



Effect of the Regrowth Age on the Chemical Composition, Digestibility and Polyphenol Content of *Jatropha Curcas*

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Abstract

The present work was developed with the objective of evaluating the effect of the regrowth age on the chemical composition, digestibility and polyphenol content of the *Jatropha curcas* in an area of Cauto Valley. Was used piece of ground of *Jatropha curcas* with two years of establishment. A randomized block design with six replicas was used. The treatments were regrowth ages of 60, 120 and 180 days. At the beginning of each seasonal period a homogeneity cut was made at 1 m above the ground level. From there the sampling was carried out in 10 random plants eliminating the edge effect in an area of 0.5 ha, according to the treatments. The chemical composition of *Jatropha curcas* the dry matter content, DNF, ADF, ADL, increase with age with its best results at 180 days with 28.82; 51.53; 33.44 and 20.22%; while the CP and cellular content decrease with its highest results at 60 days 27.78 and 58.79%. The minerals showed a very variable behavior. The highest energy contribution and digestibility percentage at 60 days of age with a decrease of 11.04; 7.40; 8.90%; 1.58 and 1.11 MJ / kg. Polyphenolic compounds increase with age with the best results at 180 days of age with 11.26, 21.10, 113.93, 111.49 and 2.46 g / Kg, respectively; for total tannins, total phenols, TCT, TBCT and FTC. The results of the present investigation evidenced the marked effect of climatic factors and age on the quality of the *Jatropha curcas*, as the maturity of the forage progressed, its nutritional contribution decreased with a decrease in the CP, the nutrient content, digestibility and energy, as well as an increase in the fibrous fraction and the content of polyphenolic compounds.

Introduction

Due to the food deficit and the global economic crisis, Latin American countries have had to venture into other feeding strategies to increase animal production in tropical conditions, by supplying livestock with more protein and minerals, which in general are found deficit in grasses Diaz . In this sense, the biomass of trees, shrubs have a leading role for their considerable protein levels and acceptable nutritional value. Due to the perspectives and benefits of this plant for tropical livestock, it is necessary to know the essential characteristics of its chemical composition, nutritional

value and its impact on the acceptability of the animal Boufennara et al. [1]. The presence of foliage of trees, shrubs and legumes in animal diets, whether in fresh or flour form, can improve production yields, which represents a practical and economical alternative to increase productivity in developing countries Boufennara et al. [1]. Most of these food sources have a well-balanced amino acid pattern in relation to the established requirements, so the total protein required in the diet is much lower than when cereals are the basis of it, and the waste is less of nitrogen during metabolism

Murgueitio et al. [2]. Among these tree species is *Jatropha curcas* with outstanding nutritional qualities, rich in protein, vitamins and minerals, it is a multipurpose species, of tropical origin, belonging to the Euphorbiaceae family and their common names vary according to the different regions where it is grown. In Cuba it is recognized as: pinija pinion, fence pinion, purgative pinion; It is called piñoncillo in Mexico, piñol in Peru and tempate in Costa Rica. It can grow both in areas of high and low annual rainfall. It is fast growing and seed production can begin even in the first year of planting. Highly resistant to drought, it can be grown in marginal areas, without competing with the production of species for human consumption Toral et al. [3]. According to the above mentioned, the aim of this work was to evaluate the effect of the regrowth age on the chemical composition, digestibility and polyphenol content of *Jatropha curcas* in an area of the Cauto Valley.

Material and Methods

Geographic LOCATION

The research was carried out by the Productive Teaching Department of Granma University (Granma, Cuba). An area of 4410 m² was used for the purposes of this experiment from 2007 to 2009. The type of soil was calcic haptustep Soil Survey Staff [4], with pH 6.2. The P₂O₅, K₂O and N total content was 2.4, 33.4 y 3.0 mg/100g of soil, respectively, with 3.6% of organic matter.

Treatments and experimental design

According to weather conditions, the period of study was divided in two seasons: rainy season (May-October) and dry season (November-April). The rainfall during the rainy season was 894 mm, the mean, minimum and maximum temperatures registered were 26.7, 22.3 and 33.9 °C, respectively and the mean, minimum and maximum relative humidity were 80.8, 51.0 and 99.2 %, respectively. The rainfall during dry season was 364 mm; the mean, minimum and maximum temperatures were 24.5, 18.3 and 31.6 °C, respectively, and the mean, minimum and maximum relative humidity were 76.2, 44.2 and 97.0%, respectively. Within each season, three regrowth ages were considered: 60, 120 and 180 days; thus, the development state varied from a vegetative growing state at 60 days up to flowering at 180 days. A random block design with three replicates per block was used (9 plots of 396 m²).

Experimental procedure

At the beginning of each season, the plants were trimmed 1 m above ground level. Later on, at each regrowth age, 10 plants were randomly selected from each plot at every regrowth age, discarding the lowest and highest plants. Stalk and petioles with diameters 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 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 stored in a dry cool place until further analyses.

Chemical analysis

Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were determined according to Goering and Van Soest [5]. AOAC [6] procedures were used to determine crude protein (CP), ash, organic matter (OM) and minerals (calcium, phosphorus, magnesium and silica) contents. Phenolic compounds were extracted and analyzed following the procedures described by Makkar [7]. Total extractable phenols (TP) were determined using the Folin- Ciolateau reagent and tannic acid as standard. Total extractable tannins(TT)were estimated after adsorption of TP to insoluble polyvinylpyrrolidone, and measuring the remaining total phenols in the supernatant (or non-precipitable phenols). Concentration of total extractable tannins (TT) was calculated through subtraction as follows $TT = TP - \text{non-precipitable phenols}$. Free condensed tannins (FCT) were measured in the extract using the butanol-HCl assay Porter et al. with the modifications of Makkar [7]. Total condensed tannins attach (TCTA) were measured in the solid residue remaining after extraction of phenolic compounds. Concentration of total condensed tannins (TT) was calculated as follows: $TT = FCT + CTA$. Concentration of phenols and tannins were expressed in g tannic acid equivalent kg⁻¹ DM.

In vitro digestibility

In vitro dry matter digestibility (IVDMD) was determined by the ANKOM procedure, using a Daisy II® incubator (ANKOM Technology, Fairport, NY-USA), as described by Robinson et al. [8]. Rumen fluid obtained from four caulated sheep was diluted (1:4 v/v) into the medium as reported by Menke and Steingass [9]. Four Merino sheep were provided permanent ruminale cannula, with weight lives off $53,8 \pm 4,08$ kg, housed in cubicles singular, consumed during the experiment medic hay (*Medicago sativa*) and Erica arboreal. Rumen fluid was obtained before the morning feed and filtered through four gauze layers. Samples of (250 ± 10 mg) diets were weighed 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 a replica at random of each one of those ages of these species and a bag like white, with the purpose of generating the correction factor for the possible entrance of particles or loss of weight of the bags. The procedure was carried out on four replicate. 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. Thereafter, was determined to estimate true digestibility (TD).

In situ digestibility

In situ dry matter digestibility (ISDMD) was determined using the nylon bag technique Ørskov [10]. Samples were ground using a 2 mm screen and about 4 g of sample was weighed into nylon bags (12 × 10 cm; pore size of 40 µm), which were introduced before the morning feeding and incubated in duplicate inside the rumen of each sheep for 72 h. Upon removal, bags were soaked in cold water for 15 minutes to stop the microbial activity, and then frozen

at -30° C for 24 h to remove any microbial cells adhering to the particles. The bags were defrosted in a fridge at 4°C, washed with cold water, oven dried at 60° C for 48 h and weighed to estimate DM disappearance. Afterwards, the residues were analyzed to calculate NDF, ADF and CP digestibility. Organic matter digestibility (OMD) was estimated according to Aumont et al. [11] and metabolizable energy (ME) and net energy for lactation (NEL) was calculated according to Caceres and Gonzalez [12].

Statistical Analysis and Calculations

An analysis of variance of double classification and comparison of submultiple ranges was used. For the normal distribution of the data, Kolmogorov-Smirnov tests Massey [13] and the homogeneity of the variances Bartlett [14] was performed.

Results

As can be seen in Table 1, the chemical composition of the *Jatropha curcas*, the dry matter content, NDF, ADF, ADL, increase with age with its best results at 180 days with 26.75; 48.69; 32.85 and 20.47%; while the CP and cellular content decrease with their highest results at 60 days 24.32 and 57.44% respectively. On the other hand, minerals showed a very variable behavior. During the dry season, the chemical composition of the *Jatropha curcas* Table 2 maintained a similar behavior to the rainy season, but with

different values, the dry matter content, NDF, ADF, ADL, increased with age with its best results. 180 days with 28.82; 51.53; 33.44 and 20.22%; while the CP and cellular content decrease with their highest results at 60 days 27.78 and 58.79% respectively. On the other hand, minerals showed a very variable behavior. The highest energy contribution and digestibility percentage at 60 days of age (Table 3) with a decrement for IVDMD, TD, ISDMD, ME and NLE of 11.04; 7.40; 8.90%; 1.58 and 1.11 MJ / kg up to 180 days, respectively, is due to differences in tissue types and characteristics independently of each plant, since depending on the constitution of the cell wall, its digestibility varies; From 100% in mesophilic cells to 0% in the xylem, this variation occurs in different tissues within a part of the plant and between similar tissues in different forage species. During the dry season, the contribution of energy and digestibility (Table 4) maintained a similar behavior to the rainy season, with a decrease of 1.52, 4.63, 44.26 percentage units for IVDMD, TD, ISDMD and 0.2, 0.15 MJ / Kg of dry matter for ME and NLE. Regarding the behavior of polyphenolic compounds during the rain (Table 5), these increase with age with the best results at 180 days with 11.26, 21.10, 113.93, 111.49 and 2.46 g / Kg, respectively; for total tannin, total phenol, TCT, TCTA and FCT. While during the low rainfall these indicators also showed an increase of 10.55, 0.56, 32.14, 31.03 and 1.12 g / Kg, respectively from 60 to 180 days for total tannins, total phenols, total condensed tannins, total bound condensed tannins and free condensed tannins (Table 6).

Table 1: Chemical composition of the *Jatropha curcas* during rainy season.

Age (day)	MD (%)	CP (%)	Ca (%)	P (%)	Mg (%)	Si (%)	NDF (%)	ADF (%)	ADL (%)	CEL (%)	HCEL (%)	CC (%)
60	17.5c	24.32a	2.41b	0.016a	0.67c	3.00b	42.56c	26.24c	6.92c	19.51c	16.12	57.44a
120	22.09b	16.64b	2.89a	0.011a	0.92a	4.62a	45.25b	28.64b	16.38b	12.26b	16.61	54.75b
180	26.75a	16.66b	2.74a	0.006b	0.86b	2.66c	48.69a	32.85a	20.47a	12.38a	15.84	51.31c
SE±	1.456	0.459	0.014	0.003	0.001	0.43	2.789	1.369	0.963	0.753	0.843	1.493

Table 2: Chemical composition of the *Jatropha curcas* during dry season.

Age (day)	MD (%)	CP (%)	Ca (%)	P (%)	Mg (%)	Si (%)	NDF (%)	ADF (%)	ADL (%)	CEL (%)	HCEL (%)	CC (%)
60	20.04c	27.78a	2.27b	0.015a	0.62a	4.95a	43.41c	24.96c	8.08c	16.34a	21.79a	58.79a
120	25.12b	21.65b	2.62a	0.010b	0.46c	4.84a	48.42b	30.93b	15.82b	15.08a	17.45b	51.55b
180	28.82a	19.84c	2.05c	0.013a	0.57b	4.27b	51.53a	33.44a	20.22a	13.14b	18.14b	48.38c
SE±	1.664	0.743	0.009	0.001	0.001	0.519	3.126	1.489	1.003	0.963	1.489	1.963

a.b.c Means with different superscript in file differ at (p<0.05)

Table 3: Digestibility and energy intake of *Jatropha curcas* during the rainy season.

Age (day)	IVDMD (%)	TD (%)	ISDMD (%)	ME (MJ/Kg)	NEL (MJ/Kg)
60	73.37a	82.77a	87.85a	10.86a	6.59a
120	70.86b	81.69b	85.17b	10.51a	6.35a
180	62.33c	75.37c	78.95c	9.28b	5.48b
SE±	1.359	0.289	0.789	0.045	0.0357

Table 4: Digestibility and energy intake of *Jatropha curcas* during the dry season.

Age (day)	IVDMD (%)	TD (%)	ISDMD (%)	ME (MJ/Kg)	NEL (MJ/Kg)
60	70.30a	82.79a	85.49a	10.41a	6.28a
120	68.92b	79.44b	44.13b	10.23ab	6.15ab
180	68.78c	78.16c	41.23c	10.21b	6.13b
SE±	0.29	0.4803	4.903	0.0407	0.0287

Table 5 : Polyphenol content of the *Jatropha curcas* during the rainy season.

Age (day)	Total tannins	Total phenol (g/Kg)	TCT	TCTA	FCT
60	6.57c	11.35c	65.11c	63.17c	1.94b
120	7.86b	12.83b	72.37b	70.78b	1.58c
180	11.26a	21.10a	113.93a	111.49a	2.46a
SE±	0.479	1.04	5.226	5.04	0.086

Table 6: Polyphenol content of the *Jatropha curcas* during the dry season.

Age (day)	Total tannins	Total phenol (g/Kg)	TCT	TCTA	FCT
60	24.15c	47.33b	65.89c	64.29b	1.59c
120	29.99b	40.19c	66.49b	64.60b	1.88b
180	34.70a	47.89a	98.03a	95.32a	2.71a
SE±	1.047	3.642	0.8507	3.531	0.1145

a.b.c Means with different superscript in file differ at (p<0.05).

Discussion

This behavior was given Tables 1 & 2 among other aspects by the aging of the plant and with it the increase of the structural components of this or that was related reduction of the synthesis of protein compounds, to the decrease of the amount of leaves,

to the increase of the fraction stem and increase in the synthesis of structural carbohydrates (cellulose, hemicellulose and lignin) Verdecia et al. [15]. The decrease in protein levels with age may be related to the reduction of the synthesis of protein compounds, due to the decrease in the number of leaves and increase in the proportion of stems Verdecia et al. [16]. The levels reached in this investigation are comparable to the concentrations in most of the trees used in the tropics Pedraza et al. [17], specifically, are higher than the values obtained Garcia et al. [18,19], when evaluating different cultivars of this species and similar to those obtained by Verdecia et al. [20] in the Cauto Valley; For this reason, the evaluated species can be used as a protein supplement in ruminant diets. The fluctuations found in the minerals (Ca, Si) in both climatic seasons of this species in the present study, in calcium are consistent with those reported for numerous [21]forage trees Garcia et al. [19]. On the other hand, the silica values are similar to those obtained by Parra et al. [22] in tropical legumes. This response pattern could be attributed according to Chavez et al. [23] and Verdecia et al. [24] to the variability of the soil-climatic conditions (solar radiation, temperature, rainfall and soil) aspects that influence the assimilation and distribution in the different parts of the plants, and changes in the concentration of these elements are encouraged. The increase in Si, ADF, ADL and cell wall with age could be related to the physiological and anatomical changes that occur as the plant ages, which causes a decrease in the proportion of cytoplasmic content, cell lumen is reduced with its components soluble and increase fibrous compounds Nogueira-Filho et al. [25]. This is accentuated as the DM increases, due to the water balance of the plant and the amount of nitrogen available in the soil, among other factors.

On the other hand, Ramirez et al. [26] stated that depending on the type of tissue, as the cell of the plant matures, the cell wall widens and, commonly, produces a secondary wall of different composition, with notable deposition of aromatic constituents, so chemical and anatomical changes that affect digestibility. This was related to the fact that in the younger ages there is greater succulence of the leaves and a high amount of tender stems Bayoli et al. [27]. Hence, at an early age these species presented high percentages of digestibility. Degradability differences Tables 3 & 4 could be associated with the characteristics of each species and genus. The relationship between chemical indicators and ruminal degradability must be kept in mind, which has been described by numerous authors for various legumes Pedraza et al. [28]. The low percentages of IVDMD and ISDMD could be attributed to the concentration of NDF, ADL and Si sufficient to modify the attack of ruminal microorganisms on plant cells, as was demonstrated by Valenciana [29] in *C. purpureus* and that could be valid for legumes. Subsequently Verdecia et al. [20] reaffirmed this principle in *Lotus uliginosus*, *Tithonia diversifolia*, *Erythrina variegata*, *Gliricidia sepium* and *Leucaena leucocephala*. In this sense, Garcia [30]states that digestibility is due to the characteristics of the material of origin, age, phenology and period of the year, since these factors have a decisive influence on the tenors of the fibrous components.

These results corroborate what was stated by Harborne [31], Labrada et al. [32] and Garcia [30], where the appearance of secondary compounds is related to the age of the material Tables 5&6, the defense mechanisms of the plant and the effects of soil and climate. Santacoloma-Varon and Granados [33] in this species found low amount of tannins, which could be related to the fact that the samples were taken during the dry season and this can affect the photosynthetic rate and consequently the synthesis of secondary metabolites. A It is necessary to take into account that, although the volume and speed of biochemical reactions may increase with temperature, most chemical reactions in plants have a characteristic thermal optimum, which decreases at both higher and lower temperatures. This is due, in the first instance, to the fact that the enzymatic activity and integrity of cell membranes are affected by extreme temperatures Sierra [34].

These results coincide with those reported by Sosa et al. [35] in *L. leucocephala*. However, the concentration of TT (21.49 g / kg) found in this group can be a positive element when fully evaluating the antinutritional characteristics of the polyphenolic fraction of this species, since they are in the range where, possibly, the ruminal ecosystem is not affected and increases the probability of overpassing protein formation and would facilitate post-renal nitrogen digestibility Aerts et al. [36], Estevez et al. [37] and Olivares et al. [38] found low levels of TT in this plant (3.01 g / kg). This behavior at an early age describes that the species with the highest proportion of fiber have a high concentration of hydroxylated metabolites, aspects that coincide with the statements made by numerous authors about the negative effect of cell wall compounds and tannins on animal nutrition Kumar [39], Makkar [7]. Chew et al. [40], found lower concentrations of TF (17 g / kg) in the leaves than in the flowers in *L. leucocephala*, which could be related to the process of photosynthesis and its possible accumulation Bhattacharya et al. [41].

Conclusion

The results of the present investigation evidenced the marked effect of climatic factors and age on the quality of the *Jatropha*

curcas since, as the maturity of the forage progressed, its nutritional contribution decreased with a decrease in the CP, the nutrient content, digestibility and energy intake, as well as an increase in the fibrous fraction and the content of polyphenolic compounds.

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