potentially degradable fraction, expressing the fraction that was degraded in time (t); c = fractional rate of degradation in which fraction b was degraded each hour.

The effective degradability of DM (EDDM) and CP (EDCP) in the rumen were calculated using the equation described by Orskov and McDonald (1979):

EDDM or EDCP = 
$$a + [(b * c) / (c + k)]$$

Where:

k = represents the fractional particles passage rate in the rumen.

### 3. Results and Discussion

The DM content of leucaena and desmodium hays are within the range recommended by Evangelista and Lima (2013), who pointed out that in the moment of hay production, the



forage must have a moisture content between 10% and 18%, so it can be preserved without risk of mold formation (Table 2). The silages of both evaluated forages also presented a DM content within the acceptable for pre-dried silages that are between 35% and 45% (Pereira and Reis, 2001).

The values of CP in desmodium are close to 14% obtained by Morgado et al. (2009) and higher than the 12.75% reported by Bezerra et al. (2010). For leucaena the CP levels obtained were similar to those described by Costa et al. (2015). The variation in CP, NDF and ADF levels for desmodium in relation to leucaena can be explained by the higher proportion of stems in desmodium, since this structure has higher concentrations of structural carbohydrates and less protein. Veloso et al. (2000) observed a leaf:stem ratio of 2.7: 1 for leucaena, while Teixeira et al. (2010) observed a ratio of 1.1: 1 for the desmodium with 102 days.

Table 2. Chemical composition (% of dry matter), potential of hydrogen (pH) and ammonia nitrogen (NH<sub>3</sub>-N) of silage and hay of leucaena (*Leucaena leucocephala*) and desmodium (*Desmodium ovalifolium*) CIAT-350

Variables	Si	lage	Нау		
	Desmodium	Leucaena	Desmodium	Leucaena	
DM (% feed)	43.61	44.66	82.53	84.34	
ОМ	84. 68	83.11	92.14	89.73	
Ashes	15.36	16.89	7.86	10.27	
СР	14.29	26.78	14.39	26.69	
NDF	65.89	39.26	58.31	32.57	
ADF	45.32	18.60	41.83	15.65	
EE	2.72	2.37	2.69	2.25	
$TDN^1$	60.39	78.50	61.33	73.90	
рН	4.58	4.66	-	-	
NH <sub>3</sub> -N (% total N)	3.38	3.09	-	-	

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergente fiber; ADF: acid detergente fiber; EE: ethereal extract; TDN: total digestible nutrients.  $^{1}$ TDN = 105. 2 - (0. 68\*NDF) for silage and TDN = 81. 41-(0. 48\*ADF) for hay (Weiss. 1993).



The pH presented by the silages of both legumes was above the range of 3.6 to 4.2 recommended by McDonald et al. (1991) as one of the requirements for good quality silage. However, in silages with higher DM content, stabilization with higher pH may occur (Pereira and Reis, 2001).

The ammoniacal nitrogen contents of silages were less than 10% of the total nitrogen, which according to McDonald et al. (1991) classifies these silages as very good quality, since this parameter indicates less proteolysis by bacteria of the genus *Clostridium* during the fermentation. It is important to note that the high DM content of the silage aided this parameter, since it provided low water availability for the *Clostridium* development (Schocken-Iturrino et al., 2005).

The IVDMD values showed interaction between the legumes and the conservation method (P<0.05) where all the treatments differed from each other. Regarding the forage evaluated, the IVDMD of leucaena presented higher values (P<0.05) than the desmodium (Table 3). Abaunza et al. (1991) and Costa and Oliveira (1999) also observed a low IVDMD of the desmodium (CIAT 350), being 38.3% and 38.7%, respectively. These results may have occurred due to the high content of tannin (17%) found in this legume that negatively affects its digestibility and decreased its palatability (Abaunza et al., 1991). There were also differences regarding the conservation method, where the highest digestibility occurred for hay (P<0.05).

Table 3. In vitro dry matter digestibility (IVDMD) and cell wall (IVCWD) (% of dry matter) of silage and hay of leucaena (*Leucaena leucocephala*) and desmodium (*Desmodium ovalifolium*) CIAT-350

Variables	Silage		Hay		SD	P value		
	DESM	LEUC	DESM	LEUC		CONS	FOR	INT
IVDMD	39.47d	70.86b	48.04c	76.70a	0.79	0.000	0.000	0.008
IVCWD	34.62d	57.90a	39.26c	51.85b	0.48	0. 131	0.000	0.000

Averages within the same row, followed by different letters differ from each other at the 5% probability level for the interaction; SD = standard deviation; DESM = Desmodium; LEUC = Leucaena; CONS = conservation method; FOR = forage; INT = interaction;

The IVCWD did not show any effect in relation to the conservation method (P>0.05), but differed among the forages tested, being higher for leucaena (P<0.05). The lowest IVCWD in desmodium occurred due to the higher ADF concentration in this forage, because this fibrous fraction is negatively correlated to the digestibility and increases with the maturity of the plant (Müller et al., 2006). There was interaction between forages and the conservation method (P<0.05), with the highest IVCWD occurring in the treatment with leucaena silage and the lowest for desmodium silage.



There were differences for the soluble fraction (a) and slowly degraded fraction of DM in rumen (b) in relation to the conservation methods (Table 4), which were higher for hay (P<0.05). As for evaluated fodder, leucaena presented values higher than desmodium (P<0.05). The degradation rate (c) was faster for leucaena (P <0.05), regardless of the conservation method (P>0.05).

Table 4. Average of least squares to the nonlinear estimates and effective degradability of dry matter (EDDM) of silage and hay of leucaena (*Leucaena leucocephala*) and desmodium (*Desmodium ovalifolium*) CIAT-350

Variables	Silage		Feno		SD	P value		
	DESM	LEUC	DESM	LEUC		CONS	FOR	INT
a (%)	19. 84c	26. 87b	19. 80c	31. 83a	0. 59	0. 014	0.000	0.014
b (%)	44. 64b	62. 73a	56. 62a	62. 26a	1. 28	0. 015	0. 001	0.006
c (%/h)	3. 70	5.20	3. 50	5.80	0.003	0. 587	0.005	0. 304
EDDM P2 (%/h)	48.62	72.10	55.77	77.35	0. 63	0. 001	0.000	0. 207
EDDM P5 (%/h)	38.66	58.77	43.09	64. 69	0. 94	0. 005	0.000	0. 475

Averages within the same row, followed by different letters differ from each other at the 5% probability level for the interaction; SD = standard deviation; DESM = Desmodium; LEUC = Leucaena; CONS = conservation method; FOR = Forage; INT = Interaction; a = rapidly soluble fraction; b = potentially degradable fraction; c = fractional rate of degradation in which fraction b; EDDM P2 and EDDM P5 = Effective degradability of dry matter at passage rate of 2 and 5%/h

The EDDM at passage rate 2 and 5% was superior for leucaena compared to desmodium (P<0.05). These values agree with Bezerra et al. (2010) who also observed reduced EDDM for the *Desmodium canum* being 46.61 and 36.05% for the passage rates of 2 and 5% respectively, while Pires et al. (2006) obtained 62.9 and 53.9% of EDDM for leucaena at the same passage rates, respectively. These results may be associated to the higher ADF concentration in the desmodium, since this fibrous fraction has lignin that is not utilized by ruminal microorganisms (Silva and Queiroz, 2002), reducing DM degradability. In relation to the conservation method, the EDDM in the passage rate of 2 and 5% were higher for hay compared to silage. There was no interaction between the conservation method and the legume species (P>0.05).

The conservation method and the different legumes did not influence the soluble fraction (a) of CP (P>0.05), with an average value of 17.47% (Table 5). Potentially degradable CP fraction (b) differed between the conservation method and the fodder species, and there was

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interaction between the factors (P<0.05). The leucaena conserved in the hay and silage form were similar to each other (P>0.05) but differed from the desmodium hay that presented intermediate value and the desmodium silage which presented lower value. Degradation rate (c) was faster for leucaena (P<0.05), regardless of the conservation method (P>0.05).

Table 5. Average of least squares to the nonlinear estimates and effective degradability of crude protein (EDCP) of silage and hay of leucaena (*Leucaena leucocephala*) and desmodium (*Desmodium ovalifolium*) CIAT-350

Variables	Silage		Hay		SD	P value		
	DESM	LEUC	DESM	LEUC		CONS	FOR	INT
a (%)	16.81	18.66	18.68	15.75	0.88	0.587	0.573	0.054
b (%)	50.13c	78.19a	68.05b	82.91a	1.53	0.002	0.000	0.013
c (%/h)	3.70	5.70	3.60	5.80	0.004	0.998	0.009	0.833
EDCP P2 (%/h)	49.04c	76.64a	62.52b	77.23a	0.69	0.001	0.000	0.001
EDCP P5 (%/h)	37.89c	60.46a	47.27b	60.08a	1.03	0.012	0.000	0.009

Averages within the same row, followed by different letters differ from each other at the 5% probability level for the interaction; SD = standard deviation; DESM = Desmodium; LEUC = Leucaena; CONS = conservation method; FOR = Forage; INT = Interaction; a = rapidly soluble fraction; b = potentially degradable fraction; c = fractional rate of degradation in which fraction b; EDCP P2 and EDCP P5 = Effective degradability of crude protein at passage rate of 2 and 5%/h

The estimated EDCP for the 2 and 5%/hour passage rates were higher for leucaena (P<0.05) compared to desmodium. These differences in degradability may be related to variations in specific characteristics of the protein present in each forage, as well as the accessibility of this protein by the digestive enzymes and the presence of tannins and other polyphenols, which may protect the protein fraction from rumen degradation (Veloso et al., 2006). Thus, the high tannin content in desmodium, which according to Abaunza et al. (1991) is around 17%, may have contributed to the lower CP degradability for this legume. There was an interaction between the conservation method and forage species, where leucaena conserved as hay and silage presented EDCP at 2 and 5%/hour similar to each other (P>0.05), but higher than the other treatments. Desmodium conserved as hay and silage differed from each other (P>0.05) with higher value obtained for the hay. Considering that the forage harvest for silage and hay production occurred at the same vegetative stage, the lower EDCP presented by desmodium silage may be an indicative of Maillard reaction occurrence. According to Pereira and Reis (2001) silages with high DM content are more likely to raise the temperature of the ensiled mass, and when this temperature exceeds 55 °C reactions can occur between soluble carbohydrates and the amino group of amino acids, resulting in a reduction in CP availability to rumen microorganisms.



# 4. Conclusion

Leucaena has a higher nutritional value than desmodium, because it has higher digestibilities of dry matter and cell wall and a higher degradability of dry matter and crude protein. The conservation of legumes as hay had a higher nutritional value than those conserved in the silage form, for most of the parameters. Studies evaluating the use of these legumes preserved as hay and silage to feed cattle are needed to define their effects on milk and meat production.

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