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Influence of chemical composition and secondary metabolites on the digestibility of *Leucaena leucocephala*.

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Abstract

Leucaena leucocephala, for the tropical region, constitutes a protein tree, which is used as the main alternative to solve the lack of food during the dry period is a challenge for researchers. The influence of chemical composition and secondary metabolites on Leucaena leucocephala digestibility was evaluated. A randomized block design with six replications was used. The statistical procedure. Multiple linear equations between in vitro and in situ digestibility, as dependent variable, and the age, NDF, ADF, ADL, Ca, Si, NDF/N, ADF/N, TT, CT and saponins, as independents variables, were established. An increase in cell wall content, its component, and a variable performance of minerals were obtained. The linear equations between digestibility's with the age, NDF, ADF and Si (R^2> 0,78) and the secondary metabolites TT, CT and saponins (R^2> 0,79) showed high fitting. The regrowth age, cell wall components, polyphenol compounds, saponins contents affected the digestibility and these aspects must be taken into consideration to use this plant in animal nutrition.

Key words: *age, chemical composition, digestibility, Leucaena leucocephala, secondary metabolites.*

Introduction

In tropical region, that do not produce grains and cereals, searching for strategies to feed the animals with local resources is one of the main key of the livestock scenario. The amounts and high prices of the energy and protein balanced feeds, their efficient and competitive utilization indicate future efforts to use local and alternative resources (Peiretti *et al,* 2010), in order that the animal could expressed their genetic potential (Zamora *et al,* 2009).

Leguminous trees, creeping legumes and shrub play an important role in animal nutrition due to their high protein content and nutritive value (Garcia, 2004).

Several species of well-adapted legumes, non-legumes fodders and shrub mean a high potential supplement with many benefits on ruminant nutrition not only significant from the nutritional point of view, but also from the factor that promotes the ruminal fermentation of the total diet. Considering their chemical composition and secondary compounds content, mainly tannins (Carmona, 2007; Kumar *et al,* 2010).

One of the most important trees in Cuba for livestock is *Leucaena leucocephala* commonly called Leucaena, forage acacia or white seethe, is a perennial legume, with a wide potential for feeding ruminants and able to grow with accompanying grasses in tropical and subtropical conditions. It develops in well-drained soils, with pH from neutral to high, below 1,400 meters of altitude and with acceptable fertility (Rivera *et al*, 2017). When handled as a shrub, this species is tolerant to drought, and is recognized for its high forage value, due to its ability to regrow, palatability and superior nutritional characteristics, which include a high content of crude protein and carbohydrates soluble, in addition to low fiber content (Cuartas *et al*, 2015; Gaviria *et al*, 2015a).

On the other hand, in recent years it has been proven that there is abundant evidence on the nutritional advantages of *L. leucocephala* for feeding ruminants and their contribution to generating. more productive and sustainable systems in tropical conditions (Murgueitio *et al*, 2015). Due to its high forage production and nutritional quality, in systems that include this Leguminous it is possible to use a higher animal load per hectare and obtain weight gains and higher productions of milk in ruminants, compared to those achieved in other forage systems tropical (Mohammed *et al*, 2015). Hence the importance for Cuban livestock and the tropics

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Therefore, the objective of this report was study the influence of chemical composition and secondary metabolites content on *Leucaena leucocephala* digestibility.

Materials and Methods

Experimental area.

The study was carried out in the experimental and production facilities at the University of Granma, eastern Cuba. The area had been established two years ago and the experimental period length was two years (2007-2009). Four sampling periods were used, two from November to April (dry season), and two from May to October (rainy season).

The soil was classified as Calcic Haplustept (Soil Survey Staff 2014), with 6.2 of pH and values of 2,4; 33,42 and 3 mg/100g of soil, and 3,6% of organic matter respectively. The average rain in the rainy season was 812,6 mm; mean, minimum and maximum temperatures was 26,73; 22,31 and 33,92 °C by this order, and mean, minimum and maximum relative humidity was 80,78; 51,02 and 96,22%, respectively. During the dry period the values were 270 mm, 24,05 °C; 18,29 °C, 31,58°C, 76,21%, 49,16% and 97,3% respectively.

Treatments, statistical design and experimental procedure.

Three ages (60, 120 and 180 days of regrowth) were arranged under a randomized blocks design with six replications.

At the beginning of each season, a homogenized cut of all the plants at 1m above the ground level was carried out. Later, at each regrowth age 10 plants were randomly selected from each plot, discarding the lowest and highest plants. Stalk and petioles with a diameter inferior to 2 cm were chosen (considered, as a whole, the useful biomass to be eaten by animals). All select materials were mixed and a homogenous sample representative of useful biomass per plot was obtained. This sample was dried at 65 ˚C in a forced-air circulation oven for 72 hours, in order to determine the dry matter content (DM). Once dried, samples were grinding and stored in a dry cool place until further analyses.

Chemical analysis was carried out for DM, P and Ca according to AOAC (2016) and NDF, ADF, lignin, hemicelluloses, cellulose, cell content and Si according to Goering and Van Soest (1967).

In vitro dry matter digestibility (IVDMD) was determined by the ANKOM procedure, using a Daisy II® incubator (ANKOM Technology, Fairport, NY-USA), as described Robinson *et al.* (1979). Rumen fluid was obtained from four canulated sheep with lives weight of 53,8 ± 4,08 kg, housed in individual pens, consuming during the experiment alfalfa hay (*Medicago sativa*) and *Erica arboreal* The ruminal liquid was obtained before the tomorrow's feed and filtered through four gauze layers and diluted (1:4 v/v) with the medium reported by Menke and Steingass (1988). Samples of the plants (250 ± 10 mg) were weighed and put into F57 Ankom bags with a pore size of 25 µm, heat-sealed and then placed into an incubation jar. In each digestion jar, they were incubated at random of each one of those ages and a standard bag without sample was used, with the purpose of generating the correction factor for the possible input of particles or loss of weight of the bags. Nine bags per treatment were used. Samples were incubated at 39 ˚C at constant levels of agitation and rotation. After 48 h of incubation, the jars were emptied and the bags were rinsed with cold water and dried in an oven at 105 °C.

In situ dry matter digestibility (ISDMD) was determined according to the nylon bag technique (Mehrez and Orskov *et al*, 1980). Samples were ground using a 2 mm screen and 4 g of sample was put into the nylon bags (12 × 10 cm; pore size of 40 µm) which were introduced in the rumen, before the morning feeding, and incubated in duplicate in each sheep for 72 h. Upon removal, bags were soaked in cold water for 15 minutes, frozen at -30 ºC for 24 h. The bags were unfreezing at 4 ºC, washed with cold water to remove any microbial cells adhering to the particles, oven dried at 60 ºC for 48 h and weighed to estimate DM disappearance.

Total Tannins (TT) were determined by the Folin-Ciocalteu method (Makkar, 2003), total condensed tannins (TCT) by nButanol/HCl/Fe^{3+} (Porter *et al*, 1986) and saponins by Obdoni and Ochuko (2001).

Statistical analysis

The normal distributions of data were analyzed by the test of Kolmogorov-Smirnov (Massey, 1951) and variance homogeneity according Bartlett (1937).

In each season, Pearson coefficient was determined between *in vitro* (IVDMD) and *in situ* (ISDMD) dry matter digestibility (dependents variables), and the age of regrowth, CP, NDF, ADF, lignin, Calcium, Silica, Cell content and fiber/nitrogen ratio (NDF/N and ADF/N), as independents variables.

Multiple lineal equations were established between the same dependent and independent variables plus TT, TCT and saponins. To select the best fit equations R^2 value, signification level of the equations and parameters, standard error of the parameters and equation, residual and concordance test between predicted and observer values were considered according to Keviste *et al,* (2002); Guerra *et al,* (2003); Torres and Ortiz (2005). Statistical analysis was carried out according to SPSS (Version 22.0).

Results

The relationship between fiber/nitrogen ratio and digestibility show that negative Pearson coefficients were found in both seasons. The highest values were obtained for ISDND and IVDMD the rainy and dry season, respectively (table 2)

Negative Pearson coefficients between IVDMD and age, NDF, ADF, lignin, calcium and silica were obtained in both seasons. Similar performance was reported for ISDMD, but the coefficients between digestibility's and CP and cell content were variables. On the other hand, the higher negative coefficients were found in ISDMD during the rainy season (table 3).

Table 1. Effect of fiber/nitrogen ratio on *Leucaena leucocephala* digestibility

Indicator	Rainy season		Dry season	
	NDF/N	**ADF/N**	**NDF/N**	**ADF/N**
IVDMD	-0,52**	-0,45**	-0,83***	-0,94***
ISDMD	-0,98***	-0,99***	-0,42**	-0,50**

All values of the coefficients were significant at **p<0.01 *** p<0.001

Table 2. Relation between the digestibility, chemical composition and age of *Leucaena leucocephala*

Rainy season								
Indicators	**Age**	**CP**	**NDF**	**ADF**	**Lignin**	**Cell content**	**Calcium**	**Silica**
IVDMD	-0,55**	0,36	-0,60**	-0,40	-0,55**	0,50**	-0,14	-0,45**
ISDMD	-0,97***	0,98***	-0,96***	-0,97***	-0,92***	0,52**	-0,45**	-0,87**
Dry season								
Indicators	**Age**	**CP**	**NDF**	**ADF**	**Lignin**	**Cell content**	**Calcium**	**Silica**
IVDMD	-0,89***	0,77**	-0,53**	-0,90***	-0,75**	0,53**	-0,24	-0,26
ISDMD	-0,51**	0,43**	-0,20	-0,43	-0,21	0,20	-0,52**	-0,59**

All values of the coefficients were significant at **p<0.01 *** p<0.001

High coefficients (R^2 >0,95) were found in the effects of secondary metabolites (saponins, TT and CT) on Leucaena digestibility (IVDMD and ISDMD) in the rainy season (table 3), But in the dry sea son noli TT and CT affected the digestibility´s with lower R^2 (0.79-0.87) in comparison to the rainy season.

Table 3. Multiple lineal equations between digestibility and some secondary metabolites of *Leucaena leucocephala.*

Rainy season							
Variables	**F ind**	**TT**	**CT**	**SAP**	**R^2**	**1- R^2**	**SE±**
DIVMS	795,04	1,85	-5,50	-7,42	0,95	0,05	0,28
DISMS	120,3	0,49	-0,39	-1,06	0,99	0,01	0,217
Dry season							
Variables	**F ind**	**TT**	**CT**		**R^2**	**1- R^2**	**SE±**
DIVMS	-11,97	-1,52	0,84		0,79	0,21	0,90
DISMS	-270,3	3,01	-6,21		0,87	0,13	1,391

All values of R^2 were significant at (p<0,001)

Discussion

The cell wall components (NDF, ADF, Lignin) NDF/N, ADF/N increased while CP, cell content IVDMD and ISDMD decreased in both season due to the amount of stem with the regrowth age (Verdecia *et al,* 2012). On the other hand, this behavior may be related to the phenological changes that happen as the plant get more nature (Nogueira-Filho *et al,* 2000). On the other hand, other authors confirm the use of this legume in the production systems of ruminants and reported lower values than those reported in this work for the FDN and FDA (Mboko *et al*, 2017; Hernández *et al*, 2018), which means the ecological plasticity of this species in the tropics, showing the importance of this work.

The nutrient content of *Leucaena leucocephala* are similar to those reported for the majority of the tropical trees (Verdecia *et al,* 2014; Verdecia *et a*l, 2020). However, the values obtained in this study were higher than the reported by Garcia *et al,* (2009) in the Cauto Valley of the Granma province. All this means the high nutritional potential of Leucaena as supplement for ruminant diets.

The variations found in Ca and Si have been also reported by many other authors (Garcia *et al,* 2008; Padron *et al,* 2008; Garcia *et al*, 2009; Chavez *et al,* 2012; Verdecia *et al,* 2013) who explain that this performance is caused by the soil particularities, the rain variations and temperatures, as the most important factors that influence on plant nutrition.

In general, the digestibility rates were clearly influenced by the age regrowth of the plant, its chemical composition and also with the amount of secondary metabolites (tables 1, 2, 3). Due to the contribution of the cell wall, its digestibility can fluctuate from 100% in the mesophyll cells to 0% in the xylem, and this can differ

between legumes trees (Pedraza *et al*, 2003). Other results reported values similar to those reported in this research, these authors conducted various studies on tropical legumes, highlighting the importance of these for the feeding of ruminants in the tropics (Alatorre *et al*, 2018).

The percentages of IVDMD and ISDMD were considered low, especially those obtained *in situ.* Tarazona *et al,* (2012) reported that the NDF, ADL and Si content con modify the attack of rumen microorganisms to the plant cells of *C. purpureus,* and this has also been observed in experiments with *Lotus uliginosus, Tithonia diversifolia, Erythrina variegata, Gliricidia sepium* y *Leucaena leucocephala* (Quirama *et al,* 2001; Verdecia *et al*, 2014). The tannins linked to the fibrous fraction, is parameters that negatively influences on the enzymes released by the microorganisms. On the other hand, Ramirez *et al,* (2002) reported that saponins may also provoke negative and significant effects on digestibility due to their defaunating power reduce the number of protozoa, cellulolytic bacteria and detergent effects have been proved.

Other studies reported a high relation between the total tannins and the condensed ones with the digestibility of the dry matter of Leucaena, with coefficients over 0.80 (Herrera *et al*, 2017), which explains the high coefficients obtained in the linear models established in this investigation.

The content of secondary compounds, like any other chemical compound, can vary according to the stage of growth of the plants, nutrition of them and environmental conditions, including by the different methods used for the determination of such compounds. This could explain the low amounts of tannins and phenols obtained in the current study, since the evaluation of these components was carried out when the plants were around two months old (Alatorre *et al*, 2018)

Conclusions

The results evidenced notable effects of the regrowth age, cell wall components, polyphenol compound and saponins on the digestibility of Leucaena. Therefore, these aspects most be considered when this forage will be used in animal feeding.

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