Effect of the re-growth age on the nutritive quality of *Neonotonia wightii* in the Cauto valley, Cuba

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Through a completely randomized design with six replicates, the effect of the re-growth age (30, 45, 60, 75 and 90 d) on the nutritive quality of *Neonotonia wightii* was assessed, as well as the relation between its digestibility, ME and tannins. The experiment was conducted during the trimesters January-February-March (dry season) and May-June-July (rainy season), on a brown soil with carbonate, without irrigation or fertilization. The DM, silica (Si), NDF, ADF and lignin increased ($P < 0.05$) with age and the highest values were obtained at 180 d, while the CP, DM *in vitro* digestibility and ME diminished ($P < 0.05$). The highest values were reached at 30 d. The total phenols, total tannins and total condensed tannins increased ($P < 0.05$) with the re-growth age in both seasons and reached the maximum concentrations at 90 d (10.7, 5.24 and 77.66 g/kg DM in May-June-July and 8.24, 3.12 and 96.64 g/kg DM in January-February-March, respectively). In both seasons, high negative correlations (between -0.82 and -0.99) were obtained between the total phenols, total tannins and total condensed tannins and the DM *in vitro* digestibility, OMD, DM *in situ* digestibility, true digestibility (TD) and ME. It is concluded that age had a marked effect on the indicators assessed, more highlighted in the rainy season, when the nutritive quality diminished due to the increase of the fiber fraction, diminishing of the protein, digestibility and ME, and due to the inverse relation between the two last indicators and the phenolic compounds. Studies on other legumes under different edafoclimatic and management conditions are suggested.

Key words: legume, phenolic compound, tannins, digestibility, energy.

The use of legumes in systems for animal feeding is a typical example of the alternative techniques referred by several authors, who defend their use due to their high nutritive value, protein and mineral richness and other nutritive principles (García *et al*. 2006).

The importance of legumes in a grassland from middle to low quality has been demonstrated when stimulating the ruminal microbial action and increasing the concentration of VFAt, N-NH_3, NNP-NA, N-microbial and N-plant and improving the animals' productivity (Elías *et al*. 2006).

The presence of toxic secondary metabolites may be a limitation to be considered in the use of legumes. They not only can interfere in the nutrients digestion and absorption processes but, before passing to the blood, they can have several systemic effects on the animals (Jackson *et al*. 1996). According to Baxter (1997), these metabolites act mainly on the digestion and absorption of proteins and influence also on the carbohydrates digestion, minerals use and vitamins bio-availability (García *et al*. 2006).

The objective of this study was to determine the effect of the re-growth age on the nutritive quality and the relation between digestibility, ME, and tannins in *Neonotonia wightii* in Cauto valley, Cuba.

Materials and Methods

Research area. The study was conducted in the animal production site of Granma University, located in the southeast of Cuba, Granma province, at 17.5 km away from Bayamo city.

A *Neonotonia wightii* grassland with two years of establishment was used. The study was carried out in January-February-March (dry season) and May-June-July (rainy season) during 2007-2008. These months were selected as they represent both seasons (Ramírez 2010).

During January-February-March, the rainfall was of 83.7 mm, and the middle, minimum and maximum temperature recorded values of 23.89, 18.28 and 31.41 ºC, respectively. The middle, minimum and maximum relative humidity was of 76.71, 43.92 and 97.13 %, respectively. In May-June-July, the rainfall reached 309.88 mm; the middle, minimum and maximum temperature recorded 27.22; 22.23 and 35.17 ºC, respectively. The middle, minimum and maximum relative humidity had averages of 79.25, 49.96 and 96.17 %, respectively.

The soil was brown with carbonate (Hernández *et al*. 1999), and pH of 6.2. The content of P_2O_5, K_2O and total N recorded values of 2.4, 33.42 and 3.0 mg/100 g of soil, respectively and 3.6 % of OM.

Treatments and experimental design. A random block design with six replicates was applied. The treatments were the re-growth age of 30, 45, 60, 75 and 90 d.

Experimental procedure. In each period, at the beginning of the assessment, a uniformity cut was conducted at 5 cm above the soil level (January and May for each trimester, respectively). Plots of 25 m^2 were marked out, with 50 cm between them, for each of the re-growth age. The soil was not irrigated or fertilized during the experiment. The samples collection was conducted throughout the total cut of the plot in each treatment. A total of 50 cm was eliminated of border effect. In each plot, 200 g of the material previously homogenized were taken and later dried at 65 ºC in a forced-air circulation oven for 72 h (Herrera 2006).

The percentages of DM, CP, MO and Si were determined according to AOAC (2000) and those of NDF, ADF and lignin, in agreement with Goering and van Soest (1970).

For the *in vitro* and true digestibility, the protocol recommended for the incubator DaisyII®, Fairport, NY-USA, (ANKOM Technology 2000) was followed. The samples were incubated for 48 h in the DaisyII®, at 39.2 ± 0.5 °C, with constant circular agitation. Later, the bags were washed with cold water to stop the fermentation and dried in an air-forced oven at 105 °C for an hour. Afterward, the NDF was determined to define the real digestibility of the feed. The *in situ* digestibility, at 72 h of incubation, was determined in agreement with the method of bag in the rumen described by Orskov *et al*. (1980). The OM digestibility was counted through Aumont *et al*. (1995). The ME was established according to Cáceres and González (2000).

The analysis of the total phenols and tannins was conducted by the method of Folin-Ciocalteau, before and after the treatment of the extracts with polyvinylpolypyrrolidone, as described by Makkar (2003). The total condensed tannins were counted by the method nbutanol/HCl/Fe_3+ (Porter *et al*. 1986).

Statistical analysis and calculations. Analysis of variance was carried out according to experimental design. The means were compared with the multiple range test of Duncan (1955). The test of Kolmogorov-Smirnov (Massey 1951) was used for the normal distribution of the data and that of Bartlett (1937) for the variances.

Linear correlation analysis was conducted between the *in vitro*, *in situ*, true, OM digestibilities and ME as dependant variables. The total phenols, total tannins and total condensed tannins were analyzed as independent variables in each season. Only the coefficients (r) were reported and the statistical software Statistics 8.0 for Windows (2007) was applied.

Results

During the trimester May-June-July, the DM, Si, NDF, ADF and lignin increased (P < 0.05) with the regrowth age and reached the highest values at 180 d (25.70, 3.99, 57.79, 31.80 and 14.08 %, respectively). In the CP, DM *in vitro* digestibility and ME, the performance was inverse (P < 0.05) and the best values were obtained at 30 d of regrowth (20.64 %, 64.37 % and 9.55 MJ/kg DM, respectively). These last indicators decreased when comparing 90 with 30 d, in 14.02 and 10.02 percent units and 1.53 MJ/kg DM, respectively. However, the firsts increased in 8.49, 2.33, 8.07, 4.33 and 8.27 percent units in that same order (table 1).

During January-February-March, the DM, CP, Si, NDF, ADF, lignin, DM *in vitro* digestibility and ME had similar response pattern than that of May-June-July. However, when comparing 90 with 30 d, the CP, DM *in vitro* digestibility and ME decreased in 7.32 y 6.99 percent units and 1.08 MJ/kg DM, respectively, while the DM, Si, NDF, ADF and lignin increased in 10.11; 1.01; 4.36; 7.86 and 8.24 percent units, respectively (table 2).

In both seasons, the total phenols, total tannins and total condensed tannins increased (P < 0.05) with the regrowth age and the highest values were recorded at 90 d (table 3).

In May-June-July (table 4) there were negative correlations (P < 0.05) between all the indicators. The coefficients (r) between the total condensed tannins and total tannins with the DM *in vitro* digestibility, OM digestibility, DM *in situ* digestibility and ME highlighted, that varied between -0.92 and -0.99 for the total condensed tannins and for the total tannins of -0.89 to -0.95. However, the relations with total phenols were lower (between -0.82 and -0.92).

During January-February-March, similar performance was recorded in the stage May-June-July, but the ratios with the total phenols were superior (table 5).

Table 1. Effect of the re-growth age on the nutritive quality of *Neonotonia wightii* on May-June-July

Age, d	DM, %	CP, %	Si, %	NDF, %	ADF, %	Lignin, %	DM *in vitro* digestibility, %	ME, MJ/kg DM
30	17.21[a]	20.64[a]	1.66[a]	49.72[a]	27.47[a]	5.81[a]	64.37[a]	9.55[a]
45	19.43[b]	18.68[b]	1.99[b]	52.92[b]	29.37[b]	7.16[b]	62.69[b]	9.34[b]
60	21.24[c]	17.68[c]	2.99[c]	53.41[c]	29.76[c]	7.97[c]	60.82[c]	9.07[c]
75	23.64[d]	14.84[d]	3.00[c]	56.27[d]	31.26[d]	12.12[d]	58.62[d]	8.70[d]
90	25.70[e]	6.62[e]	3.99[d]	57.79[e]	31.80[e]	14.08[e]	54.35[e]	8.02[e]
SE±	0.56*	0.91*	0.15*	0.52*	0.28*	0.58*	0.65*	0.10*

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

Table 2.Influence of re-growth age on the nutritive quality of *Neonotonia wightii* on January-February-March

Age, d	DM, %	CP, %	Si, %	NDF, %	ADF, %	Lignin, %	DM *in vitro* digestibility, %	ME, MJ/kg DS
30	18.22[a]	15.66[a]	1.98[a]	53.05[a]	30.24[a]	5.24[a]	63.35[a]	9.40[a]
45	20.52[b]	16.39[b]	1.84[b]	55.53[b]	32.37[b]	8.22[b]	59.71[b]	8.87[b]
60	22.47[c]	15.82[c]	1.99[a]	56.64[c]	34.70[c]	9.50[c]	58.77[c]	8.75[c]
75	25.75[d]	15.34[d]	2.99[c]	56.45[c]	36.84[d]	10.13[d]	57.25[d]	8.54[d]
90	28.33[e]	8.34[e]	2.99[c]	57.41[d]	38.10[e]	13.48[e]	56.36[e]	8.32[e]
SE±	0.67*	0.56*	0.09*	0.28*	0.53*	0.50*	0.45*	0.07*

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

Table 3. Influence of re-growth age on the total phenols and tannins of *Neonotonia wightii*

Age, d	Total phenols, g/kg	Total tannins, g/kg	Total condensed tannins, g/kg
May-June-July			
30	5.54[a]	1.90[a]	44.87[a]
45	7.22[b]	2.16[b]	51.87[b]
60	8.55[c]	3.60[c]	54.32[c]
75	8.76[d]	4.57[d]	64.17[d]
90	10.07[e]	5.24[e]	77.63[e]
SE±	0.29*	0.24*	2.11*
January-February-March			
30	6.57[a]	1.80[a]	58.14[a]
45	7.24[b]	2.03[b]	67.14[b]
60	7.48[c]	2.29[c]	71.35[c]
75	7.66[d]	2.85[d]	75.69[d]
90	8.24[e]	3.12[e]	96.64[e]
SE±	0.10*	0.09*	2.38*

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

Table 4. Correlation matrix between digestibility, energy and polyphenols in May-June-July

Variables	Total phenols	Total tannins	Total condensed tannins
DM *in vitro* digestibility	-0.93	-0.95	-0.99
OM digestibility	-0.92	-0.95	-0.99
True digestibility	-0.86	-0.89	-0.99
DM *in situ* digestibility	-0.82	-0.93	-0.92
ME	-0.91	-0.94	-0.99

Table 5. Correlation matrix between digestibility, energy and polyphenols in January-February-March

Variables	Total phenols	Total tannins	Total condensed tannins
DM *in vitro* digestibility	-0.96	-0.89	-0.87
OM digestibility	-0.97	-0.91	-0.90
True digestibility	-0.93	-0.97	-0.89
DM *in situ* digestibility	-0.97	-0.95	-0.98
ME	-0.97	-0.89	-0.89

Discussion

Due to the productive competitiveness of the agricultural sector, producers need to use efficiently the available resources. Many of them limit the forage quality and regrowth age. It is known that the cutting intervals or regrowth age influence on the quality of the harvested material as the structural changes and modifications produced in the chemical composition as the grassland age increases (Ramírez *et al*. 2012 and Verdecia *et al*. 2012).

The DM increased with the regrowth age due to the aging of the plant, among other aspects. With that, the structural components increased. The studies of Ríos (1999) showed DM values of 19.1 %, similar to those found in this research, after 45 d.

The protein decreased with the regrowth age, maybe related with the reduction of the protein compound synthesis, diminishing of the amount of leaves, increase of the stem fraction and increase of the structural carbohydrates synthesis (cellulose, hemicellulose and lignin). It is important to highlight that the protein values, in both seasons, were higher than 14 %. Similar results were obtained by Delgado *et al*. (2007).

This protein decrease is characteristic of grasses. However, the decrease in legumes is lower (Benavides 2003) and even better as happens with *Hibiscus rosasinensis*, with 17.8 % in the leaves (Bolio *et al*. 2006). This result coincides with that obtained in this study at 30 d, during the trimester May-June-July.

With age, the increase of the ADF and cell wall could be related with the physiological and anatomical changes produced with the plant aging. This diminishes the proportion of the cytoplasmic content, reduces the cell lumen with their soluble components and increases the fiber compounds (Nogueira Filho *et al*. 2000).

These increases are emphasized even more when the yield is higher, due to the hydric balance of the plant and the amount of available nitrogen on the soil, among other factors. The studies of Vanlauwe *et al*. (2005) in *Tithonia diversifolia* showed inferior lignin values to those obtained in this study, due to the climatic conditions and the nitrogen availability on the soil, among other aspects.

The increase of lignin with the plant aging could be related with the rigidness degree, the resistance of the vascular tissues, the conduction of solutes, water and mineral salts needed for their survival. It is increased with the physiological growth; more marked during the rainy season when ripening accelerates (Cornu *et al*. 1994). The NDF and ADF averaged 57 and 34 % respectively, with lignin mean concentration of 13.26 %, superior value to that recorded by Delgado *et al*. (2001) in tropical legumes.

The total digestibility of the forage mass will be in function of the relative proportion of each component and its individual digestibility. The increase of the structural components could also influence on the reduction of digestibility as ripening increased. In the tropical forages, both of grasses and legumes, the cell wall is thin during the first growth stages, with little fiber. This allows its easy breaking by the ruminal microorganisms, apart from short digestion times.

When age increases, the vascular structures of the leaves and the vascular tissue are thicker. The sclerenchyma of leaves and stems lignifies and is physically stronger and harder to reduce size (Medina *et al*. 2009).

The studies of Soto *et al*. (2009) showed values of DM *in vitro* digestibility of 36.4 and 45.8 %, at 48 and 144 h, respectively. These results are inferior to those obtained in this study, performance associated with the lignification of the cell wall. This is mainly because the forages are more fibrous and less digestible than the legumes (Urriola 1997).

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

García *et al*. (2008) obtained in ecotypes of leucaena values of total phenols and condensed tannins of 55.5 and 55.2 g/kg MS, respectively. These authors stated that the tannins content could be determined by the plant genotype and the environmental factors that cause stress, including the lack of water. These values surpass those reported in this study, due to the differences in species and conditions both experiments were conducted.

Miller and Ehlke (1996) and McMahon *et al*. (2000), when studying the concentration of condensed tannins, stated that this compound is controlled, first, by the genetic factors and second, by the environmental conditions. In general, its concentration increases with maturity and is associated with the lignin increase in the tissues. This could diminish the forage digestibility when high values are reached.

In the last decade of the 20th century, the criterion that the creeping legumes have low ruminal degradability was generalized, attributed to the generally high content of tannins, polyphenols and other secondary components of the plant metabolism (Ruiz *et al*. 1995). However, La O *et al*. (2006) determined the variability of some chemical indicators and the effective *in situ* ruminal degradability of DM and of protein in five legume species (dolicho, mucuna, centrosema, siratro and glycine). Their results showed values of DM effective degradation between 56 and 88 %.

La O *et al*. (2006), when conducting studies on tropical legumes stated that the OM digestibility of these plants diminish when the tannins contents are high. This is mainly due to the link of tannins with the different constituents of the diet and, therefore, with the diminishing of the bacterial activity during the ruminal degradation of the feeds.

Vázquez *et al*. (2009) found that the digestibility is negatively correlated (-0.793) with the phenolic compounds. These results are inferior to those found in this research (-0.82 to -0.97). The inverse relation proved between total phenols, digestibility and ME indicated that the tannins and lignin influence on the digestibility. Tannins precipitate proteins and, thus, reduce the amount of digestible protein of the feed. As consequence, the digestibility of the cell soluble matter decreases in species rich in tannins (Schofield *et al*. 2001).

There are exceptions in the relation between total phenols and digestibility. Species like *Trifolium arvense* have high content of phenolic compounds (133.7 mg/g) and similar digestibility to that of the *Ornitopus compresus*, that has 43 mg/g of phenolic compounds. These results suggest that this species could have different phenolic compounds to those that diminish digestibility. In this study, the range of values was of 8-10 g/kg for the total phenols, 3-5 g/kg for the total tannins and of 77-96 g/kg for the total condensed tannins in the period of January-February-March and May-June-July. Only in these lasts are over the minimum amounts reported by Makkar *et al*. (1988), from which the rumen fermentation is altered (40, 40 and 60 g/kg DM, respectively).

Matlebyane *et al*. (2009) observed reduction of the DM *in vitro* digestibility and OM digestibility in the foliage of *Acacia nilotica*, attributed to the presence of total phenols and tannins. Meanwhile, Norton (2000) and Tscherning *et al*. (2006) stated that high values of condensed tannins in the diet diminish the ruminants' intake and reduce protein and fiber digestibility. However, low values could have positive effects on animal production.

Tscherning *et al*. (2005 and 2006) indicated that the content of condensed tannins is the plant constituent with higher effect on the process of tissue degradation. These authors obtained high negative correlations (-0.99) between these compounds and the quality indicators of the plant, ue to the marked influence on the regulation of decomposition and DM digestibility.

The results of this research showed the marked effect of the climatic factor son the studied indicators. In both seasons, similar response patterns at the regrowth age were obtained, but with different absolute values. Nevertheless, it was proved that the nutritive quality of the plant decreased as maturity advanced. This is determined, among other aspects, by the negative relations between the phenolic compounds, digestibilities and energy. These aspects should be considered in the management of this plant when used as cattle feeding in order to improve its use efficiency.

Further studies on other legumes and under different edafoclimatic conditions are suggested to widen the knowledge on the performance of the indicators studied. Special attention should be paid to the phenolic compounds and their relation with digestibility and energy.

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