



## Seasonal water balance and response on the yield and biochemical composition of *Leucaena leucocephala* (Lam.) de Wit



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### Abstract:

The objective was to evaluate the effect of the seasonal water balance (SWB) on the yield and biochemical composition of *Leucaena leucocephala*. Three 5 x 5 m plots were established in a warm sub-humid climate (Aw<sub>1</sub>). Nine sampling cuts were made every 42 d in seven seasons of the year, classified by their SWB (mm d<sup>-1</sup>) as follows: “North winds” - 0.18 (N, Dec-Jan); Dry -2.39 (S, Feb-Apr); Onset rains 1.77 (On-R, Apr-May); Full rains 4.89 (FR, Jun-Jul); Extreme rains 8.32 (Ex-R, Jul-Aug); Late rains 3.74 (LR, Aug-Sep), and Residual rains -1.46 (Re-R, Sep-Dec). Dry matter yield and biochemical composition (DM, ashes, EE, NDF, ADF, lignin, CP, NPN, NDIP, and ADIP), based on which the rumen-soluble carbohydrate and protein fractions were estimated. A randomized, complete block statistical design with seven treatments was utilized. Means were compared by Tukey test ( $P \leq 0.05$ ). The DM yield (kg ha<sup>-1</sup>) decreased to 152<sup>d</sup> in Nw and D with SWB-, and increased to 1,497<sup>b</sup>

in FR and LR with SWB+. True soluble protein (TSP, %CP) increases to 73.1<sup>a</sup> in Nw and D with SWB- and decreases to 69.8<sup>b</sup> in FR and LR with SWB+. Non-fiber carbohydrates (NFC, %Total carbohydrates) decrease to 44.4<sup>b</sup> in Nw and D and increase to 54.7<sup>a</sup> in FR and LR. The authors concluded that the NFC decreases and the TSP increases in Nw and D with SWB- magnifying the energy/protein imbalance in the rumen for microbial protein synthesis.

**Keywords:** Shrub legume, Carbohydrate fractions, Protein fractions.

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## Introduction

In the American tropics, particularly in the coastal plains, changes in temperature and daylight hours are not as relevant to forage yield and biochemical composition as the amount and distribution of rainfall and its evaporation, which define rainy seasons with a positive water balance and dry seasons with a negative water balance. Three climate seasons have been characterized in the tropical region of the Gulf of Mexico (based on the distribution of the seasonal water balance, which integrates the effects of the rainfall, evaporation, relative humidity, cloud cover, and temperature throughout the year): drought (March to May), rains (June to November) and “north winds” (December to February)<sup>(1)</sup>. The seasonal water balance (SWB) is an agronomic tool to evaluate the amount of water (in mm d<sup>-1</sup>) available for a crop<sup>(2)</sup>. This calculation takes into account the rainfall as the input source, and the evaporation as the output. The SWB thus allows a simple evaluation of the amount of water available to the plant. In their growth phase, tropical forages are affected by daily variations in humidity with an impact on their yield and biochemical composition<sup>(3)</sup>. Thus, measuring the water balance during the growth period between forage plant cuts over an annual cycle would provide information on the yield response and its biochemical composition to estimate seasonal variations in nutritional quality for ruminants.

Al-Mefleh and Tadros<sup>(4)</sup> evaluated the yield of *Leucaena leucocephala* under different levels of evapotranspiration (ET) in arid regions. They report that irrigation at 50 % ET produces the most efficient response in terms of water management and leaf yield (676.3 kg MS ha<sup>-1</sup>). *Leucaena leucocephala* maintains green forage even through prolonged periods of negative SWB because it is a bushy legume with very deep roots as a strategy to prevent drought and exposure of the protoplasm to extremely negative water potential values<sup>(5)</sup>. Hsiao<sup>(6)</sup> points out that the parameters most sensitive to minimum water stress are cell growth, cell wall synthesis, and protein synthesis; therefore, the yields are significantly reduced. During long

droughts, *Leucaena leucocephala* diverts irreversible and costly energy, not to lignification, cutinization or silicification, but to energetically cheaper substances such as tannins and alkaloids<sup>(7)</sup>; this allows it to respond at once in terms of yield to positive SWB conditions. An essential preliminary step for estimating the nutritional quality is to determine the biochemical composition. The appropriate chemical analysis will render the prediction of the nutritional quality of the forage more accurate<sup>(8,9)</sup>. The analysis profile proposed by Higgs *et al*<sup>(10)</sup> allows very accurate estimates of the nutritional quality of ruminant feeds using the Cornell Net Carbohydrate and Protein System (CNCPS) equations. The nutritional quality of forage is defined as the availability of its nutrients to support the maintenance and production functions of the animals it feeds<sup>(11)</sup>. The chief determinants of the nutritional quality of forages are the availability of nitrogen (protein) and carbohydrates<sup>(12)</sup>. The nitrogen supply of tropical legumes has been poorly described, as the crude protein value alone is not sufficient to explain the availability of nitrogen for microbial protein synthesis by the rumen microbiome. This requires disaggregating the protein into its various biochemical fractions according to their solubility and the microbial population that uses them: Non-protein nitrogen is immediately soluble and used as NH<sub>3</sub> mainly by bacteria that degrade fibrous carbohydrates; soluble true protein, which is rapidly and intermediately soluble and is used as amino acids mainly by proteolytic and non-fibrous carbohydrate utilizing bacteria, which protein fractions are used for microbial protein synthesis (main source of protein for the ruminant); insoluble or escape protein, which is digested in the abomasum and duodenum and is absorbed as amino acids by the animal, and indigestible protein, which is excreted in the feces<sup>(13)</sup>. Until these protein fractions are described in forages, particularly in tree legumes, it will not be possible to estimate their availability to optimize the efficiency of ruminal synthesis of microbial protein.

Thus, there is a need to evaluate the quality of *Leucaena leucocephala* in terms of protein and carbohydrate fractions to determine the availability of these nutrients for the most efficient feeding of cattle in the tropics. On the other hand, according to Muñoz *et al*<sup>(1)</sup>, in the tropics, the season of the year is the main factor affecting the nutritional quality of forage. In addition, climatic factors such as variations in rainfall and temperature can modify the biochemical composition of *Leucaena leucocephala*<sup>(14)</sup>. Al-Mefleh *et al*<sup>(4)</sup> report *L. leucocephala* yields of 306, 676, 724, 839, and 1,173 DM kg ha<sup>-1</sup> at evapotranspiration (ET) of 0.25, 0.50, 0.75, 1.00, and 1.25, suggesting that at 0.50 ET *L. leucocephala* uses water more efficiently for growth. The objective of the present study was to evaluate the effect of seasonal water balance on the yield and nutritional quality of *L. leucocephala*.

## Material and methods

### Study area

The research was carried out at the “La Posta” Experimental Field (INIFAP) in Paso del Toro, Veracruz, located at 19° 02' N and 96° 08' W, and 16 m asl, with average, maximum, and minimum temperatures of 25.4, 31.3, and 19.5 °C, respectively; 1,337 mm rainfall, and evaporation of 1,379 mm evaporation<sup>(15)</sup>. The predominant soil type is vertisol, with a pH=5.4, a sandy loam texture, and 2.6 % organic matter.

### Vegetative material

The vegetative material was *Leucaena leucocephala* (Lam.) de Wit cv. Cunningham 1.5 m in height, established in three 5 x 5 m plots. Each plot had four bushes with 2 x 2 m spacing. Edible leaves and tender stems (up to 5 mm in diameter approx.) were collected. Forage samples were cut every 42 d from August 2011 to July 2012.

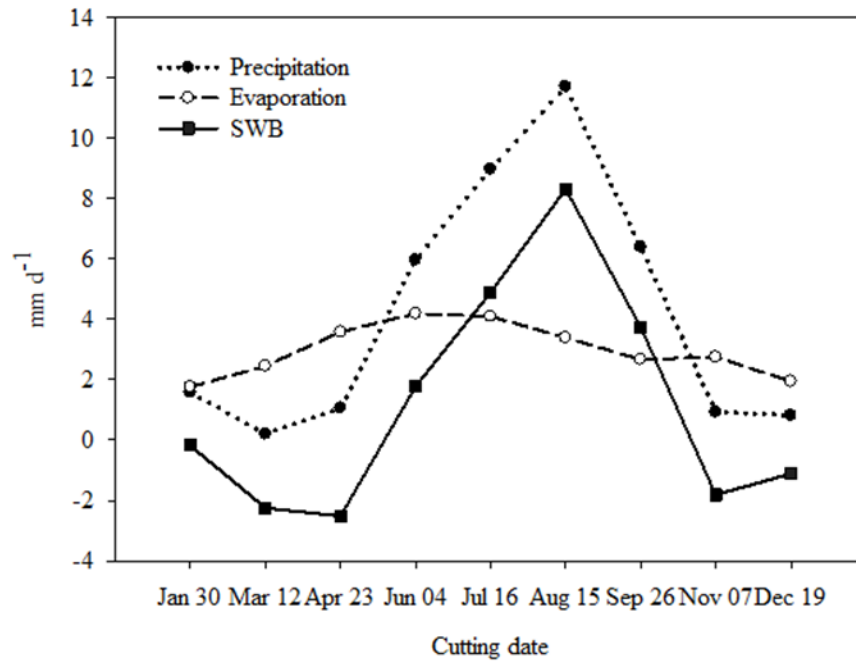
### Weather data

Rainfall and evaporation data were recorded by the climate station “El Tejar” Veracruz, Mexico<sup>(16)</sup> located 5 km from the EC. La Posta. The precipitation and evaporation data used for this study were the averages corresponding to the regrowth periods of *Leucaena leucocephala* grouped and calculated by cutting dates.

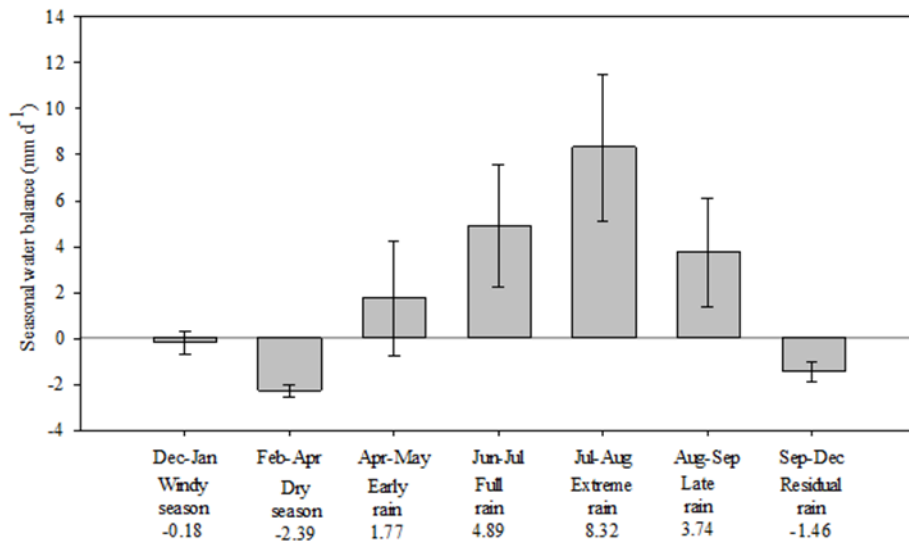
### Season of the year defined by seasonal water balance (SWB)

The SWB (mm d<sup>-1</sup>) considers rainfall (mm d<sup>-1</sup>) as the only water input, and evaporation (mm d<sup>-1</sup>) as the water output. The calculation was based on the difference between the rainfall minus the evaporation (Figure 1). The SWB by cutting date was determined on a daily basis from the day of the sampling cut until the day before the next sampling cut (42 d). The cutting dates were August 15, September 26, November 7, and December 19, 2011, and January 30, April 23, and July 16, 2012. The daily SWB results were averaged every 42 d in order to obtain a representative value for each sampling date. The SWB averages (mm d<sup>-1</sup>) were grouped into seven seasons of the year as follows: “North winds”,  $-0.18 \pm 0.67$  (Dec-Jan); Dry,  $-2.39 \pm 0.26$  (Feb-Apr); Onset rains,  $1.77 \pm 2.49$  (Apr-May); Full rains  $4.89 \pm 2.66$  (Jun-Jul); Extreme rains,  $8.32 \pm 3.20$  (Jul-Aug); Late rains,  $3.74 \pm 2.34$  (Aug-Sep), and Residual rains,  $-1.46 \pm 0.39$  (Sep-Dec). The seasons of the year are the seven treatments (Figure 2).

**Figure 1:** Seasonal water balance (SWB) by date of *Leucaena leucocephala* forage samplings during the 2011-2012 cycle



**Figure 2:** Seasonal water balance, seasons, and months of the cutting dates



## Evapotranspiration (ET)

The calculation was based on the difference between the rainfalls ( $\text{mm d}^{-1}$ ) minus the evaporation ( $\text{mm d}^{-1}$ ), with a crop coefficient of 0.82<sup>(17)</sup>.

## Dry matter yield

This parameter was estimated by harvesting the total available tree biomass at a height of 1.5 m at each cutting interval and all the foliage of the trees that constituted new growth (leaves and tender stems, less than 5 mm in diameter). The forage harvested at each cutting date per treatment was dried in a forced-air oven at 100 °C for 24 h. The dry material was weighed on an analytical balance, and the dry matter (DM) yield for each cut was determined; in order to estimate the DM yield, the number of bushes was calculated per hectare based on the distance within and between the rows, resulting in a planting density of 1,600 bushes  $\text{ha}^{-1}$ .

## Biochemical composition

The samples were dried in a forced air oven at 55 °C until constant weight. They were ground in a Wiley mill with a 2 mm mesh. Standard AOAC<sup>(18)</sup> procedures were used to measure DM, ash (Ash), ether extract (EE), and crude protein (CP) contents. Nitrogen fractions were determined using the techniques described by Licitra *et al*<sup>(13)</sup>: non-protein nitrogen (NPN); soluble protein (SP); protein in cell walls (NDIP); indigestible protein (ADIP); neutral detergent fiber (NDF), and acid detergent fiber (ADF), by Van Soest<sup>(7)</sup>, and lignin, by Goering and Van Soest<sup>(19)</sup>.

## Carbohydrate fractions

Total carbohydrates and available carbohydrate fractions were estimated with equations 1, 2 and 3.

$$\text{Total carbohydrates (TC, \%DM)} = 100 - \text{CP} - \text{EE} - \text{Ashes} - \text{Lignin} \quad (1)$$

$$\text{Non-fibrous carbohydrates (NFC, \%DM)} = 100 - \text{Ashes} - \text{EE} - \text{CP} - (\text{CP-free NDF}) \quad (2)$$

$$\text{Fibrous carbohydrates (FC, \%MS)} = (\text{CP-free NDF}) - \text{Lignin} \quad \dots (3)$$

## Protein fractions

The estimation of protein fractions is presented as an outline in Table 1.

**Table 1:** Equivalences of biochemical protein fractions to nutritional protein fractions

Biochemical fractions	Nutritional fractions
Nitrogen * 6.25	Crude protein
NPN	Non-protein nitrogen (immediate solubility)
CP – NDIP	SP is a step to obtain the true soluble protein (TSP).
SP – NPN	TSP (rapid and intermediate solubility)
NDIP - ADIP	True protein in NDF (slow solubility)
ADIP	Indigestible protein (insoluble)

NDIP= neutral detergent insoluble protein; ADIP= acid detergent insoluble protein.

## Experimental design and statistical analysis

Yield, biochemical composition, available carbohydrate, and protein fractions were subjected to analysis of variance in a randomized complete block design with seven treatments and three replicates per treatment. The GLM procedure of SAS version 9.1 was utilized<sup>(20)</sup>. The least squared means of the treatments were estimated with the LSMEANS option, while the comparisons between them were performed using Tukey's test ( $P \leq 0.05$ ). In addition, the Sigma Plot version 15.0 software<sup>(21)</sup> was used for regression analysis and charting.

## Results and discussion

### Seasonal water balance (SWB)

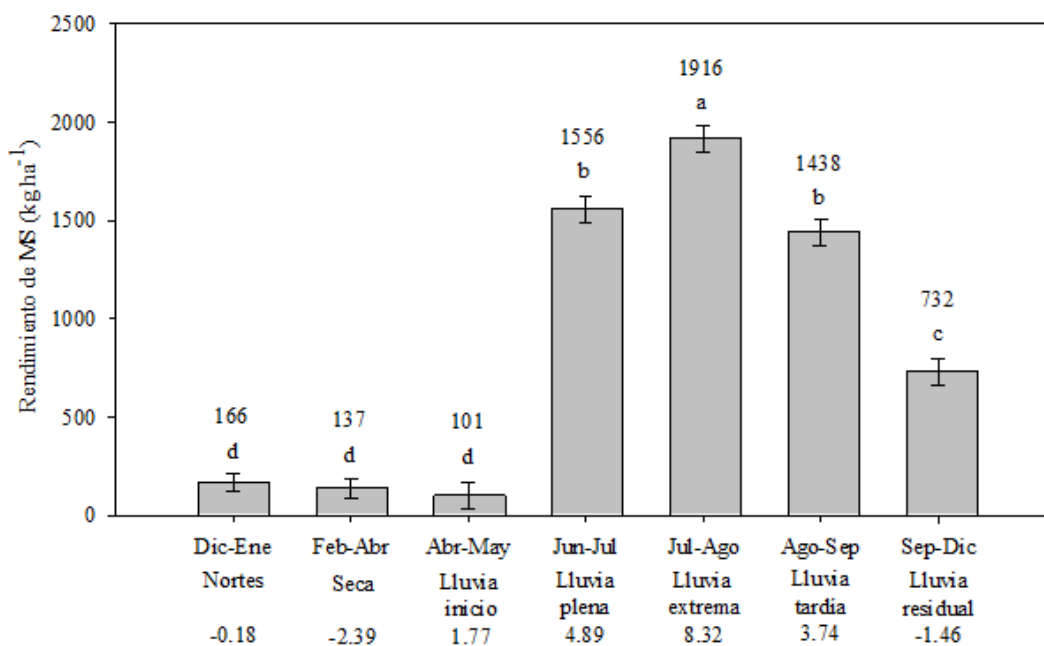
Although the SWB has a determining influence on the seasons and plant behavior, other physiographic variables condition the effect of the SWB, particularly the soil water status, temperature, humidity, and luminosity. Negative SWB during the “north winds” and Dry seasons is related to a decrease in ambient temperature and luminosity. In contrast, positive SWB in the full and late rainy seasons is associated with higher temperatures and light levels. During onset rains, although the SWB is positive, the soil remains dehydrated and compacted due to the previous six months of drought. In the case of residual rainfall, although the SWB is negative, the soil retains residual moisture, the result of six consecutive months of rainfall. Finally, in the extreme rainy season, excessive rainfall, humidity, temperature, and luminosity are observed.

Overall, the responses in yield, biochemical composition, and nutritional quality of *Leucaena leucocephala* are difficult to explain. In an organized manner, comparisons will be made between seasons of interest, as follows: “North winds” and Dry vs. full and late rains, onset vs. residual rainfall, and extreme rains will be discussed separately.

## DM yield

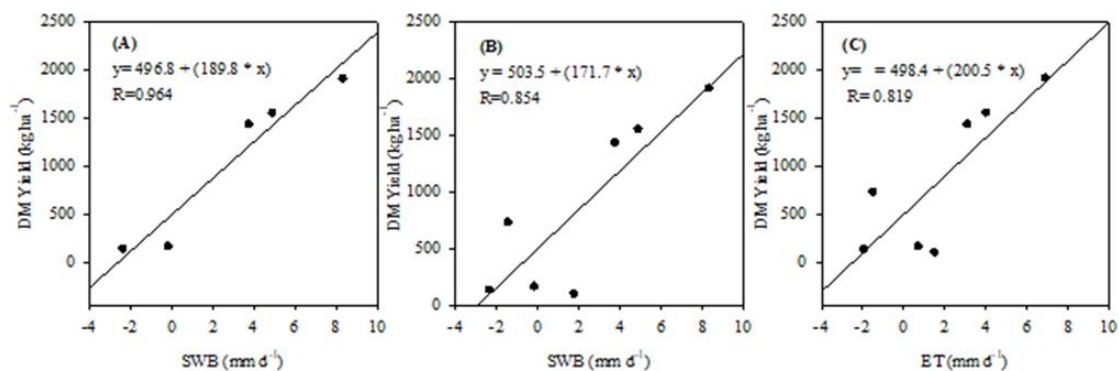
In the full and late rainy seasons, with more than 3 mm of average daily rainfall during the growth stage of *Leucaena leucocephala*, DM yields exceeded 1,000 kg ha<sup>-1</sup> (Figure 3), and in the “North winds” and dry seasons with negative SWB, DM yields were only about 150 kg ha<sup>-1</sup> (Figure 3). In the rainy season, the environmental SWB is positive (1.77 mm d<sup>-1</sup>) but allows only DM yields of 100 kg ha<sup>-1</sup>, it is presumed that soil water stress and soil compaction could be limiting yields. During the period of residual rains, although the environmental SWB is negative (-1.46 mm d<sup>-1</sup>), the residual soil moisture may allow DM yields of approximately 750 kg ha<sup>-1</sup> (-1.46 mm d<sup>-1</sup>). When the SWB is above 8 mm, yields reach almost 2,000 kg ha<sup>-1</sup>, perhaps boosted by the high temperature, humidity, and luminosity that are typical of summer.

**Figure 3:** DM yield (kg ha<sup>-1</sup>) per 42-day cut in each season of the year classified by seasonal water balance (SWB)



When the SWB is regressed on the DM yield of *Leucaena leucocephala* (Figure 4), positive growth rates are observed in connection to the SWB, except for the transition periods at the beginning (Apr-May) and end of the rainy season (Sep-Dec). Without considering these transition periods, DM yields (kg ha<sup>-1</sup>) of *Leucaena leucocephala* were 137, 166, 1,438, 1,556, and 1,916 for a SWB (mm d<sup>-1</sup>) of -2.39, -0.18, 3.74, 4.89, and 8.32. These SWB are equivalent to an evapotranspiration (ET) (mm d<sup>-1</sup>) of -1.51, 0.20, 3.24, 4.14, and 6.80 using the equation  $y = 0.7756x + 0.3436$ ;  $r^2 = 0.93$ , where  $x = \text{SWB}$  and  $y = \text{ET}$ . Al-Mefleh *et al*<sup>(3)</sup> report *L. leucocephala* yields of 306, 676, 724, 839, and 1,173 at ET of: 0.25, 0.50, 0.75, 1.00, and 1.25; ranges that fall within the ET values of 0.7 to 3.1 found in the present study.

**Figure 4:** Regression of the seasonal water balance (SWB, mm d<sup>-1</sup>) on the DM yield (kg ha<sup>-1</sup>) of *Leucaena leucocephala*



(A) The transition values (Apr-May) and (Sep-Dec), where the soil water condition influences the yield, were excluded. (B) The 7 seasons of the year classified by SWB are included. (C) The DM yield is regressed on evapotranspiration (ET, mm d<sup>-1</sup>).

### Biochemical composition

All biochemical variables are affected by SWB. There is a tendency for biochemical variables to be diluted with SWB+ and concentrated with SWB- (Table 2). This behavior may be associated with the DM yield of the plant, except for EE and lignin, which are concentrated with SWB+ and diluted with SWB-. It is likely that these variables are in line with the plant's metabolism and serve as carbon and hydrogen sequestration. Total nitrogen (%DM) is considerably high in the North winds and Dry seasons with a SWB-, unlike in the Full and Late rains' season with SWB+. This behavior may be associated with a higher growth and metabolism with a SWB+ that dilutes and depletes this nutrient in contrast to a SWB- with a lower growth and metabolic rate, which concentrates and reserves nitrogen<sup>(22)</sup>. Water deficit (SWB-) generally improves forage quality because nutrients are concentrated, the development of the plant slows down or ceases, and the transpiration is reduced<sup>(22)</sup>.

**Table 2:** Biochemical composition of *Leucaena leucocephala* (Lam.) de Wit cv. Cunningham by time of year classified by seasonal water balance (SWB)

Month	Dec-Jan	Feb-Apr	Apr-May	Jun-Jul	Jul-Aug	Aug-Sep	Sep-Dec	MSE	P Value
Season	North winds	Dry	On-R	Fu-R	Ex-R	La-R	Re-R		
<b>BHE, mm d<sup>-1</sup></b>	<b>-0.18</b>	<b>-2.39</b>	<b>1.77</b>	<b>4.89</b>	<b>8.32</b>	<b>3.7</b>	<b>-1.46</b>		
Ash	7.7 <sup>a</sup>	7.2 <sup>ab</sup>	6.7 <sup>abc</sup>	5.5 <sup>c</sup>	6.9 <sup>abc</sup>	5.9 <sup>bc</sup>	7.4 <sup>ab</sup>	0.64	0.0016
EE	3.2 <sup>bc</sup>	2.6 <sup>c</sup>	5.7 <sup>a</sup>	3.5 <sup>bc</sup>	4.5 <sup>ab</sup>	3.8 <sup>bc</sup>	2.8 <sup>c</sup>	0.53	<.0001
CP	28.6 <sup>a</sup>	28.4 <sup>a</sup>	25.8 <sup>b</sup>	20.4 <sup>d</sup>	27.0 <sup>b</sup>	22.7 <sup>c</sup>	21.8 <sup>cd</sup>	0.57	<.0001
NPN	6.5 <sup>a</sup>	5.8 <sup>ab</sup>	4.7 <sup>ab</sup>	5.3 <sup>ab</sup>	6.1 <sup>a</sup>	5.6 <sup>ab</sup>	4.1 <sup>b</sup>	0.78	0.0080
NDF	40.8 <sup>b</sup>	37.9 <sup>c</sup>	33.2 <sup>d</sup>	35.8 <sup>cd</sup>	36.3 <sup>c</sup>	41.7 <sup>ab</sup>	44.2 <sup>a</sup>	1.17	<.0001
ADF	23.8 <sup>a</sup>	24.2 <sup>a</sup>	24.8 <sup>a</sup>	27.5 <sup>a</sup>	25.9 <sup>a</sup>	22.2 <sup>a</sup>	25.9 <sup>a</sup>	2.87	0.3691
NDIP	1.6 <sup>b</sup>	1.5 <sup>b</sup>	0.8 <sup>c</sup>	0.7 <sup>c</sup>	1.6 <sup>b</sup>	1.4 <sup>b</sup>	2.5 <sup>a</sup>	0.16	<.0001
ADIP	0.4 <sup>bc</sup>	0.5 <sup>abc</sup>	0.7 <sup>a</sup>	0.4 <sup>bc</sup>	0.8 <sup>a</sup>	0.3 <sup>c</sup>	0.8 <sup>a</sup>	0.13	0.0009
Lig	9.9 <sup>c</sup>	7.6 <sup>c</sup>	11.3 <sup>b</sup>	12.1 <sup>ab</sup>	11.5 <sup>b</sup>	11.6 <sup>b</sup>	14.9 <sup>a</sup>	1.30	<.0001

On-R onset rains; F-RF= full rains; Ex-R= Extreme rains; La-R= late rains; Re-R= residual rains.

Ash= ashes; EE= ether extract; CP= crude protein; NPN= non-protein nitrogen; NDF= neutral detergent fiber;

ADF= acid detergent fiber; NDIP= neutral detergent insoluble protein; ADIP= acid detergent insoluble protein; Lig= lignin. MSE= mean squared error.

<sup>abcd</sup> Different letters in the same row indicate Tukey statistical difference. ( $P \leq 0.05$ ).

NDF tends to concentrate with SWB- and dilute with SWB+, contrary to what happens with lignin, which is diluted with SWB- and concentrated with SWB+. If lignin were expressed as a percentage of NDF, the synergism would enhance the indigestibility of NDF under SWB+ conditions. Several authors agree that lack of water tends to slow down the development of the plant and thus delay maturity, whereby digestibility is increased and DM yield is reduced<sup>(23,24,25)</sup>.

## Carbohydrate fractions

Nutrient availability is also affected by the SWB. Although carbohydrates and proteins have contrasting behavior, because proteins are synthesized from carbohydrates (the higher the protein concentration, the lower the concentration of carbohydrates, particularly non-fibrous ones). Under conditions of full (4.9 mm d<sup>-1</sup>) and late rains (3.7 mm), total carbohydrates (TC) show the highest values. In these same periods, CP showed the lowest values (Table 3). In North winds, *Leucaena leucocephala* has the lowest values for TC and the highest for CP. In extreme conditions such as the end of the dry season (Apr-May) and the “peak” of the rainy season (Jul-Aug), the TC drop significantly, with no change in CP. Perhaps the carbons seek another route, such as cutin or tannins. While, under the conditions of late and residual rains, CP decreases significantly, with no changes in TC during the same period, perhaps due to the phenological stage (maturity) of the plant. The composition of the TC is also modified

by the SWB. The NFC:FC ratio is approximately 50:50. Although in the periods of onset, full and extreme rains the NFC (%TC) are more abundant at a 60:40 ratio, proportionally and inversely, the FC (%TC) are significantly higher in winter and dry compared to onset, full, and extreme rains. As these fibrous carbohydrates (mainly cellulose and hemicellulose) coincide with a lower proportion of lignin, they are expected to be more available for ruminal fermentation.

**Table 3:** Nutritional quality of *Leucaena leucocephala* (Lam.) de Wit cv. Cunningham by time of year classified by seasonal water balance (SWB)

Month	Dec-Jan	Feb-Apr	Apr-May	Jun-Jul	Jul-Aug	Aug-Sep	Sep-Dec		
Season	North winds	Dry	On-R	Fu-R	Ex-R	La-R	Re-R	MSE	P value
SWB (mmd <sup>-1</sup> )	-0.18	-2.39	1.77	4.89	8.32	3.7	-1.46		
<b>Carbohydrates</b>									
TC, DM	50.6 <sup>b</sup>	54.1 <sup>ab</sup>	50.6 <sup>b</sup>	58.4 <sup>a</sup>	50.1 <sup>b</sup>	56.0 <sup>a</sup>	53.0 <sup>ab</sup>	2.18	0.0004
CNF, DM	21.3 <sup>c</sup>	25.4 <sup>bc</sup>	29.5 <sup>b</sup>	35.5 <sup>a</sup>	26.9 <sup>b</sup>	27.3 <sup>b</sup>	26.3 <sup>b</sup>	1.91	<.000 1
FC, DM	29.3 <sup>a</sup>	28.7 <sup>ab</sup>	21.0 <sup>c</sup>	22.9 <sup>c</sup>	23.2 <sup>c</sup>	28.7 <sup>ab</sup>	26.7 <sup>b</sup>	0.91	<.000 1
NFC, TC	41.9 <sup>e</sup>	46.9 <sup>de</sup>	58.4 <sup>ab</sup>	60.7 <sup>a</sup>	53.7 <sup>bc</sup>	48.7 <sup>cd</sup>	49.6 <sup>cd</sup>	2.15	<.000 1
FC, TC	58.1 <sup>a</sup>	53.1 <sup>ab</sup>	41.6 <sup>de</sup>	39.3 <sup>e</sup>	46.3 <sup>cd</sup>	51.3 <sup>bc</sup>	50.4 <sup>bc</sup>	2.15	<.000 1
<b>Proteins</b>									
CP, DM	28.6 <sup>a</sup>	28.4 <sup>a</sup>	25.8 <sup>b</sup>	20.4 <sup>d</sup>	27.0 <sup>b</sup>	22.7 <sup>c</sup>	21.8 <sup>cd</sup>	0.57	<.000 1
NPN, CP	22.6 <sup>ab</sup>	20.3 <sup>ab</sup>	18.2 <sup>b</sup>	25.9 <sup>a</sup>	22.6 <sup>ab</sup>	24.8 <sup>ab</sup>	18.9 <sup>b</sup>	2.75	0.0156
TSP, CP	71.8 <sup>ab</sup>	74.4 <sup>ab</sup>	78.5 <sup>a</sup>	70.7 <sup>b</sup>	71.5 <sup>ab</sup>	68.9 <sup>b</sup>	69.4 <sup>b</sup>	2.96	0.0082
F, CP	4.2 <sup>b</sup>	3.5 <sup>b</sup>	0.7 <sup>d</sup>	1.4 <sup>cd</sup>	3.1 <sup>bcd</sup>	4.8 <sup>b</sup>	8.2 <sup>a</sup>	3.73	<.000 1
IP, CP	1.4 <sup>c</sup>	1.8 <sup>bc</sup>	2.6 <sup>bc</sup>	2.0 <sup>b</sup>	2.8 <sup>ab</sup>	1.4 <sup>c</sup>	3.5 <sup>a</sup>	0.47	<.000 1

On-R= Onset rains; Fu-R= Full rains; Ex-R= extreme rains; La-R= late rains; Re-R= residual rains. DM=dry matter; TC=total carbohydrates; NFC= non-fibrous carbohydrates; FC= fibrous carbohydrates; CP= crude protein; NPN= non-protein nitrogen; TSP= True soluble protein; PF= protein associated with potentially digestible fiber; IP= indigestible protein. MSE= mean squared error.

<sup>abcd</sup> Different letters in the same row indicate Tukey statistical difference. ( $P \leq 0.05$ ).

### Protein fractions

The nitrogen fractions were not very susceptible to the effects of SWB (Table 3). Although TSP and NPN consistently represent 70 % and 20 % of the CP in *Leucaena leucocephala*,

and these nitrogen fractions are completely degraded in the rumen, they practically do not fluctuate throughout the year. Tadros *et al*<sup>(26)</sup> also showed that the CP of *Leucaena leucocephala* leaves tended to increase with irrigation, although the values did not differ among irrigation levels (ET of 0.25, 0.50, 0.75, 1.00, and 1.25)<sup>(26)</sup>. Particularly in the season of residual rains, the lowest CP content coincides with the lowest proportion of soluble protein (TSP and NPN), while a considerable amount of the insoluble portion (PF) is indigestible (IP), causing the protein during these four months to be rated as having the worst nutritional quality. The high rumen nitrogen availability and low soluble or non-fibrous carbohydrate content ( $\leq 50$  % of TC) occurring during the North winds and Dry seasons imply an energy limitation for rumen N incorporation into microbial protein synthesis. This leads to limiting the use of *Leucaena leucocephala* in ruminant feed to avoid exceeding blood urea N or supplementing with soluble carbohydrates in the diet to increase microbial protein synthesis and minimize N waste in urea. Castro-González *et al*<sup>(27)</sup> supplemented heifers consuming Taiwan grass (*Cenchrus purpureus*) with *Leucaena leucocephala* to increase ruminal microbial protein synthesis (MRPS) and found that maximum efficiency is attained with 20 % *L. leucocephala* in the ration. Higher amounts of *L. leucocephala* increase the excretion of N-ureic acid. For higher levels of *L. leucocephala* in the ration, Castro-González *et al*<sup>(27)</sup> suggest including NFC sources to capture the N provided by *L. leucocephala* and avoid N losses in the urine.

Under tropical conditions, the main factor limiting the yield and determining the concentration and variation of forage nutrients is the SWB, which defines the rainy and dry seasons. In this study, the North winds and dry conditions, as well as full and late rains, clearly show the effects of the SWB on the yields, biochemical composition, and nutritional quality of *Leucaena leucocephala*; however, there are certain conditions –e.g., at the end of the dry or rainy seasons– under which soil hydration significantly influences the forage yield and nutritional quality, while the peak summer conditions, with excessive rainfall, humidity, temperature and luminosity, render the plant's response in terms of yield and quality practically unpredictable. This condition is also associated with the meteorological phenomenon known in the region as “*Canícula*” (midsummer heat), which begins a few weeks after the summer solstice and lasts 40 d. These extreme weather conditions are difficult for both people and animals even when they can seek shade. But plants are buried in the soil and need to develop defense mechanisms for their survival that could enhance or undermine the nutritional quality. In early growing *Leucaena leucocephala* plants with irrigation on a daily or weekly basis or every other day. Jabasingh<sup>(28)</sup> reported (for fresh weight g<sup>-1</sup>) 28.6, 25.6, and 24.3 mg CP; 11.6, 17.8, and 48.8 mg soluble carbohydrates; and 11.7, 16.6, and 20 mg proline, respectively; thus, drought tolerance may be due to a gradual saving of soluble carbohydrates. Previous research points to the protective role that soluble carbohydrates play at the level of the membrane systems in general and the mitochondrial membrane in particular<sup>(29)</sup>. Carbohydrates and proline, together with other substances, contribute to the phenomenon of hydrolytic modification, which protects membranes and enzyme systems by

reducing their hydrolytic potential to compensate for the low water potential found in the leaves<sup>(30)</sup>; this refers to fluctuations in energy reserves between the rainy and dry seasons.

## Conclusions and implications

The NFC decreases and the TSP increases during the North winds and Dry seasons with SWB-. Conversely, in the season of full and late rains with SWB+, the NFC increases and the TSP decreases. Apparently, in the late dry season and late rainy season the soil water conditions are more important determinants of *Leucaena leucocephala*'s yield and nutritional quality than seasonal water balance. Extreme midsummer heat conditions allow maximum DM yields without deterioration of the nutritional quality—an indication that *Leucaena leucocephala* has more potential as a protein forage for ruminants. The biochemical composition is not indicative of nutritional quality because the fractions that make up the carbohydrates and proteins in forage are dynamic, and their combination affects the availability of these nutrients to the animal.

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The authors declare that they have no conflict of interest.

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