



## **Yield and chemical composition of foliage and branches of tropical tree legumes with different trunk diameters**

Rendimiento y composición química de follaje y ramas de leguminosas arbóreas tropicales con diferentes diámetros del tronco

Rendimiento e composição química de folhagens e ramos de leguminosas arbóreas tropicais com diferentes diâmetros de tronco

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## **Crop production**

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# **Keywords:**

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## **Abstract**

*Erythrina americana* Miller and *Gliricidia sepium* (Jacq.) Walp., are tree legumes presents on livestock farms in the tropical region, mainly on living fences. The goal of the study was to determine the influence of tree legume species (TLS), and of the trunk diameter at base tree (TDB), on production and chemical composition of foliage and branches at a similar regrowth age. The study was conducted in Tabasco, Mexico. Seventy-nine trees were used in a completely randomised design with a 2 X 5 factorial arrangement. The factors were TLS (*E. americana* y *G. sepium*), and TDB at 0.20 m height above ground level (D-5: 0.050-0.059 m; D-6: 0.060-0.069 m; D-7: 0.070-0.079 m; D-8: 0.080-0.089 m y D-9: 0.090-0.099 m). The production per tree of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and ash, of foliage and branches were the response variables. The STL x DBT interaction did not affect  $(P>0.05)$  the response variables studied in the foliage and branches. In foliage, the TLS and TDB affected the production per tree of DM, CP, ADF, and ash (P<0.01). *G. sepium* produced a greater amount of DM and the different components assessed than *E. americana*. An increase in TDB resulted in higher DM production and the components studied in foliage and branches. *G. sepium* produces foliage with higher CP and lower structural carbohydrate content than *E. americana* foliage.



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## **Resumen**

*Erythrina americana* Miller y *Gliricidia sepium* (Jacq.) Walp., son leguminosas arbóreas presentes en fincas ganaderas de la región tropical, principalmente en los cercos vivos. El objetivo del estudio fue determinar la influencia de la especie de leguminosa arbórea (ELA) y del diámetro del tronco en la base del árbol (DTB), sobre la producción y composición química del follaje y las ramas a una edad de rebrote similar. El estudio fue realizado en Tabasco, México. Se utilizaron 79 árboles en un diseño completamente al azar con un arreglo factorial 2 X 5. Los factores fueron ELA (*E. americana* y *G. sepium*) y DTB a 0,20 m de altura sobre el nivel del suelo (D-5: 0,050-0,059 m; D-6: 0,060-0,069 m; D-7: 0,070-0,079 m; D-8: 0,080-0,089 m y D-9: 0,090-0,099 m). La producción por árbol de materia seca (MS), proteína cruda (PC), fibra detergente neutro (FDN), fibra detergente ácido (FDA) y ceniza, de follaje y ramas fueron las variables respuesta. La interacción STL x DBT no afectó (P>0,05) las variables respuesta estudiadas en follaje y ramas. En follaje, la ELA y DTB afectaron la producción por árbol de MS, PC, FDA y ceniza (P<0,01). *G. sepium* produjo mayor cantidad de MS y de los diferentes componentes evaluados con respecto a *E. americana.* Un incremento en DTB resultó en mayor producción de MS y de los componentes estudiados en follaje y ramas. *G. sepium* produce un follaje con mayor PC y menor contenido en carbohidratos estructurales con respecto al follaje de *E. americana*.

Palabras clave: proteína cruda, materia seca, trópico húmedo, rendimento cercos vivos.

### **Resumo**

*Erythrina americana* Miller e *Gliricidia sepium* (Jacq.) Walp., som leguminosas arbóreas presentes em fincas ganadeiras da região tropical, principalmente em cercos vivos. O objetivo do estudo foi determinar a influência da espécie de leguminosa arbórea (ELA) e do diâmetro do tronco na base da árvore (DTB), sobre a produção e composição química da folhagem e dos ramos em numa idade de rebentação semelhante. O estudo foi realizado em Tabasco, no México. Foram utilizadas 79 árvores num desenho completamente aleatório com um arranjo fatorial 2 X 5. Os fatores estudados foram ELA (*E. americana* e *G. sepium*) e DTB a 0,20 m de altura sobre o nível do solo (D-5: 0,050-0,059 m; D-6: 0,060-0,069 m; D-7: 0,070- 0,079 m; D-8: 0,080-0,089 m ano; D-9: 0,090-0,099m). A produção por árvore de matéria seca (MS), proteína bruta (PB), fibra em detergente neutro (FDN), fibra em detergente ácido (FDA) e cinzas, de folhagem e ramos foram as variáveis resposta. A interação ELA x DTB não afetou (P>0,05) as variáveis de resposta estudadas na folhagem e nos ramos. Na folhagem, ELA e DTB afetaram a produção de árvores de MS, PB, FDA e cinzas (P<0,01). *G. sepium* produzido em grande quantidade de MS e dos diferentes componentes avaliados em relação a *E. americana*. Um incremento no DTB resultou em uma maior produção de MS e dos componentes estudados em folhagem e ramos. *A G. sepium* produz uma folhagem com maior teor de PB e menor teor de hidratos de carbono estruturais do que a folhagem da *E. americana*.

**Palavras-chave:** proteína bruta, matéria seca, trópico húmido, rendimento cercos vivos.

# **Introduction**

In the tropical region of Mexico, smallholder sheep farming systems are predominant, therefore these acquires social and productive importance (Pérez-Bautista *et al*., 2024). In these systems, the farmers with a sheep inventory less than 51 animals use grazing as the basis of the feeding, in pastures with tropical grasses, which present several variations in their dry matter (DM) production and crude protein (CP) content. The above, due to changes in climatic variables during the year, which leads to alterations in DM consumption that can cause the sheep to not meet their nutrient needs and thus not express their productive potential (Zamora-Zepeda *et al*., 2015).

The smallholder sheep farms are characterized by a limited use of technology in feed management, and use of available or potentially available natural resources to compensate for the variation in DM and CP production from pasture grass (Pérez-Bautista *et al*., 2024) Therefore, the use of a feed supplement is important and necessary for grazing sheep, in order to compensate the nutrients not provided by the pasture through their voluntary consumption during feeding.

The presence of diverse fodder trees as part of the living fences and disperses within the pastures, represents an opportunity to use their foliage as feed supplement. Some forage trees species presents in sheep farms are *Erythrina* spp., *Gliricidia sepium* (Jacq.) Walp., *Guazuma ulmifolia* Lam., *Leucaena leucocephala* (Lam.) de Wit and *Spathodea campanulata* P. Beauv (Castillo-Linares *et al*., 2024).

Among these forage trees, *Erythrina* spp., and *G. sepium* presents in fences facilitates their management and exploitation as foliage suppliers (Morantes-Toloza and Renjifo, 2018; Oliva-Hernández *et al*., 2021a). However, it is necessary to generate knowledge about their productive potential of DM and CP, to optimize their use in small ruminants feeding and establish agronomic management strategies. Based on the above scenario, the goal of this study was to determine the influence of tree legume species and of the trunk diameter at tree base, on yield and chemical composition of foliage and branches at a similar regrowth age.

## **Materials and methods**

### **Location and climatic characteristics of the study area**

The study was conducted at living fences of Sheep Unit of the National Institute for Forestry, Agriculture and Livestock Research (for its Spanish acronym, INIFAP), located in Huimanguillo municipality, in Tabasco, Mexico (17°51'4''N and 93°23'47''W). The climate is warm humid, with rainfall all year round (Af), mean annual rainfall 2,386.0 mm, mean annual environmental temperature 26.2 °C, thermal oscillation 10.6 °C, evaporation 954.1 mm and photoperiod 12 h (INEGI, 2017). The study was conducted during the transition from rainy season to drought (December 2022 to March 2023) in a warm humid climate.

#### **Trees, pruning and foliage**

The trees used in the study were part of a living fence with 250 trees (planted between 2012 and 2015 using stakes) located at perimeter of the pastures managed for sheep grazing. These trees had a controlled pruning management at 90-day intervals, so before study, these had received six pruning of all branches.

The trees selected for the study had straight trunks and without branches. The distance between the trees was 0.8 m for *E. americana* and 0.9 m for *G. sepium*; and with a trunk diameter measured at 0.20 m above the ground, between 0.05 m and 0.10 m, in order to have homogeneous trees in their physical characteristics. A total of 79 trees met these characteristics, of which 31 were *E. americana* and 48 were *G. sepium* (table 1). The trees were full pruning, cutting all branches with pruning shears, without irrigation or fertilization applied to the soil during the study period.

#### **Table 1. Morphological characteristics of two species of tree legumes exposed to a pruning process at 90-day intervals.**



N= number of observations; ¥ Mean **±** standard error.

#### **Trunk diameter**

To determine the influence of trunk diameter on the response variables studied, the trees were classified into five categories according to the trunk diameter at the base (TDB), measured at 0.20 m height above the ground: D-5: diameter between 0.050 and 0. 059 m ( $N = 13$  trees); D-6: between 0.060 and 0.069 m ( $N = 16$  trees); D-7: between 0.070 and 0.079 m (N = 21 trees); D-8: between 0.080 and  $0.089$  m (N = 15 trees); and D-9: between 0.090 and 0.099 m (N  $= 14$  trees).

#### **Branch and leaf yield**

The trees were evaluated for a single pruning in a single season, and regrowth age chosen to this study was of 90 days. The production of foliage per tree of both tree legumes species, by destructive method (cutting all branches and separating the foliage) was determined. The foliage was separated from the branches, them, which included leaves and petioles without tender stems. The foliage and branches were weighed green (wet basis) with a scale of 5 kg capacity and an accuracy of 10 g (Nuevo León SA de CV; model L; NOM-1895, Mexico).

#### **Chemical composition of foliage and branches**

Whole branches were randomly selected from ten randomly selected trees of each species studied to obtain samples of foliage (leaves and petioles) and branches. The samples were dried at 70 °C, crushed in a Wiley mill, and sieved with a 1 mm mesh, then the dry matter (DM), crude protein (CP), and ash contents were determined for each sample using methods of the Association of Official Agricultural Chemists (AOAC International, 2023). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) fractions were determined using the techniques of Van Soest *et al*. (1991).

#### **Response variables**

Per each tree was measured the yield of foliage and branches (kg.tree-1). With the amount of branches and foliage per tree, and

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the data of DM, CP, NDF, ADF and ash content, was estimated the production  $(g,tree^{-1})$  of DM, CP, NDF, ADF and ash from foliage and branches. The foliage:branch ratio was determined on a wet and dry basis by dividing the weight of the foliage by the weight of the branches.

#### **Experimental design and statistical analysis**

The study carried out in completely randomized experimental design under 2 x 5 factorial arrangement. The first study factor was the species of tree legume (STL: *E. americana* and *G. sepium*); the second factor was the trunk diameter at the base (TDB) measured at 0.20 m height above the ground, and the STL x TDB interaction. The experimental unit was the tree. The analyses was carried out through SAS v 9.3 statistical package (SAS Institute, 2002), and the normality and homoscedasticity of the data were checking with Shapiro-Wilk and Levene tests, respectively. The dependent variables studied had non-normal distribution or homoscedasticity, so all variables were transformed with the natural logarithm function, thus achieving normal distribution and homoscedasticity of the data. The influence of the study factors on the response variables was analysed statistically using the general linear model (GLM) procedure of SAS. T-test with least mean square (LMS) analysis was used to comparing means using pdiff option of SAS. LMS were statistically significant when P≤0.05.

## **Results and discussion**

The STL  $x$  DBT interaction did not affect (P $> 0.05$ ) the response variables studied in the foliage and branches of *E. americana* and *G. sepium*, so it becomes more important to focus the discussion of the results towards the influence of the main effects, STL and DBT, on the response variables.

#### **Chemical composition of foliage and branches**

 DM and ash content in both foliage and branches, were not affected  $(P>0.05)$  by the tree legume species. A similar response was shown in the CP content in branches  $(P>0.05)$ . However, the foliage of *G. sepium* presented higher CP content and lower NDF and ADF content in relation to *E. americana* (P<0.05). In addition, the values of NDF and ADF in branches were higher (P<0.05) in *G. sepium* than in *E. americana* (table 2).

 The CP content of *E. americana* and *G. sepium* foliage was lower than reported for *L. leucocephala* (21.6 % CP). However, the foliage of *G. sepium* contained higher CP than that reported for *G. ulmifolia* (15.3 % CP), while the foliage of *E. americana* contained less CP than that of *G. ulmifolia*. Nevertheless, the foliage of *G. sepium* has lower NDF content than that of *L. leucocephala* (43.8 %) and *G. ulmifolia*  (53.2 %). While the foliage of *E. americana* has a similar NDF content to that of *G. ulmifolia*, but higher than that of *L. leucocephala*  (Hernández-Morales *et al*., 2018). A lower NDF and ADF content in forage is associated with higher dry matter degradation, which favors higher dry matter intake in ruminants, a condition that is favorable for promoting the use of *G. sepium* and *E. americana* foliage as a feed supplement for grazing small ruminants (Bayhan, 2023).

**Table 2. Chemical composition of foliage and branches of the tree legumes assessed with a regrowth age of 90 days, during the transition from rainy season to drought in warm humid climate.**



NDF=neutral detergent fiber; ADF=acid detergent fiber; CP=crude protein.

<sup>a, b</sup>Least squares means  $\pm$  standard error with different superscript within the same row are significantly different (T-test, P<0.05).

The branches of *G. sepium* and *E. americana* at a regrowth age of 90 days contain less CP and more structural carbohydrates compared to the foliage, and the first one are not easily consumed by small ruminants because of their size, difficulty to fractionate them through a bite because they are less available than the foliage (Santana-Pérez *et al*., 2019).

The branches of *G. sepium* have a higher NDF and ADF content compared to those of *E. americana*, and it is likely that these differences explain the higher resistance of *G. sepium* branches compared to those of *E. americana* when pruned with scissors. In this respect, the wood of *G. sepium* has a higher density (0.74 g.cm-3) compared to *E. americana* (0.197 g.cm3 ) (Ordoñez Diaz *et al*., 2015; Ricker *et al*., 2024). However, no studies could be identified where branch density was assessed at a regrowth age of 90 days.

#### **Influence of tree legume species on the production of foliage and branches**

With exception of foliage:branch ratio, the tree legume species affected  $(P<0.01)$  dry matter (DM) production from foliage and branches (table 3). The production of foliage and branches was higher in *G. sepium* than in *E*. *americana. G. sepium* was observed to produce 37 % more DM per tree from foliage and 47 % more DM per tree from branches.

The foliage yield per tree of *G. sepium* was higher than the 98 g  $DM.$ tree<sup>-1</sup> reported in this specie at a density of 20,000 plants.ha<sup>-1</sup>, a regrowth age of 75 days, and 0.90 m height (Ramos-Trejo *et al*., 2016). However, it was lower than the 405 g DM.tree<sup>-1</sup> indicated in the same type of tree, but with a lower planting density  $(6,670 \text{ trees.ha}^{-1})$ and 84 days of regrowth age (Melchor *et al*., 2005)8, 12 and 24 weeks.

In the case of *E. americana*, the foliage production was lower than reported for the same type of tree with a regrowth age of 90 days, but harvested during the dry season  $(257 \text{ g} \text{ DM.} \text{tree}^{-1})$  (Oliva Hernández *et al*., 2021a). And higher than observed in *E. variegata*  $(21 \text{ g} \text{ DM.} \text{tree}^{-1})$  at a density of 7,182 plants.ha<sup>-1</sup>, 3-4 cm diameter of the planted stake, a stake height of 0.35-0.40 m and a regrowth age of six months (Kongmanila *et al*., 2012).

### **Table 3. Production of foliage and branches per tree of the tree legumes assessed with a regrowth age of 90 days, during the transition from rainy season to drought in warm humid climate.**



N= number of observations; a, bLeast squares means  $\pm$  standard error with different superscript within the same row are significantly different (T-test, P<0.01).

The wide variation in DM production per tree between studies could be attributed to tree species, density, age at regrowth, tree height, season, and soil type. In addition, most of the studies on tree legume yields have not been carried out in live fences, but in plots specifically designed for this purpose.

The foliage and branches of *G. sepium*, showed a higher amount per tree (g) of DM, CP, ADF and ash content, in relation to *E. americana* (table 4). However, NDF from foliage was not affected (P>0.05) by the specie of tree legume.

**Table 4. Production per tree (g) of crude protein, neutral detergent fiber (NDF), acid detergent fiber (ADF) and ash of foliage and branches of the tree legumes assessed with a regrowth age of 90 days, during the transition from rainy season to drought in warm humid climate.**

	<b>Species</b>			
<b>Response variable</b>	Erytrhina americana $(N=31)$	Gliricidia sepium $(N=48)$		
Foliage $(g.\text{tree}^{-1})$				
CP	$16.8^b \pm 2.4$	$42.1^a \pm 1.9$		
<b>NDF</b>	$69.4 \pm 5.5$	$78.9 \pm 4.4$		
ADF	$45.7^{\rm b} \pm 4.0$	$62.1^{\circ} \pm 3.2$		
Ash	$6.4^{\rm b} \pm 1.1$	$12.1^a \pm 0.9$		
Branches $(g.\text{tree}^{-1})$				
CP	$6.2^b \pm 0.9$	$9.7^{\circ} \pm 0.7$		
<b>NDF</b>	$43.3^{b} \pm 8.2$	$91.6^a \pm 6.6$		
ADF	$37.3^b \pm 7.1$	$78.4^{\circ} \pm 5.7$		
Ash	$4.4^{\rm b} \pm 0.9$	$9.8^{\circ} \pm 0.7$		

N= number of observations; <sup>a, b</sup>Least squares means  $\pm$  standard error with different superscript within the same row are significantly different (T-test,  $P<0.01$ ).

The foliage production per tree and their chemical composition (CP, NDF and ADF) allowed identifying the superiority of *G.* 

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*sepium* over *E. americana* in the quantity and quality of foliage with opportunity to supplement the feed of small ruminants (Castillo Linares *et al*., 2021).

In *G. sepium*, the branches production per tree was higher than that indicated in the same type of tree  $(38 \text{ g DM.} \text{tree}^{-1})$ , but with a lower regrowth age (Ramos-Trejo *et al*., 2016). Although, lower than 351 g DM.tree-1 indicated in *G. sepium* with 84 days of regrowth age (Melchor *et al*., 2005). In *E. americana*, the branches production per tree was higher than that observed in *E. variegata* plants at a regrowth age of six months (Kongmanila *et al*., 2012). Differences in production of DM per tree among studies can be attributed to, tree species, density, height, plant age, regrowth age, number, length and diameter of growing branches, season and soil type (Canul-Solís *et al*., 2018).

The production of branches per tree and their chemical composition (CP, NDF and ADF) allowed identifying higher branch production efficiency and nitrogen, structural carbohydrate and mineral content in *G. sepium* over *E. americana*. Branches at 90 days of regrowth age do not represent an option as food for small ruminants, nor as material to be used as firewood. However, the production of aerial biomass is an indicator of its potential to sequester atmospheric carbon.

*G. sepium* had a carbon content in foliage and branches of 48.7 and 46.8 %, respectively (Gómez-Castro *et al*., 2010). Therefore, it is necessary to evaluate, in future studies, the benefit of *G. sepium* and *E. americana* trees as contributors to atmospheric carbon sequestration in their aerial biomass (foliage and branches), in a scenario of successive pruning at 90-day intervals.

## **Influence of trunk diameter on foliage and branch production**

Foliage: branch ratio was not affected by trunk diameter (P>0.05). However, tree diameter affected production per tree (g) of DM, CP, NDF, ADF and ash in foliage (P<0.01) and branches (P<0.05) (tables 5 and 6). An increase in diameter allowed a higher yield per tree (g) of DM in foliage and branches. In trees with a D-9, a greater amount of foliage was harvested in relation to D-5, D-6 and D-8 diameters, but had not a similar trend to foliage per tree production. Trees with D-5 presented the lowest production of branches per tree, although similar to that recorded in D-6 and D-8 (table 5).

The production per tree (g) of CP, NDF, ADF and ash in branches was affected by trunk diameter (P<0.01), where branches of trees with D-5 presented the lowest production of CP, NDF, ADF, and ash content, although equivalent to that detected in D-6 and D-8 (table 6).

In general, an increase in trunk diameter at 0.20 m height above the ground of *G. sepium* and *E. Americana*, favored an increase in the foliage and branches yield per tree. A similar tendency has been indicated in studies with *G. sepium* with trunk diameters at 0.20 m above the ground between 0.064 and 0.22 m, and at a breast height (DBH) between 0.05 and 0.23 m (Gómez-Castro *et al*., 2010; Mulyana *et al*., 2020). The diameter at 0.20 m from the trunk base in *E. americana* explained 34 % of the variation in foliage yield tree-1 (Oliva-Hernández *et al*., 2021b).

An increase of trunk diameter implies a greater surface area of xylem, a tissue that conducts water, inorganic and organic compounds from the root to the leaves. As well as phloem, a tissue that allows the massive distribution of water and the transport of amino acids, hormones and carbohydrates produced in the synthesis sites or those mobilized, from the storage sites to the dump or demand organs of the tree (Zúñiga-Sánchez *et al*., 2017).

**Table 5. Foliage and branches production per tree, considering trunk diameter of the tree legumes assessed, with a regrowth age of 90 days, during the transition from rainy season to drought in warm humid climate.** 

rainy scason to arought in warm numiu chinate.						
	Trunk diameter at 0.20 m above the ground level (m)					
<b>Response variable</b>	$D-5$	$D-6$	$D-7$	$D-8$	$D-9$	
	$(N=13)$	$(N=16)$	$(N=21)$	$(N=15)$	$(N=14)$	
Foliage $(g.\text{tree}^{-1})$						
Production on wet basis	$551^\circ \pm 91$	$676^{\circ} \pm 84$	$891^{ab} \pm 71$	$778^{bc}$ ± 83	$959^{\circ}$ ± 88	
Production on dry basis	$130^{\circ} \pm 21$	$158^{\circ} \pm 20$	$209^{ab} \pm 17$	$182^{bc} \pm 20$	$225^a \pm 20$	
Branches (g.tree <sup>-1</sup> )						
Production on wet basis	$297^{\rm b} \pm 79$	$388^{ab} \pm 73$	$545^{\circ} \pm 62$	$417^{ab} + 73$	$523^a \pm 76$	
Production on dry basis	$65^{\rm b} \pm 17$	$85^{ab} \pm 16$	$119^a \pm 13$	$91^{ab} \pm 16$	$114^a \pm 17$	
Foliage:branch ratio on wet basis	$2.0 \pm 0.1$	$2.0 \pm 0.1$	$1.8 \pm 0.1$	$2.1 \pm 0.1$	$1.9 \pm 0.1$	
Foliage:branch ratio on dry basis	$2.2 \pm 0.1$	$2.1 \pm 0.1$	$2.0 \pm 0.1$	$2.3 \pm 0.1$	$2.0 \pm 0.1$	

D-5= 0.050-0.059; D-6= 0.060-0.069; D-7= 0.070-0.079; D-8= 0.080-0.089; and D-9= 0.090- 0.099; N= number of observations; a, b, cLeast squares means **±** standard error with different superscript within the same row are significantly different (T-test, P<0.05).

An increase in the surface area of nutrient-conducting tissues may explain the positive relationship between tree diameter and DM production per tree. Otherwise, *G. sepium* and *Erythrina spp*. can reach DBH´s up to 0.88 m and 0.72 m, respectively (Ramírez-Meneses *et al*., 2013). However, the growth rate and lifespan of *G. sepium* and *E. americana* are unknown; when these trees are used as live posts in fences their height is controlled (less than one meter) and they are pruned continuously at 90-day intervals.

## **Conclusions**

*E. americana* and *G. sepium*, as well as trunk diameter, affected the yield and chemical composition of foliage and branches. However, the STL x DBT interaction did not affect the response variables studied in the foliage and branches. Under similar conditions of regrowth age, soil type and climatic season, *G. sepium* produces foliage with higher CP and lower structural carbohydrate content than *E. americana* foliage, giving it an advantage as feed supplement for small ruminants. Within the studied limits of diameter at the base of the tree, an increase in diameter allowed obtaining a greater production of DM and its components studied in both, foliage and branches.

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**Table 6. Production per tree of CP, NDF, ADF and ash of foliage and branches, considering trunk diameter of the tree legumes assessed with a regrowth age of 90 days, during the transition from rainy season to drought in warm humid climate.** 



CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber**;** D-5= 0.050-0.059; D-6= 0.060-0.069; D-7= 0.070-0.079; D-8= 0.080-0.089; and D-9= 0.090- 0.099; N= number of observations; a, bLeast squares means **±** standard error with different superscript within the same row indicate that they are different (T-test, P<0.01).

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