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# Tannin concentration and degradation rate *in vitro* of *Morus alba* and *Hibiscus rosa-sinensis*

## Concentración de taninos y tasa de degradación *in vitro* de *Morus alba* e *Hibiscus rosa-sinensis*

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## Abstract

The study evaluated the influence of age (30, 60, 90 and 120 days) on dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), free condensed tannins (FCT), fiber-bound condensed tannins (FBCT), protein-bound condensed tannin content (PBCT), *in vitro* degradation rate of DM ($DM_{kd}$), CP ($CP_{kd}$) and NDF ($NDF_{kd}$) of *Morus alba* (MA) and *Hibiscus rosa-sinensis* (HRS). For MA leaves, there was a quadratic ($p < 0.01$) relationship between CP content and age: the CP content of 60, 90 and 120 days-old leaves was similar, but it was lower than that of 30 days-old leaves. For HRS leaves, CP content decreased linearly ($p < 0.01$) as age increased, and NDF content increased linearly ($p < 0.01$). For both MA and HRS, there were cubit ($p < 0.01$) effects of age on FCT, FBCT and PBCT content, in which the highest values were found in 120-d-old leaves and the lowest in 30 and 90 d-old leaves. Furthermore, old leaves had a linear decrease in $DM_{kd}$, $CP_{kd}$, and $NDF_{kd}$. $DM_{kd}$, $CP_{kd}$, and $NDF_{kd}$ negatively correlated ($p < 0.001$) with FCT, FBCT, and PBCT content. For both MA and HRS, 90 days-old leaves had higher nutritional value than 120 days-old leaves.

**Keywords:** *Morus alba*; *Hibiscus rosa-sinensis*; protein; tannins; degradation.

## Resumen

En el estudio se evaluó la influencia de la edad (30, 60, y 120 días) de la materia seca (MS), proteína cruda (PC), fibra detergente neutro (FDN), taninos condensados libres (TCL), taninos condensados ligados a la fibra (TCFB), contenido de taninos condensados ligados a proteínas (TCPB), velocidad de degradación *in vitro* de la MS (VDkd), PC (PCkd) y FDN (FDNkd) de Morus alba (MA) e Hibiscus rosa-sinensis (HRS). Para las hojas de MA, hubo una relación cuadrática ($p < 0.01$) entre el contenido de PC y la edad: el contenido de PC de las hojas de 60, 90 y 120 días fue similar, pero menor que el de las hojas de 30 días. Para las hojas de HRS, el contenido de PC disminuyó linealmente ($p < 0.01$) a medida que aumentaba la edad, y el contenido de FND aumentó linealmente ($p < 0.01$). Tanto para MA como para HRS hubo efectos cúbicos ($p < 0.01$) en la edad sobre el contenido de TCL, TCFB y TCPB, en los cuales los valores más altos se encontraron en hojas de 120 días y las más bajas en hojas de 30 y 90 días. Además, las hojas viejas tuvieron una disminución lineal en MSkd, PCkd y FDNkd. MSkd, PCkd y FDNkd se correlacionaron negativamente ($p < 0.001$) con el contenido de TCL, TCFB y TCPB. Tanto para MA como para HRS, las hojas de 90 días tuvieron un valor nutricional mayor que las hojas de 120 días.

**Palabras clave:** *Morus alba; Hibiscus rosa-sinensis*; proteína; taninos; degradación.

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## Introduction

Forage is the most important source of feed for ruminants in tropical areas. In these regions, forage yield and nutritive value vary throughout the year due to climate and inadequate land management. In this scenario, the seasons of the year are an important issue: during the rainy season there is surplus forage; whereas during the dry season, forage availability is low (Hernández, Martínez, Mena, Pérez & Enriquez, 2002). However, good land management can increase yield and nutritive value of bushes and shrubs. In this respect, the use of alternative species of trees and shrubs (*Hibiscus rosa-sinensis* and *Morus alba*) can provide high amounts of feed biomass (30 t/ha and 21 t/ha per year) and high protein content, more than 19% compared with 8% in tropical grasses. This makes them an option as ruminant feed during the dry season. Furthermore, the yield and nutritive value of these plants is persistent (Bolio, Lara, Magaña & Sanginés-García, 2006; Kandylis, Hadjigeorgiou & Harizanis, 2009). Both *Morus alba* and *Hibiscus rosa-sinensis* are suited to the tropics. They capture great quantities of solar energy and, with adequate moisture, they grow fast and can produce high amounts of biomass during the year. They also reduce erosion and improve soil structure and quality, while allowing other crops to grow under their foliage (Ainalis & Tsiouvaras, 1998). Information in Mexico on MA and HRS is disperse and based on agricultural studies that do not consider nutritional value for animals or the secondary metabolites that affect digestibility. The condensed tannins and secondary metabolites of some plants may modify nutritional value since they bond with proteins and other macro molecules. This is manifested in reduced digestibility of nitrogenous nutriments (Mueller-Harvey, 2001; Silanikove, Perevoltsky & Provenza, 2001) and other cell components. In ruminants, these changes in digestibility are important at the ruminal level because ruminal microorganisms are unable to metabolize those complexes (López, Tejada, Vázquez, Garza & Shimada, 2004; McSweeney, Palmer, McNeil & Krause, 2001). The aim of the present study was to determine changes in MA and HRS dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), free condensed tannins (FCT), condensed tannins attached to proteins (PBCT), and condensed tannins attached to fiber (FBCT), in four growth stages, and how they affect digestion rate.

## Materials and Methods

The present experiment was conducted during the period of February to September 2017 in the animal nutrition laboratory of the Colegio of Postgraduados, in Cárdenas, Tabasco, located between 18° 17′ and 17° 50′ N and 93° 17′ and 99° 08′ W. The climate is classified as Am(f) W(i), with abundant rain during the summer. Fields of MA and HRS were established in June 2017. The plant material used was cuttings of 20 cm length. The cuttings were planted one meter apart from each other in furrows separated one meter. Samples were taken at four different stages of growth (30, 60, 90, 120 days of age). From the forage collected, a 5 kg sample was taken (100% leaf), washed and placed in bags with tags. Samples were dried in a forced air oven at 55 °C for 48 h and then ground in a mill Wiley (1 mm screen diameter). The following variables were analyzed: DM and CP, according to the Association of Official Analytical Chemists (AOAC) (1990), and NDF, according to Van Soest, Robertson & Lewis (1991). The potential digestible fraction of each component was calculated; 72 h was used as a reference time, and ruminal degradation constants of the fractions were determined as the end point of digestion with a prime order kinetic model (Mertens, 1973). The degradation rate of each component was obtained by associating the incubation time with non-degraded substrate. Disappearance of potentially degradable components was expressed in a logarithm base with the aim of fitting the results to a straight line and establishing a linear regression between the non-degraded substrate and time. Thus, the regression coefficient line obtained was from the degradation rate ($K_d$), applying the natural logarithm to the potentially digestible fraction that was not digested at the different incubation times (3 h, 6 h, 9 h, 12 h, 24 h, 48 h and 72 h).

To determine condensed tannins, the technique described by Terrill, Rowan, Douglas & Barrey (1992) was used with the modifications applied by López *et al*. (2004). Free condensed tannin (FCT) extraction was carried on in triplicate. One gram of the ground forage sample was weighed and placed in a flask on a centrifuge with 20 mL of a 0.1% ascorbic acid solution in acetone-water 7:3 v/v. The flasks were shaken and centrifuged for 15 min. Ethylic ether (30 mL) was added to the supernatant, which was shaken and left in repose. Two phases were formed; the lower phase was suctioned at 35 ºC to eliminate residues from the solvents and centrifuged at 18 000 G for 15 min. The supernatant was diluted in 50 mL of distilled water for evaluation. To extract the PBCT, the residues from the first extraction were added to 15 mL of dodecyl sodium sulfate (DSS), 10 g $L^{-1}$, 2-mercaptoethanol, 50 g $L^{-1}$, and 10 Mm of tris chloride to obtain pH 8.0 with hydrochloric acid. The forage residues in DSS were placed in a water bath and kept at the point of ebullition for 45 minutes. The flasks were cooled and centrifuged at 18 000 G for 15 min. The supernatant was placed in another flask and centrifuged. This process was repeated, and the supernatant combination was centrifuged at 18 000 G for 15 min to eliminate the phenol residues. The solution was diluted to a volume of 50 mL with DSS and stored in amber colored glass containers. To extract the FBCT, 500 mg of PBCT solid residue was placed in a flask with 1 mL of DSS and 6 mL of butane-hydrochloric acid. The flask was put into a boiling water bath for 75 min and cooled down. The solution was filtered through filter paper to separate the non-tannin residues and read in a spectrophotometer at 550 nm. In addition, controls were run with a butane-HCL solution. Standards were prepared, and the curves were made using purified tannins from *Flemigia macrofila*, according to Hagerman & Butler (1991). Tannin concentrations per mL were 0 µg, 100 µg, 200 µg, 300 µg, 400 µg and 500 µg from a standard solution of 550 µg $mL^{-1}$ tannin. To determine the FBCT and PBCT, the curve was constructed with DSS. Curve estimation was analyzed with the absorbance values given by the concentration of condensed tannin from *Flemigia macrophylla* and from catechin.

A complete randomized design with a 2x4 factorial was used: two shrubs and four harvest periods. The statistical analysis of the data was performed using PROC MIXED (SAS, 2002). Canonical correlation was applied to degradation rate with respect to tannin concentrations. Means were also compared with the Tukey test.

## Results and Discussion

The results are shown in table 1. For MA leaves, there was a quadratic ($p < 0.01$) relation between CP content and age; that is, the CP content of 60, 90, and 120 days-old leaves was similar, but lower than that in 30 days-old leaves. For HRS leaves, as age increased, CP content decreased linearly ($p < 0.01$), and NDF content increased linearly ($p < 0.01$). For both MA and HRS, there were cubic ($p < 0.01$) effects of age on FCT, FBCT, and PBCT content, in which the highest values were found at 120 days and the lowest at 30 and 90 days. As the age of leaves increased, there was a linear decrease in $DM_{kd}$, $CP_{kd}$, and $NDF_{kd}$. The correlation analysis (table 2) indicated that $DM_{kd}$, $CP_{kd}$, and $NDF_{kd}$ were negatively correlated ($p < 0.001$) with FCT, FBCT, and PBCT content. For both MA and HRS, 90 days-old leaves had higher nutritional value than 120 days-old leaves.

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**Table 1.** Chemical composition and degradation rate of *Morus alba*.

| | Age (days) | | | | SEM | *p*-value | | |
|---|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | | L | Q | C |
| | **Chemical composition, g/kg DM** | | | | | | | |
| DM $^{L}$ | 229$^{d}$ | 278$^{c}$ | 295$^{b}$ | 495$^{a}$ | 13.6 | 0.003 | 0.06 | 0.07 |
| CP $^{Q}$ | 219a | 171$^{b}$ | 169$^{b}$ | 165$^{b}$ | 7.9 | 0.06 | 0.01 | 0.05 |
| NDF | 438 | 439 | 442 | 458 | 21.1 | 0.49 | 0.54 | 0.42 |
| FCT $^{C}$ | 2$^{b}$ | 4$^{a}$ | 3$^{ab}$ | 3$^{ab}$ | 0.1 | 0.06 | 0.05 | 0.002 |
| FBCT $^{C}$ | 3$^{b}$ | 7$^{a}$ | 4$^{b}$ | 6$^{a}$ | 0.1 | 0.14 | 0.05 | 0.004 |
| PBCT $^{C}$ | 6 | 8 | 5 | 9 | 0.4 | 0.11 | 0.07 | 0.009 |
| | **Degradation rate g/h** | | | | | | | |
| DM $^{L}$ | 73 | 57 | 50 | 40 | 2.4 | 0.004 | 0.05 | 0.17 |
| CP $^{L}$ | 63 | 55 | 50 | 40 | 3.1 | 0.006 | 0.06 | 0.08 |
| NDF $^{L}$ | 53 | 47 | 38 | 34 | 2.7 | 0.001 | 0.07 | 0.06 |

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; FCT, free condensed tannins; FBCT, free-bound condensed tannins; PBCT, protein-bound condensed tannins; SEM, standard error of means; L, linear; Q, quadratic; C, cubic effects of increasing age. $^{a-d}$, different letter in row means difference at $p < 0.05$.
**Source:** Authors' own elaboration.

**Table 2.** Chemical composition and degradation rate of *Hibicus rosa-sinensis*.

| | Age (days) | | | | SEM | *p*-value | | |
|---|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | | L | Q | C |
| | **Chemical composition, g/kg DM** | | | | | | | |
| DM $^{L}$ | 200$^{d}$ | 283$^{c}$ | 293$^{b}$ | 336$^{a}$ | 9.8 | 0.001 | 0.07 | 0.06 |
| CP $^{L}$ | 196$^{a}$ | 173$^{b}$ | 163$^{c}$ | 153$^{d}$ | 5.8 | 0.003 | 0.06 | 0.05 |
| NDF $^{L}$ | 326$^{d}$ | 365$^{c}$ | 385$^{b}$ | 406$^{a}$ | 11.9 | 0.004 | 0.54 | 0.42 |
| FCT $^{C}$ | 3$^{b}$ | 5$^{a}$ | 4$^{ab}$ | 5$^{a}$ | 0.1 | 0.08 | 0.07 | 0.005 |
| FBCT $^{C}$ | 3$^{b}$ | 4$^{ab}$ | 5$^{a}$ | 5$^{a}$ | 0.1 | 0.10 | 0.07 | 0.003 |
| PBCT $^{C}$ | 6$^{b}$ | 8$^{a}$ | 6$^{b}$ | 9$^{a}$ | 0.2 | 0.09 | 0.06 | 0.001 |
| | **Degradation rate g/h** | | | | | | | |
| DM $^{L}$ | 60$^{a}$ | 57$^{ab}$ | 54$^{bc}$ | 51$^{c}$ | 1.2 | 0.002 | 0.07 | 0.42 |
| CP $^{L}$ | 80$^{a}$ | 70$^{b}$ | 60$^{c}$ | 53$^{d}$ | 3.2 | 0.001 | 0.07 | 0.06 |
| NDF $^{L}$ | 48$^{a}$ | 44$^{ab}$ | 41$^{bc}$ | 38$^{c}$ | 2.3 | 0.002 | 0.06 | 0.05 |

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; FCT, free condensed tannins; FBCT, free-bound condensed tannins; PBCT, protein-bound condensed tannins; SEM, standard error of means; L, linear; Q, quadratic; C, cubic effects of increasing age. $^{a-d}$, different letter in row means difference at $p < 0.05$.
**Source:** Authors' own elaboration.

The nutritional value of shrubs and trees depends on environmental conditions and handling at harvest (Hernández *et al*., 2002). Good management of harvest frequency is very important to achieve high yields. Research on MA has shown that yield increases from 11 t DM ha/year to 16 t DM ha/year when the age at harvest is extended from 45 to 90 days (Sánchez, 2002). Similar results were obtained in the present study. For CP content, high values were found at 30 days: 219 g /kg and 196 g /kg DM for MA and HRS. These results agree with those reported for the same species by Bolio *et al*. (2006) and Kandylis *et al*. (2009). The differences in NDF between the species analyzed are due to plant morphological traits, age of re-sprouting and type of soil, as well as to harvesting techniques. At 30 days, 100% of the new sprouts were harvested; this, in turn, led to an increase in CP content and digestibility (Sánchez, 2002). In this study, concentrations of 2 g kg$^{-1}$ DM FCT and 3 g kg$^{-1}$ DM FCT were found in MA and HRS when harvested at 30 days. These concentrations are lower than those reported by Barry & McNabb (1999), who mention that forage with 5 g kg$^{-1}$ DM FCT can be used for animal feed, whereas FCT concentrations higher than 60 g kg DM can affect the ruminal microorganism and metabolism and lead to animal intoxication (Reed, 1995). Moreover, the low $K_d$ of CP was obtained by extending the harvest period from 30 to 60, 90 and 120 days in both species, this was probably due to the increased levels of PBCT and FCT in both species, which in turn, according to Reed (1995), can cause a reaction in the ruminal medium and affect protein degradation.

## Conclusion

The highest DM content was found in 90 and 120 days-old leaves of both MA and HRS species. The protein content and free condensed tannins decreased with age of MA and HRS. Ninety-day-old leaves of both MA and HRS had higher nutritional value.

## Referencias

Ainalis, A. B., & Tsiouvaras, C. N. (1998). Forage production of woody fodder species and herbaceous vegetation in a silvopastoral system in northern Greece. *Agroforestry Systems, 42*(1), 1-11. doi: https://doi.org/10.1023/A:1006112330453

Association of Official Analytical Chemists (AOAC). (1990). Official Methods of Analysis.. Washington, D.C. USA: Association of Official Analytical Chemists.

Barry, T. N., & McNabb, W. C. (1999). The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *British Journal of Nutrition, 81*(4), 263-272. doi: https://doi.org/10.1017/S0007114599000501

Bolio, O. R. E., Lara, L. P. E., Magaña, M. M. A., & Sanginés-García, J. R. (2006). Rose of china (Hibicus rosa-sinensis) forage production in accordance with harvest frequency and plant density. *Técnica Pecuaria en México, 44*(3), 379-388.

Hagerman, A. E., & Butler, L. G. (1991). Tannins and lignins. In: G. A. Rosenthal, & M. R. Berenbaum (Eds.). *Herbivores: Their interactions with secondary plant metabolites*. (pp. 360-388). USA: Academic Press. doi: https://doi.org/10.1016/C2009-0-03210-3

Hernández, G. A., Martínez, H. P. A., Mena, U. M., Pérez, P. J., & Enríquez, Q. J. F. (2002). Regrowth dynamics of Insurgente grass (Brachiaria brizantha Hochst. Stapf.) grazed at different allowances in the rainy season. *Técnica Pecuaria en México, 40*(2), 193-205.

Kandylis, K., Hadjigeorgiou, I., & Harizanis, P. (2009). The nutritive value of mulberry leaves (Morus alba) as a feed supplement for sheep. *Tropical Animal Health and Production, 41*(1), 17-24. doi: https://doi.org/10.1007/s11250-008-9149-y

López, J., Tejada, I., Vázquez Peláez, C., Garza, J. D., & Shimada, A. (2004). Condensed tannins in humid tropical fodder crops and their in vitro biological activity. Part 1. *Journal of The Science of Food and Agriculture, 84*(4), 291-294. doi: https://doi.org/10.1002/jsfa.1651

Mertens, D. R. (1973). Applications of theoretical mathematical models to cell wall digestion and forage intake in ruminants (doctoral thesis). Cornell University., Ithaca, N.Y. USA.

McSweeney, S. C., Palmer, B., McNeil, D. M., & Krause, D. O. (2001). Microbial interactions with tannins: nutritional consequences for ruminants. *Animal Feed Science and Technology, 91*(1-2), 83-93. doi: https://doi.org/10.1016/S0377-8401(01)00232-2

Mueller-Harvey, I. (2001). Analysis of hydrolysable tannins. *Animal Feed Science and Technology, 91*(1-2), 3-20. doi: https://doi.org/10.1016/S0377-8401(01)00227-9

Reed, J. D. (1995). Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science, 73*(5), 1516-1528. doi: https://doi.org/10.2527/1995.7351516x

Statistical Analysis Software (SAS). (2002). *SAS User´s Guide: Statistics (version 9.0)*. NC, USA: SAS Institute Inc.

Sánchez, M. D. (2002). *Mulberry for Animal Production: Proceedings of an electronic conference carried out between May and August 2000*. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO).

Silanikove, N., Perevolotsky, A., & Provenza, F. D. (2001). Use of tannin-binding chemicals to assay for tannins and their negative postingestive effects in ruminants. *Animal Feed Science and Technology, 91*(1-2), 69-81. doi: https://doi.org/10.1016/S0377-8401(01)00234-6

Terrill, T. H., Rowan, A. M., Douglas, G. D., & Barrey, T. N. (1992). Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grain. *Journal of the Science of Food and Agriculture, 58*(3), 321-329. doi: https://doi.org/10.1002/jsfa.2740580306

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Symposium: Carbohydrate methodology, metabolism and nutritional implications in dairy cattle. *Journal of Dairy Science, 74*(10), 3583-3597. doi: https://doi.org/10.3168/jds.S0022-0302(91)78551-2