

Maturity dependent variation in composition and characteristics of potentially digestible tissues of leucena

Variação dependente da maturidade na composição e características dos tecidos potencialmente digestíveis da Leucena

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Abstract

The objective of this study was to evaluate the effect of cutting age on the production, chemical composition, degradation kinetics and anatomy of Leucena (*Leucaena leucocephala*). The experimental design was a completely randomized design with a factorial arrangement 2x4 (two types of tissues and four cutting ages) for the production data of dry matter and 3x4 (three degrees of degrees of digestion of tissues and four cutting ages). Observed effect ($P < 0.05$) for the total production of DM of Leucena in function of different ages. The tissue types grain and non-grain showed maximum production at 70 days of age, with production of 2333,00 and 716.60 kg DM ha⁻¹, respectively. The parameters of degradation of DM evaluated decreased significantly with the increase in the maturity of the plant, in the same way the chemical composition presented behavior inherent to the advance of age. The effective degradability DM also decreased with the increase in the rate of passage (2, 5, and 8% h⁻¹). The highest rate of degradation (c) was obtained for 30 days. With the advance in plant maturity increases the proportion of vascular tissue lignificad influencing parameters of ruminal degradation of Leucena. The ages assessed influenced the chemical composition of the Leucena ($P < 0.05$), where the levels of dry matter, crude protein, acid detergent fiber and lignin and ash showed increasing linear behavior. The cutting age of 70 days offers an optimal point regarding the proportion of anatomical tissues correlated with the degradation and chemical composition of the Leucena.

Key words: Anatomy. Legume. Regrowth. Nutritional value.

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Resumo

Objetivou-se com este estudo avaliar o efeito da idade de corte sobre a produção, composição química, cinética de degradação e anatomia da Leucena (*Leucaena leucocephala*). Utilizou-se o delineamento inteiramente casualizado com arranjo fatorial 2x4 (dois tipos de tecidos e quatro idades de corte) para os dados de produção de matéria seca e 3x4 (três graus de digestão dos tecidos e quatro idades de corte). Verificou-se efeito ($P<0,05$) para a produção total de MS da Leucena em função das diferentes idades de corte. Os tecidos tipos forrageiros e não forrageiros apresentaram produção máxima aos 70 dias de idade, com produção de 2333,00 e 716,60 kg de MS ha⁻¹, respectivamente. Os parâmetros de degradação da MS avaliadas diminuíram significativamente com o aumento na maturidade da planta, da mesma forma a composição química apresentou comportamento inerente ao avançar da idade. A degradabilidade efetiva da MS também diminuíram com o aumento na taxa de passagem (2, 5 e 8% h⁻¹). A maior taxa de degradação (c) foi obtida aos 30 dias. Com o avanço na maturidade da planta aumenta a proporção de tecido vascular lignificado influenciando nos parâmetros de degradação ruminal da Leucena. As idades avaliadas influenciaram a composição química da Leucena ($P<0,05$), onde os teores de matéria seca, proteína bruta, fibra em detergente ácido e Lignina e cinzas apresentaram comportamento linear crescente. A idade de corte de 70 dias apresenta um ponto ótimo quanto à proporção de tecidos anatômicos correlacionados com a degradação e composição química da Leucena.

Palavra-chave: Anatomia. Leguminosa. Rebrotar. Valor nutritivo.

Introduction

The leucena (*Leucaena leucocephala*) is a perennial leguminous shrub, originating from Central America and now found throughout the tropical region. It has high productivity, which can vary from two to eight tons of dry matter per year with a protein content of over 20% (BARRETO et al., 2010). It is also preferentially consumed by ruminants. However, similar to other harvested plants, the nutritional content in leucena decreases over time due to physiological changes within the plant tissue, making it less attractive as a source of nutrition.

Understanding plant anatomy allows us to identify different structures within plant tissues and their distribution in the plant. For this reason, anatomical characterization of forages can contribute to a better understanding of the factors involved in the digestion of plant tissues by ruminants (FERREIRA et al., 2013), especially the behavior of the tissues of forage plants at different ages, as well as the level of insertion of the sheet, as these are factors that contribute to changes in the chemical composition of forages (FERREIRA et al., 2005).

The factors that affect the nutritional quality of forage plants can be anatomical, physical, or chemical in origin, in addition to factors related to the structure of the vegetation community. The management of cutting plants using a harvester is a factor that modifies both the productivity and the quality of the forage. Leucena, for example, has the ability to easily adapt to various ecosystems. More frequent cuts result in lower dry matter production but greater nutritional value in comparison to less frequent cuts, which provide higher dry matter production, but are of lesser quality (VERAS et al., 2010). With this, the correlation between harvest intervals and the nutritional quality of the plant can provide a more efficient method of management for this forage crop.

The potential digestibility of plant tissue is determined by the ease of access of microorganisms to the cellular contents of said tissue. In addition to that, plant anatomy influences the nutritive value of the species, which holds true for forages. This influence is exerted mainly through the proportion of different tissue types that are more or less digestible, and the thickness of the cell wall (CARVALHO; PIRES, 2008) found in the plant.

For this reason, the objective of this study was to evaluate the effect of cutting age on the production, chemical composition, degradation kinetics, and anatomy of leucena (*Leucaena leucocephala*).

Material and Methods

The experiment was carried out at the Forage Crops Section at CCAA/UFMA (03°44'33" S, 43°21'21" W). According to the Köppen classification, the climate in the region is a hot wet tropical type (Aw) with average annual temperature higher than 27 °C and average annual precipitation of 1,835 mm, rainy periods between January and June, and dry periods from July to December (Maranhão 2002). The area used was approximately 0.017 hectares, established in the year of 2012, with leucaena (*Leucaena leucocephala*), subdivided in four paddocks, which were imposed on the four ages of cuts, being 30, 50, 70 and 90 days.

Samples were collected for each treatment, according to methodology described by Sanchês et al. (2019). For the cross-sectional cut treatment, 10 stems were selected. Firstly, leaflets were cut. Next, the secondary rachis of the stem was cut, and finally the primary rachis.

The cuts were done freehand, with a steel blade and the aid of a styrofoam bracket. Then the samples were bleached in a 50% sodium hypochlorite solution. The time taken for this bleaching procedure corresponded with the toughness of the material; tougher plants were left in the solution for longer. After that, the samples were washed in distilled water and stained with safranin and/or toluidine blue and/or crystal violet, to provide an improved view of their structures using optical microscopy. After staining, they were washed with distilled water and attached to semipermeable slides. The sections were fixed on the slide using a drop of glycerin and a coverslip. The anatomical evaluation used images captured using a scientific digital camera for microscopes, the EUREKAM® 3.0 MP Opticam, coupled to the BELL® photonics optical

microscope. 10 semipermeable slides with differed leaf parts were evaluated (such as follicle, primary and secondary stem). Anatomical structures in the digital images were measured in mm, using the software BELL® Views. The anatomical structures analyzed were classified according to their digestion potential into low, medium, and high digestion potential categories. Structures with low digestibility are the sclerenchyma, the cuticle and the xylem; for medium digestion potential, the epidermis, and for high digestibility, the parenchyma, the mesophyll and the phloem. The leaves were considered as forage and the stalks as non-forage.

Samples of the material removed via cutting were collected and were then processed further in order to evaluate the bromatological composition of the plant tissue. For this purpose, the samples were first dried in a 55 °C forced-air oven until the mass was constant, then ground using a 5 mm sieve Willey grinder. Samples were analyzed to measure dry matter (DM, using method 930.15) and crude protein (method 954.01), according to the procedure described in AOAC (2003). Analyses of lignin and ash content were carried out according to Detmann et al. (2012) (INCT-CA F- 005/1 Method; INCT-CA M-001/1 Method, respectively). Analyses of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were carried out according to the procedures proposed by Van Soest et al. (1994).

For in situ degradability analysis, the samples were weighed and dried in a forced air circulation oven for 72 hours at 55 °C. They were then weighed to obtain their dry mass, and ground in a Willey knife mill for further chemical analysis into the *in situ* degradability of the DM. To evaluate the *in situ* degradability of DM, 12 x 8 cm nylon bags with 50 µm porosity were used (ØRSKOV; McDONALD, 1979), containing 4 g of sample in accordance with the 42 mg/cm² ratio adopted by Campos et al. (2011). The bags and their contents were then incubated in the rumen of a fistulated sheep for 72 hours (NRC, 2007). The *in situ* degradation parameters (a, b, and c) and the potential degradability of DM were

estimated using the model proposed by Sampaio et al. (1995), by simplifying the exponential model of Ørskov and McDonald (1979).

After the incubation period, the bags were removed for washing and were then dried in a forced air oven at 55 ° C for 48 hours. To determine the loss of the plant material after the treatment, control bags were kept in a water bath for one hour at a temperature of 39 ° C. After this time, the bags received the same analyses and tests as the bags that were incubated. The percentage of DM disappearance was measured by the proportion of food that disappeared in the bags after rumen incubation relative to the control. The parameters were evaluated using the model by of Ørskov and McDonald (1979), adapted by Sampaio (1994).

The data were then subjected to normality and homoscedasticity tests before the analysis of variance was performed. The variables related to dry matter production were compared using the Duncan test at 5% probability by the PROC GLM procedure of the statistical program SAS® (SAS, 2005), using the following statistical model:

$$\text{Model 1: } Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

Where Y_{ijk} is the dependent variable corresponding to experimental observation; μ is the general average; α_i is the fixed effect of age of plant when cut ('cutting age'); β_j is the fixed effect of the tissue type (forage or non-forage); $(\alpha\beta)_{ij}$ is the interaction effect between cutting age and tissue type; and e_{ijk} is the random error, assuming a normal distribution.

Tissue digestion variables were also compared by the 5% probability Duncan test by the PROC GLM procedure of the SAS® statistical program

(SAS, 2005) using the following model. statistical:

$$\text{Model 2: } Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

Where Y_{ijk} is the dependent variable corresponding to experimental observation; μ is the general average; α_i is the fixed effect of cutting ages; β_j is the fixed effect of the degree of tissue digestion; $(\alpha\beta)_{ij}$ is the interaction effect between cutting age and the degree of tissue digestion; and e_{ijk} is the random error, assuming a normal distribution. For the chemical composition data, a regression analysis was performed.

Results and Discussion

A significant effect ($P < 0.05$) was found for total leucena DM production as a function of different cutting ages (Table 1) in both tissue types. For forage tissue type, a maximum yield of 233.33 kg DM ha⁻¹ was observed when cut at 70 days, followed by 50 days (1700 kg DM ha⁻¹), but did not differ from production at 30 days (1415 DM ha⁻¹), which in turn did not differ from the cut Leucena at 90 days of age (800 kg of DM ha⁻¹). For non-forage tissue type, it was observed that the highest production of this type of tissue also occurred at 70 days (716.60 kg DM ha⁻¹).

A significant correlation ($P < 0.0001$) exists between cutting age, extent of tissue digestion and the interaction between both factors was observed (Table 2). The plants, independent of cutting ages, presented higher amount of tissues of high digestion degree (mm). It is interesting to note that, within the digestive potential, only medium and high potential tissues were influenced by cutting ages, where the smallest amount was observed at 30 days, being 52.21 and 86.77 mm, respectively.

Table 1. Dry matter (DM) kg ha⁻¹ yield of *Leucaena leucocephala* under different cutting ages.

Tissues	Dry matter yield (DM) kg ha ⁻¹				Means	P-value		
	30	50	70	90		Age	Tissue	A x T
Forage	1415.00Abc	1700.00Ab	2333.00Aa	800.00Ac	1562.00	<0.001	<0.001	<0.001
No Forage	183.30Bb	316.70Bb	716.70Ba	312.5Bb	382.30			
Means	799.15	1008.35	1524.85	556.25				

Means followed by equal lowercase letters in rows and uppercase in columns do not differ from each other by the Duncan test at P> 0.05.

Table 2. Sum (mm) of tissues present in the transverse sections of the leaves (follicle, primary and secondary rachis) of *Leucaena leucocephala* under different cutting ages, classified according to their digestion potential.

Age (days)	Degree of tissue digestion (mm)				CV (%)	P-value		
	Low	Medium	high	Means		Age	Tissues	A*T
30	52.21Bb	0.36Ac	86.57Ba	46.38	16.55	<0.0001	<0.0001	<0.0001
50	100.67Ab	0.31Ac	166.81Aa	92.59				
70	100.17Ab	0.30Ac	177.30Aa	89.26				
90	93.06Ab	0.30Ac	138.71Aa	77.36				
Means	86.53	0.32	142.35					

Means followed by equal lowercase letters in rows and uppercase in columns do not differ from each other by the Duncan test at P> 0.05.

The fact that low and high digestibility tissues have a smaller proportion in the 30-day-old plant-cutting may be linked to the growth of the legume, which after 30 days of regrowth would still be in its growing phase, not yet reaching high productivity and therefore having a smaller amount of digestible tissues compared to other cutting ages, where a large amount of phloem is observed, both in the leaflets (A) and in the primary (B) and secondary rachis(C). In the medium category of digestibility (the epidermis), the absence of this effect may be related, because the cutting age is not proportionately related to the cutting age as compared with other tissue types (vascularized tissues, which with maturity tend to increasingly lignify). In this sense, lignin seems to be the main chemical limitation to digestion of these cells, which may hinder the access of microorganisms to cellular content (AKIN; CHESSON, 1989). According to Brito and Deschamps (2001), the parenchymatic tissue presents high rates of degradation and occupies a

large part of the area in the different organs and fractions and is therefore very important in forage quality.

Therefore, it can be said that low and high digestive potential tissues are linked to forage production, because with plant growth, the amounts of these tissues increased, according to plant production potential, both vascularized tissues (low potential). Digestion caused by lignification of structures with advancing age when filling tissues, such as the parenchyma (high digestive potential) that make up, for example, the mesophyll.

Although grasses have lower lignin contents than legumes, a negative correlation between lignin content and digestibility is stronger in grasses due to a higher concentration of hemicellulose compared to legumes. As lignin covalently binds to hemicellulose, its effect on the digestibility of grasses would be more detrimental than on legumes (VAN SOEST, 1994).

For the cutting ages of 50, 70, and 90 days, no difference was found in tissue digestibility. A likely cause is that owing to having larger amounts of different tissue types, including tissues with low potential digestibility that are made up of fiber bundles and xylem present thick and lignified wall, both in leaf and stem, and the walls of these cells are considered indigestible in grass and leguminous (CARVALHO; PIRES, 2008), which may affect the nutritional value of the legume, which presented most of the forage tissue at 70 days.

Thus, even if the legume is in a more advanced stage of growth, as is the case of the 90-day-old cut, it can still be a forage option for ruminants, possessing greater potential for digestibility than grasses. In addition, legume mesophyll cells are more dispersed with more intercellular spaces, allowing easier access for microorganisms and thereby facilitating digestion (CARVALHO; PIRES, 2008). In this study, at 90 days a higher proportion of xylem and sclerenchyma was observed, potentially due to the lignified vascular tissue being basically composed of xylem and sclerenchyma. Paciullo et al. (2001) found that sclerenchyma and xylem

cell wall thickness were negatively correlated with digestibility, and concluded that the proportion of mesophyll, xylem and sclerenchyma relative to cell wall thickness can be combined with chemical composition analyses to improve the nutritional value estimation of fodder.

There was a decrease in the soluble fraction correlated to an increase in leucena's cutting age (Table 3). These results were expected, because as it matures, the plant tends to accumulate support structures that result in greater cell density and, consequently, a decrease in its degradability. The 30-day-old cut leucena presented the highest amount of "a", a 10.40% water solubility, indicating a high solubility. As a 30-day-old leucena is a young and still developing plant, most of its carbohydrates are readily available for rumen microbiota. Regarding the other cutting ages, it was observed that there was a tendency for the soluble fraction to decrease with advancing age. These values are in agreement with the results of the bromatological composition. According to Carvalho et al. (2007), the fraction "a" of dry matter represents the portion of the food that is readily available to rumen microorganisms.

Table 3. In situ ruminal degradation parameters of Leucena (*Leucaena leucocephala*) dry matter under different cutting ages.

Age (Days)	a (%)	b (%)	c (% h ⁻¹)	A	R ²	Effective degradation (%)		
						2 % h ⁻¹	5 % h ⁻¹	8 % h ⁻¹
30	10.40	78.50	1.46	88.90	90.78	28.68	18.90	15.94
50	7.78	76.73	1.26	82.51	97.99	35.86	22.34	17.61
70	6.69	73.17	0.73	81.86	89.13	26.98	16.35	13.03
90	6.43	57.93	0.61	64.36	90.57	30.87	19.52	15.37

Potential degradability (A), rumen degradable fraction (B), degradation rate (C), coefficient of determination (R²) and Effective Digestibility (for passage rates of 2, 5 and 8% h⁻¹), relative to the models degradation of DM according to forage.

The degradable fraction in the rumen (b) presented lower values in response to an increase in cutting age, at 76.73%; 73.17% and 57.93%, respectively. The degradation rate (c) was highest at the lowest cutting age evaluated (30 days), as it was the youngest plant (1.46%). This treatment presented

younger plants and material readily available for rumen microbiota, data that are According to the chemical composition (Table 4), it is clear that the speed at which this plant material degrades is greatly influenced by its maturity. However, according to Sampaio (1994), dry matter degradation rates of less

than 2% per hour indicate poor quality food, as they require a longer time in the rumen to be degraded, such as most tropical roughages. Here, all samples showed degradation rates below 2% per hour.

Table 4. Chemical composition of *Leucaena leucocephala* under different cutting ages.

Variables	Age (Days)				s.e.m	R ²	equations (Effects of age)
	30	50	70	90			
Dry matter	19,78	28,20	33,41	45,47	8,13	0,9759	$\hat{Y} = 11,145 + 8,228x$
Crude protein	22,90	21,01	17,76	12,31	7,39	0,9503	$\hat{Y} = 27,25 - 3,50x$
Neutral detergent fiber	51,39	45,70	49,64	51,70	7,30	0,8544	$\hat{Y} = 44,67 + 1,975x$
Acid detergent fiber	29,08	24,15	27,66	29,61	7,91	0,9759	$\hat{Y} = 21,92 + 1,889x$
Hemicellulose	22,50	21,03	21,98	21,48	14,83	0,9484	$\hat{Y} = 18,775 + 0,989x$
Cellulose	4,67	5,11	7,41	5,24	49,82	0,9346	$\hat{Y} = 3,105 + 1,301x$
Lignin	24,42	18,95	21,10	24,42	9,17	0,9777	$\hat{Y} = 14,465 + 2,381x$
Ash	6,09	4,81	4,90	3,67	3,28	0,8764	$\hat{Y} = 6,66 - 0,717x$

The highest potential degradability (A) was observed at 30 days for cutting age, influenced by its high degradation rate (1.46%) and ruminal degradable fraction (10.40%), reaching 88.90% of its potential degradability. At 50 days of cutting age samples were degraded by 82.51% of "A", and at 90 days the effect of plant maturity was more pronounced, with potential degradability reaching only 64.36%. The decrease in potential degradability here was seen to be related to increasing maturity of the plant.

According to Carvalho et al. (2007), measuring rumen degradability without considering different ruminal passage rates may result in an overestimation of the extent of degradation, as food particles must move to the next stomach compartment before they are completely degraded. It was found that effective degradability decreased significantly with an increase of the passage rate (2, 5, and 8% h⁻¹), a result that can be explained by the increase in the speed of passage of the food through the gastrointestinal tract of the animal consequently decreasing the time for rumen microbiota to degrade the plant matter. According to Pereira et al. (2005), as the passage rate increases, there is a greater loss of carbohydrates and ruminal fermentation proteins,

increasing the flow of these nutrients to the small intestine. Cutting age, however, did not have a significant impact on degradability. As seen in Tables 1 and 2, advancing plant maturity increases the proportion of lignified vascular tissue, affecting leucena's ruminal degradation parameters.

The cutting ages were found to have influenced the chemical composition of leucena (P <0.05), where the contents of DM, CP, ADF, Lignin and ashes presented a positive linear correlation with cutting age, increasing as cutting age increases (Table 4). For example, the DM was highest at 90 days after regrowth (45.47%) and lowest at 30 days after regrowth (19.78%).

Cutting age and CP content showed a negative correlation, where the highest CP contents were observed (22.90 and 21.01%, respectively) at 30 and 50 days after regrowth, while the plants cut at 90 days had the lowest content (12.31%). Costa et al. (2003) stated that thin leaves and branches of leucena are very nutritious, with a crude protein content of around 25%, while in older leaves and branches this content fall to 15-20%. Protein, followed by energy, is the nutrient most needed by ruminants. (RODRIGUES JÚNIOR et al., 2015). According to Carvalho et al. (2011), 7% of CP is the minimum

content of this nutrient necessary to ensure adequate fermentation of fibrous carbohydrates in the rumen. In this study, all cutting ages met this 7% limit, a common characteristic of legumes.

A similar relationship was observed for mineral matter content, where older plants cut at 90 days of age presented lower amount of minerals (3.67%). Determination of ash content gives an indication of the concentration of the grass's mineral nutrients. In this experiment, leaf and stem ash contents had a significant linear relationship ($P < 0.05$) with decreased cutting ages. In the same manner, analyzing the impact on nutrient fractions due to cutting frequencies showed no difference at 30 days cutting age. Castro et al. (1999), stated that with plant maturity, there is commonly a decrease in mineral content owing to the effect of dilution by the plant on the dry matter produced. There is also reduction in leaf/stem ratio, an increase in senescent leaves, diversity in the pattern of absorption of the elements during the plant cycle, and their redistribution among the various plant organs, leading to a decrease in the concentration of minerals in the plant (RODRIGUES JÚNIOR et al., 2015). Magalhães et al. (2000) found that when evaluating leucena plants, they found 9.5% MM content in a plant that was 4 years into its regrowth.

The acid detergent fiber (ADF) content corresponds with an increase in plant age ($P < 0.05$), probably as a result of the increased proportion of cell wall constituents and a lower cutoff frequency. According to Rodrigues Júnior et al. (2015), the ADF content is related to the lignin content of foods, which determine its digestibility. The lower the ADF content, the lower the lignin content and, consequently, the more digestible the food.

Lignin content increased with the age of the leucena plant, and these values corroborate the potentially digestible tissue data seen in Table 2. It can be inferred that the lignin present in the leucena plant increased over time, and therefore the arrangement in the structure of these cells promote an increase in plant resistance material, decreasing

the nutritive value with an increase in cutting age. According to Santos et al. (2001), lignin is an amorphous structural component that appears to have a "cementing" function in cell wall carbohydrate bonds; appears impregnated with cellulose and hemicellulose forming a lignocellulosic complex, making these carbohydrates unavailable for degradation by microorganisms. In this way, the maturation of the plant causes an increase of this component, influencing the qualitative aspects of the forages. For hemicellulose and cellulose content, no significant difference was observed ($P > 0.05$). However, cellulose values were lower than those found by Magalhães et al. (2000), who observed a 16.2% cellulose content.

Conclusion

The most frequently cut leucena had higher amounts of highly digestible tissues. For this reason, it is recommended to cut leucena after 70 days of regrowth, in order to maximize production of forage material. Despite leucena containing a higher nutritional value at 30 days after regrowth, the low volume of production precludes the 30-day regrowth period from being an effective method for maximizing forage yield.

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