Effect of re-growth age in the content of secondary metabolites from *Neonotonia wightii* in the Valle del Cauto, Cuba

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The effect of regrowth age (30, 45, 60, 75 and 90 d) in the content of secondary metabolites from *Neonotonia wightii* was evaluated through a random blocks design, with six replications. The experiment took place from January to March (dry season) and from May to July (rainy season), in a carbonated brown soil, without irrigation and fertilization. Total tannins, total phenols, total condensed tannins, total mixed condensed tannins, free condensed tannins, flavonoids, saponins, alkaloids, triterpenes and steroids increased ($P < 0.05$) with age and the highest values were obtained at 90 d (5.24; 10.07; 77.67; 76.72; 0.95; 3.29; 0.53; 0.30; 8.58 and 0.63 g/kg DM during May-June-July and 3.12; 8.24; 96.64; 94.68; 1.96; 3.99; 0.52; 0.30; 8.51 and 0.69 g/kg DM during January-February-March, respectively). It can be concluded that regrowth age and climatic factors had a marked effect on the concentration of secondary metabolites. It was demonstrated that while the plant was maturing, the tenors of secondary metabolites increased. Only total condensed tannins and total mixed condensed tannins surpassed the limits in which the ruminant fermentation could be affected. It is recommended not to use this plant with an regrowth age higher than 75 d old. A similar study is suggested to be conducted with this and other legumes, in different environmental conditions, and to determine the relation between these metabolites and the digestibility and intake of these plants.

Key words: *legumes, maturity stage, tannins, flavonoids, saponins, alkaloids*

The legume species, in a large amount, constitute an important feeding resource for ruminants due to their high content of crude protein and minerals. However, many plant species show secondary metabolites, like tannins, saponins, phenolic compounds and alkaloids, which can have a negative effect on the nutritional value of these forages. These substances are produced by the plant and deal with the defense against fungi, bacteria and virus and with the protection against ultraviolet radiation. Besides, it works as a mechanism for avoiding tissue dehydration. The presence of these components gives an unpleasant taste to plants, which, in many cases, leads to a low acceptance for its intake by the herbivorous animals (Santacoloma-Varón y Granados 2010).

In a general sense, the nitrogen-fixer species synthesize higher diversity of secondary structures, as a more advanced survival strategy regarding other plants belonging to the other botanical families, in which the desired effect can be achieved with the formation of few strong toxins (García 2004). This process has been improved for millions of years of plant evolution together with herbivorous animals, and constitutes an essential aspect for understanding chemo-taxonomical differences between plant species and their consequences in the nutritional level (Baldizán *et al*. 2006).

According to Baxter (1997), these metabolites act mainly in protein absorption and digestion, but also influence on carbohydrates digestion, use of minerals and vitamin bioavailability (García *et al*. 2006 and 2008 and Pinto *et al*. 2010).

The objective of this study was to determine the effect of regrowth age in the content of secondary metabolites from *Neonotonia wightii* in the Valle del Cauto, Cuba.

Materials and Methods

Research area. The study was carried out in the animal production area from Universidad de Granma, located at the South East of Cuba, Granma province, at 17.5 km from Bayamo city.

A *Neonotonia wightii* meadow, with two years of establishment and 98 % of purity, was used. The study was carried out from January to March (dry season) and from May to July (rainy season), during 2007-2008. These months were selected because they are representatives from both seasonal periods in the region (Ramírez 2010).

During January-February-March, precipitations were of 83.7 mm. Mean, minimum and maximum temperature registered values of 23.89, 18.28 and 31.41 ºC, respectively, and mean, minimum and maximum relative humidity values of 76.71, 43.92 and 97.13 %, respectively. During May-June-July, precipitations were of 309.88 mm; mean, minimum and maximum temperature reached values of 27.22; 22.23 and 35.17 ºC, respectively, and mean, minimum and maximum relative humidity values of 79.25, 49.96 y 96.17 %, respectively.

The soil was carbonated brown (Hernández *et al*. 1999), with a pH of 6.2. The total P_2O_5, K_2O and

N content was 2.4, 33.42 and 3.0 mg/100 g of soil, respectively and 3.6 % of organic matter (Dirección Provincial de Suelos y Fertilizantes de Granma 2007).

Treatments and experimental design. A random blocks design with six replications was applied. Treatments were the regrowth ages of 30, 45, 60, 75 and 90 d.

Experimental procedure. At the beginning of the evaluation in each period, a uniformity cut was made at 5 cm over the soil level (January and May for each trimester, respectively). Parcels of 25 m^2 were delimited, with 50 cm between them, for each of the regrowth ages. The field was not irrigated or fertilized during the experiment. The simple taking was performed through a total cut of the parcel in each treatment, removing 50 cm of border effect. In each parcel, 200 g of previously homogenized material were taken. This material was dried at environmental temperature, within a dark and ventilated room during 12 d. Later, it was grounded until it reached the size of a 1mm particle and it was stored in amber flasks until the moment of analysis. Previous each analysis, the material was homogenized and dried at 65 ºC in a forced air oven for 72 h (Herrera 2006) for removing residual humidity.

Chemical analysis. The analysis of total phenols and total tannins was carried out through the method of Folin-Ciocalteau, before and after the concentrate treatment of extracts with polyvinylpolypyrrolidone (Makkar 2003). Total condensed tannins, free condensed tannins and total mixed condensed tannins were determined according to the method of nbutanol/HCl/Fe3+ (Porter *et al*. 1986); flavonoids were stated according to Boham and Kocipai-Abyazan (1994); saponins, through the method described by Obdoni and Ochuko (2001); triterpenes, according to Jie-Ping and Chao-Hong (2006); steroids, according to the method described by Galindo *et al*. (1989), and total alkaloids, according to Muzquiz *et al*. (1994).

For the stachyose, raffinose and verbacose oligosaccharides, 1 g of the dry and ground sample was taken, and it was added 9mL of methanol 70%. It was heated for 20 minutes at 100 ºC, cooled at environment temperature and filtered with Whatman 42 paper. An amount of 0.2 mL of a solution of Carrez I (K_4Fe (CN) at 15% in distilled water) and Carrez II (Zn (CH_3COO) at 30 % in distilled water) were added, and it was filled until 10 mL with a solution of methanol 70 %. It was centrifuged at 4000 rpm for 15 min. A total of 5 mL were taken from the higher part and 5 mL of dichloromethane were added, and they were vigorously agitated in the vortex. The two phases were let to be defined and the superior one was taken with a pipette. Two more extractions with dichloromethane were carried out, like the one previously described. The sample was concentrated in a rotatory evaporator, and passed through a filter with 0.45 µm of pores. A HPLC (WATERS) of 2410, refraction index detector, was used for detection, through the use of the Empower Pro 2002 program. The chromatographic method used was the isocratic (constant flow) of 1 mL/min, with a mobile phase of water + sulfuric acid 0.01 N, 50 ºC of column temperature. The column used was of ionic inclusion: BioRad Amino HPX-87M of 300 mm x 7.8 mm.

Calculation and statistical analysis. An analysis of variance was performed, according to the experimental design and mean values were compared through the multiple range test of Duncan (1955). The test of Kolmogorov-Smirnov (Massey 1951) was used for the normal distribution of data and the test of Bartlett (1937) was used for the variances. The statistical package Statistica 8.0 for Windows (2007) was applied.

Results

During the trimester of May-June-July (table 1), concentrations of total tannins (TT), total phenols, total condensed tannins, total mixed condensed tannins and free condensed tannins increased ($P < 0.05$) with the regrowth age and reached the highest values at 90 d (5.24; 10.07; 77.67; 76.72 y 0.95 g/kg DM, respectively). There was an increase for all the indicators, when comparing 90 to 30 d, in 3.34; 4.53; 32.8; 32.15 and 0.65 g/kg DM.

During the trimester of January-February-March, total tannins (TT), total phenols (TP), total condensed tannins (TCT), total mixed condensed tannins (TMCT) and free condensed tannins (FCT) presented a similar pattern of age response to the previous period. For all the indicators, they increased when comparing 90 with

Table 1. Effect of re-growth age on the content of phenols and tannins of *Neonotonia wightii* in May-June-July

Age, d	Total tannins, g/kg	Total phenols, g/kg	Total condensed tannins, g/kg	Total mixed condensed tannins, g/kg	Free condensed tannins, g/kg
30	1.90[a]	5.54[a]	44.87[a]	44.57[a]	0.30[a]
45	2.16[b]	7.22[b]	51.87[b]	51.37[b]	0.50[b]
60	3.60[c]	8.55[c]	54.32[c]	53.68[c]	0.55[c]
75	4.57[d]	8.76[d]	64.17[d]	63.38[d]	0.79[d]
90	5.24[e]	10.07[e]	77.67[e]	76.72[e]	0.95[e]
EE±	0.24*	0.29*	2.11*	2.07*	0.04*

* $P < 0.05$. [abcde] Different letters in the same column differ at $P < 0.05$ (Duncan 1955)

30 d, in 1.32, 1.67, 36.50, 37.18 and 1.32 g/kg DM, respectively (table 2).

During the trimester of May-June-July (table 3), contents of flavonoids, saponins, triterpenes, and steroids increased with age ($P < 0.05$). The highest values were reached at 90 d. The same happened with alkaloids until 60 d of re-growth, for later to remain constant. The stachyose and raffinose decreased ($P < 0.05$) with age, while the verbacose decreased until 75 d, for later to remain constant. The higher tenors were obtained at 30 d of re-growth.

During the trimester of January-February-March, contents of flavonoids, saponins, alkaloids, triterpenes and steroids had a similar age response pattern, regarding May-June-July. The highest values were recorded at 90 d. However, oligosaccharides presented a different behavior: verbacose and raffinose increased ($P < 0.05$) with age, while stachyose increased until 60 d, and later started to decrease (table 4).

Discussion

The increase of total phenols, total tannins and total condensed tannins is related to the increase of biomass maturity and to the increase of lignin concentration (Makkar 2003). Wambui *et al*. (2006), when evaluating the chemical composition of *Tithonia diversifolia*, found values of 10.6 and 5.6 g/kg DM for total phenols and total tannins, respectively. These results are similar to those obtained in this study during the trimester of May-June-July.

Miller and Ehlke (1996) and McMahon *et al*. (2000), when studying the concentration of condensed tannins, stated that this compound is controlled, firstly, by genetic factors, and finally, by environmental variations. In

Table. 2. Effect of the regrowth age on the content of phenols and tannins of *Neonotonia wightii* in January-February-March

Age, d	Total tannins, g/kg	Total phenols, g/kg	Total condensed tannins, g/kg	Total mixed condensed tannins, g/kg	Free condensed tannins, g/kg
30	1.80[a]	6.57[a]	58.14[a]	57.50[a]	0.64[a]
45	2.03[b]	7.24[b]	67.14[b]	66.38[b]	0.75[b]
60	2.29[c]	7.48[c]	71.35[c]	70.49[c]	0.87[c]
75	2.85[d]	7.66[d]	75.69[d]	74.57[d]	1.13[d]
90	3.12[e]	8.24[e]	96.64[e]	94.68[e]	1.96[e]
EE±	0.09*	0.10*	2.38*	2.29*	0.09*

[abcde] Different letters in the same column differ at $P < 0.05$ (Duncan 1955) * $P < 0.05$

Tabla 3. Effect of the re-growth age on the content of oligosaccharides, flavonoids, saponins, alkaloids, triterpenes and steroids of *Neonotonia wightii* in May-June-July

Age, d	Verbacose, g/kg	Stachyose, g/kg	Raffinose, g/kg	Flavonoids, g/kg	Saponins, g/kg	Alkaloids, g/kg	Triterpenes, g/kg	Steroids, g/kg
30	0.0130[a]	0.0076[a]	0.0200[a]	1.18[a]	0.22[a]	0.20[a]	7.14[a]	0.42[a]
45	0.0073[b]	0.0068[b]	0.0140[b]	1.39[b]	0.30[b]	0.20[a]	7.52[b]	0.47[b]
60	0.0064[c]	0.0060[c]	0.0090[c]	1.94[c]	0.40[c]	0.30[b]	7.67[c]	0.52[c]
75	0.0044[d]	0.0130[d]	0.0073[d]	2.47[d]	0.49[d]	0.30[b]	7.99[d]	0.56[d]
90	0.0041[d]	0.0110[e]	0.0083[e]	3.29[e]	0.53[e]	0.30[b]	8.58[e]	0.63[e]
EE±	0.0006*	0.0005*	0.0009*	0.14*	0.02*	0.0094*	0.01*	0.01*

* $P < 0.05$. [abcde] Different letters in the same column differ at $P < 0.05$ (Duncan 1955)

Table 4. Effect of the re-growth age on the content of oligosaccharides, flavonoids, saponins, alkaloids, triterpenes and steroids of *Neonotonia wightii* in January-February-March.

Age, d	Verbacose, g/kg	Stachyose, g/kg	Raffinose, g/kg	Flavonoids, g/kg	Saponins, g/kg	Alkaloids, g/kg	Triterpenes, g/kg	Steroids, g/kg
30	0.0032[a]	0.0058[a]	0.0058[a]	1.30[a]	0.44[a]	0.20[a]	7.36[a]	0.45[a]
45	0.0033[b]	0.0090[b]	0.0091[b]	1.70[b]	0.21[b]	0.20[a]	7.54[b]	0.51[b]
60	0.0044[c]	0.0071[c]	0.0100[c]	2.09[c]	0.31[c]	0.30[b]	7.77[c]	0.56[c]
75	0.0058[d]	0.0059[d]	0.0130[d]	2.86[d]	0.42[d]	0.30[b]	8.05[d]	0.63[d]
90	0.0066[e]	0.0055[e]	0.0150[e]	3.99[e]	0.52[e]	0.30[b]	8.51[e]	0.69[e]
EE±	0.0003*	0.0002*	0.0006*	0.18*	0.02*	0.01*	0.08*	0.02*

* $P < 0.05$. [abcde] Different letters in the same column differ at $P < 0.05$ (Duncan 1955)

general, its concentration increased with maturity and it is related to the increase of lignin in the tissues, which can provoke a decrease of forage digestibility, when high values are reached. Besides, these authors pointed that high concentrations of total condensed tannins have been associated with the increase of lignin, because they found contents of 106 g/kg in those tannins and 132-152 g/kg of lignin in *Lotus pedunculatus*. The positive relation between them is owed to the biosynthesis common route of these two compounds.

Values of condensed tannins between 22.2 and 237.5 g/kg have been reported in forage legumes. Out of them, 90% are total mixed tannins and around 70 % are related to protein. That happens because if the concentration of total condensed tannins is high, the amount of them related to proteins will be high too, due to their affinity with this nutrient (Jackson *et al*. 1996). Terrill *et al*. (1992) obtained values of 22.2 and 33.6 g/kg in total phenols, and of 105-238 g/kg in total mixed condensed tannins, and they related them linked with the reduction of cell wall digestibility.

The differences presented by total condensed tannins, total mixed condensed tannins and free condensed tannins between the experimental periods, in this study, can be attributed to the variations of environmental conditions, plant nutrition and the probable reactions of tannins with other present compounds, among other factors (Makkar and Singh 1991). This was evident in a study carried out by Lees *et al*. (1995) in clones of *Lotus uliginosus*. These authors informed that total condensed tannins increased from 30 to 100 g/kg, when the temperature varied from 30 to 20 °C, respectively. Afterwards, concentrations from 80 to 110 g/kg were found in soils of low fertility in *Lotus pedunculatus*. They get to be four times higher than when the plants were developed in fertile soils (McMahon *et al*. 2000).

Except for condensed tannins, the values were under the amounts informed in the edible fraction of some legumes used in agro-silvopastoral systems in tropical areas (García 2004). In this study, the concentration of these compounds (except condensed tannins at 90 and 60 d in the periods May-June July and January-February-March, respectively) was inferior to the minimum concentration (total phenols, 40; total tannins, 40 and condensed tannins, 60 g/kg DM) informed by Makkar (2003), when the fermentation in the rumen started to be affected.

Chew *et al*. (2011) pointed the antioxidant and bactericide characteristics, among others, of some legumes used as medicinal plants in Malaysia, and related them with the possible content of phenolic contents. Therefore, it would be important to study this probable relation in plants used in Cuba for animal nutrition and human health.

The results found in verbacose and in stachyose turned to be inferior to those pointed by Vijayakumari *et al*. (2002) in *Dolichos lablab* (0.17 and 0.13 g/kg of stachyose and verbacose, respectively). In these non-reducing sugars, of low molecular weight, are reserve compounds, having variable amounts in plant organs and many plant seeds, included the legumes (Kadlec 2000). These oligosaccharides are also called "factors of flatulence" because they release considerable amounts of gases when they are fermented by the intestinal microflora.

The values of flavonoids found in this study were inferior to those of Verdecia *et al*. (2012) in *Leucaena leucocepha*, and are located in the range informed by Edeoga *et al*. (2005) for medicinal plants in Nigeria (from 1.5 to 9.8 g/kg). However, the relatively high concentrations of flavonoids are because this is one of the phenolic groups that offer protection to plants against solar radiation (Cerovic *et al*. 2002), due to its action as sun blocking in leaf epidermis, and protects the interior of the cell against the possible high radiations, which can be very harmful (Jordan 2002, Bassman 2004 and Morales *et al*. 2010).

There is a generalized agreement in scientific literature about that flavonoids concentration and the great number of polyphenolic compounds present drastic variations, with management and environmental factors (García 2004).

Although not all saponins are deleterious metabolites, after certain concentrations they act as intake inhibitors, have foaming properties, present a bitter flavor and constitute powerful toxic concentrations for digestive metabolism (García 2004 and Baldizán *et al*. 2006). Nevertheless, concentrations found in the evaluated specie were similar to those found in soybean meal and in the foliage of other plants consumed by cattle, without difficulties, under natural conditions (Sotelo *et al*. 1995 and Makkar 2003).

Tenors of alkaloids (0.5-1g/kg DM), informed by Sotelo *et al*. (1996) and García *et al*. (2008), are considered as intermediate in some forage legumes. Taking into account the previously mentioned, this group of compounds should not cause changes in the proper development of ruminants. Therefore, the results of 0.30 g/kg in this study may be considered as low, and support the hypothesis that sources of non proteic nitrogen are spread in most of plant organisms, mainly in dicotyledonous plants (Sotelo *et al*. 1996). The values from the evaluated plant turned to be similar to the typical concentrations of many species that does not cause toxicity.

Medina *et al*. (2009) informed 10-14 g/kg of triterpenes and stated the difficulty of comparing them because of the low availability of literature that describes this metabolite. However, Mossi *et al*. (2009), when studying the content of triterpenes in *Maytenus ilicifolia* in 16 zones of Brazil, found concentrations from 8.67 to 28.56 g/kg, but they did not find a correlation between the tenor of triterpenes and environmental variables. Therefore, these authors concluded that the variation of

this type of metabolite depends on the characteristics of each population.

Semmar *et al*. (2011) stated the variability and ecological meaning of these secondary metabolites in biosystems, while Karolewski *et al*. (2011) informed that the variability of phenolic compounds was mainly determined by age and their location in different parts of the plant, aspects reflected in this study.

The results of this research evidenced the marked effect of age and climatic factors on the production of secondary metabolites because, in both seasons, similar response patterns to the re-growth age were obtained, but with specific values in each season. However, it was demonstrated that while the plant was maturing, the concentration of secondary metabolites increased. Only total condensed tannins and total mixed condensed tannins surpassed the limits in which the ruminant fermentation can be affected. This aspect has to be considered in the management of this specie, when it is used in animal feeding. Nevertheless, it would not be appropriate, according to the values of the indicators analyzed in this study, to use *Neonotonia wightii* with a re-growth age superior to 75 d, although future multidisciplinary studies are needed for endorsing the optimal re-growth age

Conclusions

Further researches about this specie and about other legumes in different edapho-climatic conditions, with the division of seasons (rainy and dry) in trimesters, are suggested, because of the variations produced in the environmental conditions in each of them. This way, knowledge about the behavior of secondary metabolites would be widen, with an emphasis on phenolic compounds. It would be advisable to study the possible relation between these metabolites and digestibility and intake of this plant.

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References

Baldizán, A., Domínguez, C., García, D.E., Chacón, E. & Aguilar, L. 2006. Metabolitos secundarios y patrón de selección de dietas en el bosque deciduo tropical de los llanos centrales venezolanos. Zootecnia Trop. 24:213

Bartlett, M. 1937. Properties of sufficiency and statistical tests. Proceedings of the Royal Society of London. Ser. A; 160:268

Bassman, J.H. 2004. Ecosystem consequences of enhanced solar ultraviolet radiation: secondary plant metabolites as mediators of multiple trophic interactions in terrestrial plant communities. Photochem. Photobiol. 79:382

Baxter, N. J. 1997. Multiple interactions between poliphenols and salivary proline rich protein result in complexation and precipitation. J. Biochem. 36:503

Boham B.A. & Kocipai-Abyazan R. 1994. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycynium*. Pac. Sci. 48:458

Cerovic, Z.G., Ounis, A., Cartelat, A., Latouche, G., Goulas, Y., Meyer, S. & Moya, I. 2002. The use of chlorophyll fluorescence excitation spectra for the non-destructive *in situ* assessment of UV-absorbing compounds in leaves. Plant Cell Environ. 25:1663

Chew, Y., Chan, E., Tan, P., Lim, Y., Stanslas, J. & Goh, J. 2011. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medical plants in Peninsular Malaysia. Available: htto://www.biomedcentral.com/1472-6882/11/12. [Consulted: December 12th, 2012]

Dirección de Suelos y Fertilizantes de Granma. 2007. Caracterización de los suelos de la provincia Granma. Ministerio de la Agricultura. Cuba

Duncan, D. B. (1955). Multiple range and multiple F test. Biometrics 11:1

Edeoga, H.O., Okwu, D.E. & Mbaebie, B.O. 2005. Phytochemical constituents of some Nigerian medicinal plants. African J. Bio Tech. 4:685

Galindo, W., Rosales, M., Murgueitio, E. & Larrahondo, J. 1989. Sustancias antinutricionales en las hojas de árboles forrajeros. Livest. Res. Rural Devel.1:36

García, D.E. 2004. Principales factores antinutricionales de las leguminosas forrajeras y sus formas de cuantificación. Pastos y Forrajes. 27:101

García, D.E., Medina, M.G., Humbría, J., Domínguez, C.E., Baldizán, A., Cova, L.J. & Soca, M. 2006. Composición proximal, niveles de metabolitos secundarios y valor nutritivo del follaje de algunos árboles forrajeros tropicales. Arch. Zootecnia 55:373

García, D.E., Medina, M.G., Clavero, T., Cova, L. J., Domínguez, C. & Baldizán, A. 2008. Caracterización nutritiva del follaje de seis especies forrajeras con énfasis en sus perfiles polifenólicos. Rev. Científica FCV-LUZ 18:188

Hernández, A., Pérez, J.M. & Boch, D. 1999. Nueva versión de la clasificación genética de los suelos de Cuba. Ministerio de la Agricultura. Ciudad de la Habana, Cuba. p.64

Herrera, R.S. 2006. Fisiología, calidad y muestreos. En: Fisiología, producción de biomasa y sistemas silvopastoriles en pastos tropicales. Abono orgánico y biogás. Eds. Herrera, R.S., Rodríguez, I. y Febles, G. EDICA. Instituto de Ciencia Animal, La Habana, Cuba, p.89

Jackson, F., Barry, T., Lascano, C. & Palmer, B. 1996. The extractable and bound condensed tannin content of leaves for tropical tree, shrub and forage legumes. J. Sci. Food Agric. 17:103

Jie-Ping, F. & Chao-Hong, H. 2006. Simultaneous quantification of three major bioactive triterpenese acids in the leaves of *Diospyros kaki* by high-perfomance liquid chromatography method. J. Pharmaceutical and Biomedical Analysis. 41: 950

Jordan, B.R. 2002. Molecular response of plant cells to UV-B stress. Funct. Plant Biol. 29:909

Kadlec, P. 2000. Carbohydrate chemistry. In: Carbohydrates in grain legumes seeds, Improving nutritional quality and agronomic characteristics. Hedley, C.L. (Ed.), CABI Publishing, Norwich, UK, p.15

Karolewski, P., Jagodziński, A.M. & Grzebyta, J. 2011. Influence of tree age, needle age and location in the crown on the phenolic compounds content in needles of young Scots pines. Sylwan 155:797

Lees, G. L., Gruber, M. Y. & Suttill, N. H. 1995. Condensed tannins in sainfoin. II. Occurrence and changes during leaf development. Can. J. Bot. 73: 1540–1547

Makkar, H.P.S. 2003. Quantification of tannins in tree and shrub foliage. Kluwer Academic Publishers. Netherlands. A laboratory manual. 102 p.

Makkar, H.P.S. & Singh, B. 1991. Distribution of condensed tannins (proanthocyanidins) in various fibre fractions in young and mature leaves of some oak species. Anim. Feed Sci. Tech. 32:253

Massey, F. J. 1951. The Kolmogorov-Smirnov test for goodness of fit. J. Amer. Stat. Assoc. 68:78

McMahon, L., McAllister, T., Berg, B., Majak, W., Achanrya, S. & Popp, J. 2000. A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. Can. J. Plant Sci. 80:469

Medina, M., García, D., González, M., Cova, L. & Moratinos, P. 2009. Variables morfo-estructurales y de calidad de la biomasa de *Tithonia diversifolia* en la etapa inicial de crecimiento. Zoot. Trop. 27:121

Miller, P. R. & Ehlke, N. J. 1996. Condensed tannins in birdsfoot trefoil: genetic relationships with forage yield and quality in NC-83 germplasm. Euphytica 92:383

Morales, L.O., Tegelberg, R., Brosché, M., Keinänen, M., Lindfors, A. & Aphalo, P.J. 2010. Effects of solar UV-A and UV-B radiation on gene expression and phenolic accumulation in *Betula pendula* leaves. Tree Physiology. 30: 923

Mossi, A.J., Mazutti, M., Paroul, N., Corazza, M.L., Dariva, C., Cansian, R.L. & Oliveira, J.V. 2009. Chemical variation of tannins and triterpenees in Brazilian populations of *Maytenus ilicifolia* Mart. Ex Reiss. Braz. J. Biol. 69:339.

Muzquiz, M., Cuadrado, C., Ayet, G., De la Cuadra, C., Burbano, C. & Osagie, A. 1994. Variation of alkaloid components of lupin seeds in 49 genotypes of *Lupinus albus* L. from different countries and location. J. Agric. Food Chem. 42:1447

Obdoni, B.O. & Ochuko, P.O. 2001. Phytochemical Studies and Comparative Efficacy of the Crude Extract of some Homostatic Plants Plants in Edo and Delta States of Nigeria. Glob. J. Pure Appl. Sci. 8:203

Pinto, R., Hernández, D., Gómez, H., Cobos, M.A., Quiroga, R & Pezo, D. 2010. Árboles forrajeros de tres regiones ganaderas de Chiapas, México: Usos y características nutricionales. Universidad y Ciencia Trópico Húmedo 26:19

Porter, L., Hrstich, L. & Chan, B. 1986. The conversion of procyanidins and prodelphinidins to cianidin and delphidin. Phychem. 25:223

Ramírez, J.L. 2010. Rendimiento y valor nutritivo de cinco gramíneas en el Valle del Cauto. Tesis Dr. Universidad de Granma, Cuba

Santacoloma-Varón, L.E & Granados, J. 2010. Evaluación del contenido de metabolitos secundarios en dos especies de plantas forrajeras encontradas en dos pisos térmicos de Colombia. Revista de Investigación Agraria y Ambiental. 1:31

Semmar, N., Nouira, S. & Farman, M. 2011. Variability and ecological significances of secondary metabolites in terrestrial biosystems. Environmental Res. J. 5:213

Sotelo, A., Contrera, E. & Flores, S. 1995. Nutritional value and content of antinutritional compounds and toxics in ten wild legumes of Yucatan Peninsula. Plant Food. 47:115.

Sotelo, A. Soto, M. & Lucas, B. 1996. Comparative studies of the alkaloids composition of two Mexican Erythrina species and nutritive value of the detoxified seeds. J. Agric. Food Chem. 41: 2340

Verdecia, D., Herrera, R.S., Ramírez, J.L., Leonard, I., Álvarez, Y., Bazán, Y., Arceo, Y., Bodas, R., Andrés, S., Álvarez, J., Giradles, F. & López, S. 2012. Valor nutritivo de *Leucaena leucocephala* con énfasis en el contenido de metabolitos secundarios. REDVET, 13 (11)

Vijayakumari, K., Smitha, K.B. & Janardhanan, K. 2002. Biochemical characterization of the tribal pulse, *Mucuna utilis* Wall ex.Wight seeds. J. Food Sci. Tech. 39:3650

Wambui, C., Abdulrazak, S. & Noordin, Q. 2006. The effect of supplementing urea treated maize stover with Tithonia, Calliandra and Sesbania to growing goats. Livestock Res. Rural Devel. 18:117

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