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Article

Biochemical composition of tropical grasses receiving nitrogen fertilization with irrigation by season of the year

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Abstract:

The objective was to evaluate the effect of nitrogen fertilization and irrigation (FI) by season of the year on the dry matter yield (DMY) and biochemical composition of tropical grasses. Five grass varieties were used. Each with three replications with FI and three replications without FI. The pastures were cut every 35 d. The dose of nitrogen fertilization was 138 kg/ha/yr, equivalent to 300 kg of urea. The plots with FI were irrigated from December to May. The seasons were: rainy (R, Jun-Nov), "north winds" (N, Dec-Feb), and dry (D, Mar-May). DMY was determined, and the available carbohydrate and protein fractions were estimated. The statistical design was completely randomized in a split-split-plot arrangement. Means were compared by Tukey (*P*≤0.05). Without FI, in R, DMY is 53 % of the amount produced with FI, and in N and D, it is only 26 and 15 %, respectively. Non-protein nitrogen and rumen-soluble true protein are the protein fractions that are most increased with FI. Total carbohydrates (TC), non-fibrous carbohydrates (NFC), and digestible neutral detergent fiber (NDF) are higher in R and D, and the pastures without FI showed the highest increases in TC in D, NFC in R and D, and digestible NDF in D. Indigestible fractions increase during the "north wind" season, and regarding pastures with FI, they also increase during the dry season. The effect of FI on carbohydrate fractions is climate-dependent.

Keywords: Forages, Nutritional quality, Fertigation.

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Introduction

Tropical soils, due to their low nitrification capacity, among other factors, are characterized by containing low levels of nitrogen (0.10 %)(1) for growing grasses used in cattle production. It is estimated that nitrogen deficiency limits forage production and quality because it is the major constituent of chlorophyll, amino acids, adenosine triphosphate (ATP), and genetic materials(2). Some authors(3,4) recognize that nitrogen fertilization can increase the percentage of proteins and dry matter yield (DMY) t ha^{-1}. Therefore, nitrogen fertilization improves the stocking rate and animal yield per area according to the photosynthetic capacity of the plant(4).

On the other hand, the seasonality in the distribution of rainfall is the greatest limitation for pasture yield, and in this sense, forage species with greater productivity and adapted to the different seasons of the year are required(5). In the tropical regions of Mexico, due to the seasonality in forage production dependent on precipitation, the productive parameters of the cattle system, particularly the dual-purpose system, are low since 90 % of their diet is based on grasses(6). For nitrogen fertilization to work during the dry season, water must be provided; and it has been reported(1) that 35 % soil moisture is required for maximum nitrification. Fertilization with irrigation during the six-month dry period (December to May) in the Mexican tropics is currently considered an unsustainable technology from an economic point of view, but if the trend is towards intensifying the production system, importing forage resources may be less sustainable. The objective was to evaluate the effect of nitrogen fertilization and irrigation (FI) on the dry matter yield (DMY) and biochemical composition of tropical grasses during the "north wind", dry, and rainy seasons in warm subhumid climates.

Material and methods

Study area. The experiment was conducted at the “La Posta” Experimental Field (INIFAP), Paso del Toro, Veracruz, located at 19º 02´ NL, 96º 08´ WL, and at 16 m asl. Subhumid tropical climate type Aw_1[7]. Annual average of: rainfall, 1,328 mm; temperature, 25 °C; relative humidity, 81 %. The soil is classified as vertisol, with an acidic pH of 5.4, clayey texture, with 2.6 % organic matter and 9.5 ppm NO_3.

Vegetative material. The 5 grasses used were: *Cenchrus purpureus* (Maralfalfa), *Megathyrsus maximus* (Mombasa), *Urochloa brizantha* (Insurgente), *Urochloa humidicola* (Rendle) CIAT 6133, and *Urochloa ruziziensis* x *Urochloa brizantha* (Mulato I). For each grass, six plots (3 x 2 m) were established: three plots with FI and three plots without FI, with a sampling area of 1 m^2 per plot. The pastures were cut every 35 d, from August 2011 to July 2012. After each cut, the FI plots were fertilized with 13.8 kg/ha of nitrogen divided into 10 applications for a total of 138 kg/ha/yr. Plots with FI were irrigated only from December to May (“north wind” and dry seasons). Plots without FI never received irrigation or fertilization.

Seasons of the year. They were classified as rainy (June-November), “northern winds” (December-February), and dry (March-May) according to Muñoz *et al*[8].

Dry matter yield (DMY). It was determined by harvesting the available forage in a 1 m quadrant at 15 cm height for *Urochloa* and 30 cm height for *Megathyrsus* and *Cenchrus* and weighing it in the field with a portable digital scale. Fresh forage was expressed as (kg ha^{-1}). Samples of 500 g were then taken, dried at 105 °C in a forced-air oven for 24 h to determine DM content, and subsequently discarded.

Biochemical composition. Other 500 g samples were dried at 55 °C in a forced-air oven to a constant weight and ground in a Wiley mill with a 2 mm mesh. Standard procedures of AOAC[9] were used to measure the contents of DM (AOAC 934.01), ash (AOAC 942.05), ethereal extract (EE), and crude protein (AOAC 990.03) by Macrokjeldahl. Nitrogen fractions were determined by the techniques described by Licitra *et al*[10]: non-protein nitrogen (NPN), soluble protein (SP), cell wall protein (NDIP), indigestible protein (ADIP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) by Ankom technology[11]. Lignin by Goering and Van Soest[12].

Carbohydrate fractions. Total carbohydrates and available carbohydrate fractions were estimated with the following equations:

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

Non-fibrous carbohydrates (NFC, %DM)= 100 – ash – EE – CP – (CP-free NDF) (2)
Fibrous carbohydrates (FC, %DM)= (CP-free NDF) – lignin (3)
Indigestible NDF (%DM)= (CP-free NDF* (lignin, %CP-free NDF)* 2.4)/100 (4)
Digestible NDF (%DM)= CP-free NDF - indigestible-NDF (5)

Protein fractions. The estimation of protein fractions is presented in Table 1.

Table 1: Equivalences of protein biochemical fractions with protein nutritional fractions

Biochemical fractions	Nutritional fractions
Nitrogen * 6.25	Crude protein, CP
NPN	Non-protein nitrogen (immediate solubility)
CP – NDIP	SP is a step to obtain soluble true protein (RSTP)
SP – NPN	RSTP (fast and intermediate solubility)
NDIP – ADIP	RITP (slow solubility or insoluble)
ADIP	IP (insoluble protein)

NDIP= NDF-insoluble protein; SP= soluble protein; RSTP= rumen-soluble true protein; RITP= partially rumen-insoluble true protein; ADIP= ADF-insoluble protein; IP= insoluble protein.

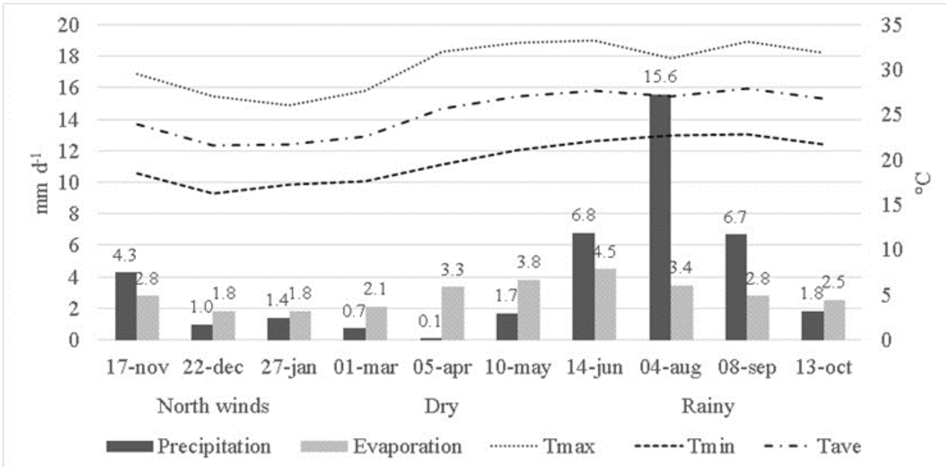
Experimental design and statistical analysis. The variables of DMY, biochemical composition, carbohydrate fractions, and available proteins were subjected to an analysis of variance with a completely randomized design in a split-plot arrangement, with the large plot being the season of the year and the small plot being fertigation (FI). The variables were statistically analyzed using the generalized linear models procedure (PROC GLM) of the SAS program, version 9.1 (SAS, 2003)[13]. The least squares means of the treatments were estimated with the LSMEANS option, whereas the comparisons between them ($P\leq0.05$) were made with Tukey's test.

Results and discussion

Climatic conditions

The rainfall and temperatures that prevailed in 2011 and 2012 during the experimental period are shown in Figure 1. There were 830 mm in the rainy season, 203 mm in the "north wind" season, and 189 mm in the dry season, accumulated in periods of 140 d (June to November), 105 d (November to March), and 105 d (March to June), respectively, which was reflected in the production of the evaluated grasses. Variations in temperature were also significant, especially from December to February, during which time low temperatures sometimes reached 17.5 °C, which limit the growth of tropical grasses.

Figure 1: Climatic conditions during 2011 and 2012 in Paso del Toro, Veracruz. Source: CONAGUA



Tables 2 and 3 show the adjusted means and statistical significance for the effects of the season of the year, fertigation, and their interactions for the variables studied. In the variables in which there was no interaction (*P*≥0.05), the main effects will be discussed in Table 4. The variables in which the interaction was (*P*≤0.05), the interactions will be discussed in Tables 5 and 6.

Table 2: Means and statistical significance of the effect of the season of the year, fertigation, and their interaction on the dry matter yield (DMY, kg ha^{-1}) and biochemical composition of tropical grasses

Variable, %	Mean	Season (S)	Fertigation (FI)	S*FI
DMY	1784.9	***	***	***
DM	22.3	***	ns	ns
ASH	10.6	***	***	***
EE	3.0	***	**	***
NDF	65.9	***	ns	***
ADF	38.4	***	*	***
LIG	9.983	***	***	***
CP	10.6	***	***	ns
NPN	2.9	***	***	ns
NDIP	3.8	***	***	ns
ADIP	1.1	***	ns	ns

DMY= dry matter yield; DM= dry matter; ASH= ashes; EE= ethereal extract; NDF= neutral detergent fiber; ADF= acid detergent fiber; LIG= lignin; CP= crude protein; NPN= non-protein nitrogen; NDIP= NDF insoluble protein; ADIP= ADF insoluble protein.
ns= not significant; **P*≤0.05; ***P*≤0.01; ****P*≤0.001.

Table 3: Means and statistical significance of the effect of the season of the year, fertigation, and their interaction on carbohydrate, fiber, and crude protein fractions and nitrogen fractions in tropical grasses

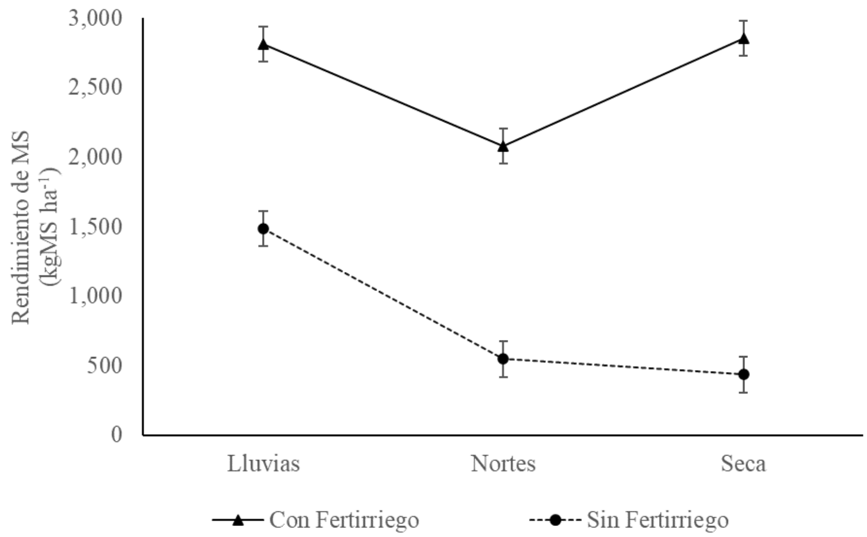
Variable, %	Mean	Season (S)	Fertigation (FI)	S*FI
Carbohydrates and fiber fractions				
TC	69.3	***	***	**
NFC	13.7	***	***	***
FC	55.6	***	**	ns
LIG, % NDF	10.0	***	***	***
Indigestible NDF	15.7	***	***	***
Digestible NDF	46.4	***	***	***
Nitrogen fractions				
NPN	2.9	***	***	ns
RSTP	3.9	***	***	ns
RITP	2.6	***	***	ns
IP	1.1	***	*	ns

TC= total carbohydrates; NFC=non-fibrous carbohydrates; FC= fibrous carbohydrates; LIG= lignin; NDF= neutral detergent fiber; NPN= non-protein nitrogen; RSTP= rumen-soluble true protein; RITP= rumen-insoluble true protein; IP= insoluble protein;
ns= not significant, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Dry matter yield (DMY)

FI significantly favors DMY, with nitrogen and water availability being the main constraints on pasture growth in the tropics (Figure 2). With FI, the DMY of the dry season is similar to that of the rainy season, and although the DMY in the "north wind" season was lower than those of the rainy and dry seasons, it was higher than any season without FI. Without FI, the DMY produced during the rainy season was 53 % of the amount produced with FI, and during the "north wind" and dry seasons, it was only 26 and 15 % of the amount produced with FI, respectively. In pastures without FI, the seasonal effect dominates the DMY because the productive response is a function of the availability of water, as could be demonstrated in the "north wind" and dry seasons, where the water balance is negative. De Dios-León *et al*[14] found that of the total dry matter production, 79 % occurs in the rainy season (June to October), 13 % during the "north wind" season (November to February), and 8 % in the dry season (March to May).

Figure 2: Effect of fertigation and season of the year on dry matter yield (DMY)



Protein biochemical composition and protein fractions

Table 4 exhibits the main effects of the season of the year and fertigation on protein biochemical composition and protein fractions (%DM).

Crude protein (CP). Grasses with FI have the highest concentration of CP. It is well known that nitrogen fertilization increases the concentration of CP in forages[15,16]. In the “north wind” season, pastures have the highest protein concentration and during the rainy season, the lowest. It coincides with the highest DMY in the rainy season and the lowest in the “north wind” season (Figure 2). Perhaps it is a dilution factor since DMY (among other factors) dilutes the concentration of CP in forages[17]. In pastures without FI, the impact on CP during the rainy season is that it places it at the critical nutritional limit of 7 %, resulting in the depression of digestibility and voluntary consumption in cattle[18]. The paradox in the tropics is that, in this time of excess forage, it has low protein quantity. Protein content and dry matter production are negatively correlated[17].

NDF-insoluble protein (NDIP) and ADF-insoluble protein (ADIP). The difference between these two fractions represents the protein bound to fiber (basically extensins), which is of slow solubility in the rumen and is positively influenced by the FI and negatively influenced by the rains, canceling out the interaction between the FI and the season. The ADIP fraction, which represents the indigestibility of the protein, is only affected by the season of the year, with protein being more digestible in the “north wind” season.

Non-protein nitrogen (NPN). NPN is the most dynamic protein fraction, representing the highest level in the "north wind" season and the lowest in the rainy season (Table 4). In the dry season, there was no statistical difference due to the effect of FI. Previous studies have indicated that when photosynthesis is limited, nitrogen fertilization increases nitrate accumulation in the plant(19,20).

Table 4: Main effects of the season of the year and fertigation on protein biochemical composition and protein fractions (%DM)

Variable	Season			SEM	Fertigation		SEM
	Rainy	"North winds"	Dry		With	Without	
Biochemical composition:							
CP	8.6^{c}	13.3^{a}	10.8^{b}	0.215	12.3^{a}	9.6^{b}	0.163
NDIP	3.2^{b}	4.2^{a}	4.1^{a}	0.120	4.1^{a}	3.5^{b}	0.091
ADIP	1.2^{a}	0.7^{b}	1.2^{a}	0.075	1.2^{a}	1.0^{a}	0.057
Protein fractions:							
NPN	2.2^{c}	3.6^{a}	2.9^{b}	0.150	3.4^{a}	2.5^{b}	0.114
RSTP	3.1^{c}	5.3^{a}	3.8^{b}	0.161	4.7^{a}	3.4^{b}	0.122
RITP	1.9^{c}	3.4^{a}	2.8^{b}	0.115	2.9^{a}	2.5^{b}	0.087
IP	1.2^{a}	0.7^{b}	1.2^{a}	0.075	1.2^{a}	1.0^{b}	0.057

CP= crude protein; NDIP= NDF-insoluble protein; ADIP= ADF-insoluble protein; NPN= non-protein nitrogen; RSTP= rumen-soluble true protein; RITP= rumen-insoluble true protein; IP= indigestible protein; SEM= standard error of the mean.

[abc] Different letters in the same row indicate significant differences (*P*<0.05).

Rumen-soluble true protein (RSTP). RSTP is the soluble fraction of nitrogen that responds to fertilization regardless of the season of the year. The biggest impact of FI on RSTP occurs during the "north wind" season (Table 4). A high concentration of RSTP is desirable since it is rapidly degradable in the rumen and can result in better animal behavior(21). Higher RSTP results in greater microbial protein synthesis, which is optimized as long as NFCs do not limit microbial growth.

Rumen-insoluble true protein (RITP). No apparent changes were found in the protein fraction of slow degradation due to the effect of FI (Table 4). Other authors(16,22) also found no differences in the protein fraction bound to fiber due to the effect of nitrogen fertilization. However, the season of the year shows a marked difference between the rainy season *vs*. the "north wind" and dry seasons. Rhaony *et al*(22) also found that this protein fraction is higher in low rainfall conditions. Apparently, water scarcity increases this fraction, but since low yield is associated with water and/or nitrogen deficiency, it is likely that extensins proportionally concentrate in the cell wall raising the RITP fraction.

Insoluble protein (IP). FI shows no tendency to influence IP (Table 4). Other authors[23,24] did not find that N fertilization affects IP either. Nevertheless, a linear decrease in the IP fraction with nitrogen fertilization is reported[22]. The "north wind" season expresses a significant decrease in IP, without this being associated with lignin content. It is recalled that part of this fraction is bound to lignin. This suggests that, in the rainy and dry seasons, other factors could be influencing the insolubility of the protein, such as cross-links with tannin-like substances[25] or glycosylation or Maillard reactions[26]. These interfere with the digestive enzymes of the rumen microbiota, making this nitrogenous fraction unavailable to the animal[27,28]. Other nutritional fractions that are not sensitive to the FI* season interaction are dry matter (DM) and fibrous carbohydrates (FC) from forage.

Dry matter (DM). DM content is not sensitive (22.0^a *vs*. $22.7^a \pm 0.38$ for with and without FI, respectively), but it does react to the season of the year, with values of 21.6^b, 20.1^b, and $25.4^a \pm 0.49$ for the rainy, "north wind", and dry seasons, respectively. In the dry season, the DM content of the grasses is higher as a manifestation of the state of dehydration of the plant given by the negative water balance that occurs both in the soil and in the air.

Fibrous carbohydrates (FC). FCs decrease with FI (54.5^b *vs*. $55.5^a \pm 0.27$) probably because, phenologically, pastures are more immature since fertilization delays maturation[5], but in the rainy season, FCs increase compared to the "north wind" and dry seasons (58.3^a *vs*. 52.9^b and $53.7^b \pm 0.35$, respectively) since, in summer, the metabolism of C4 grasses is accelerated, and soluble carbohydrates migrate to the cell wall[18]. The energy contribution of FC for the animal will depend on their concentration and their potential digestibility. The main factor of indigestibility is the concentration of lignin. In the rainy season, the higher concentration of FC is combined with a lower proportion of lignin, therefore a greater amount of digestible NDF and a higher energy contribution, without relevance of nitrogen fertilization (Table 6). Nonetheless, during the "north wind" and dry seasons when FI is recommended, there is a significant drop in digestible NDF due to lignin accumulation as a percentage of NDF.

Non-protein biochemical composition

Ashes. They are more concentrated in pastures without FI, especially in the "north wind" season (Table 5). This implies that the mineral concentration could not necessarily be associated with the level of hydration of the plant but with the decrease in the synthesis of organic nutrients, that is, less organic matter. It is also associated with the fact that in the "north wind" season, the regrowth of plants depends on the mobilization of other minerals present in the soil and the reserve of non-structural carbohydrates in the root.

Ethereal extract. It is diluted in the rainy season in pastures with and without FI. The excess of ambient humidity could explain this decrease in the EE as there is no need for more cutin as protection against wind dehydration caused by strong north winds in the "north wind" season or heat dehydration in the dry season[18].

NDF. It is very dynamic: in the summer rains, without limitations of water, temperature, and solar radiation, the accumulation of NDF is elevated regardless of fertilization (P>0.05); however, in the "north wind" season, with less water availability and lower temperature and luminosity than in the summer rains, the concentration of NDF is lower in pastures with FI as a sign of delay in maturity; the same phenomenon occurs in the dry season with pastures without FI, with the availability of water and nitrogen being the limiting factors.

Lignin. It supports this condition by being higher in the most mature pastures, as happens in the dry season with FI and in the "north wind" season without FI.

Table 5: Effect of the interaction of fertigation (FI) and season of the year on non-protein biochemical composition (% DM)

Variable	Rainy	"North winds"	Dry	SEM
ASH, with FI	9.3^{d}	11.1^{b}	10.6^{bc}	0.157
ASH, without FI	10.3^{c}	12.7^{a}	10.9^{b}	
EE, with FI	2.7^{c}	3.7^{a}	3.1^{b}	0.096
EE, without FI	2.7^{c}	3.0^{b}	3.2^{b}	
NDF, with FI	67.9^{a}	63.3^{d}	65.4^{b}	0.411
NDF, without FI	67.4^{a}	65.2^{bc}	63.8^{cd}	
ADF, with FI	39.9^{a}	37.0^{c}	38.3^{b}	0.301
ADF, without FI	39.5^{a}	38.3^{b}	35.8^{c}	
LIG, with FI	6.1^{d}	6.8^{bc}	7.9^{a}	0.175
LIG, without FI	6.2^{cd}	7.4^{ab}	5.6^{d}	

SEM= standard error of the mean; ASH=ashes; EE= ethereal extract; NDF= neutral detergent fiber; ADF= acid detergent fiber; LIG= lignin.

^{abc} Different letters in the same row indicate significant differences (P<0.05).

Carbohydrate fractions

Total carbohydrates (TC). The highest TC content occurs in the rainy season regardless of FI and coincides with higher NDF and ADF in this season (Table 6). Van Soest[18] mentions that under tropical summer conditions with high temperature and precipitation, TCs accumulate in the cell wall. During the “north wind” season, the TCs decrease, without difference due to FI, but pastures with FI have less NDF. Other authors[29] report that the fibrous components of forage decrease with increasing levels of nitrogen because it stimulates the growth of new tissue, but the dry season with FI has more NDF, ADF, and lignin. Although FI tends to decrease TC, the season of the year is the one that models this effect by acting on fiber fractions. The results found here coincide with Van Soest’s[18] concepts, where: in summer (rains), the higher the temperature and precipitation, the more TC and more NDF; in winter (“north winds”), the lower the temperature and precipitation, the less TC and less NDF; and in spring (dry), the higher the temperature and lower the precipitation, the less TC and more NDF.

Table 6: Effect of the interaction of fertigation (FI) and season of the year on carbohydrate and fiber fractions (% DM)

Variable	Rainy	“North winds”	Dry	SEM
TC, with FI	72.1^{ab}	63.4^{d}	66.2^{c}	0.484
TC, without FI	73.4^{a}	65.1^{cd}	70.7^{b}	
NFC, with FI	13.7^{b}	11.3^{c}	13.3^{b}	0.329
NFC, without FI	15.2^{a}	11.3^{c}	16.2^{a}	
LIG, % NDF, with FI	9.5^{c}	11.5^{b}	13.1^{a}	0.297
LIG, % NDF, without FI	9.6^{c}	12.1^{ab}	9.3^{c}	
Indigestible NDF, with FI	14.7^{d}	16.2^{bc}	19.0^{a}	0.420
Indigestible NDF, without FI	14.8^{cd}	17.9^{ab}	13.4^{d}	
Digestible NDF, with FI	49.8^{a}	42.6^{c}	41.9^{c}	0.617
Digestible NDF, without FI	49.6^{a}	43.5^{c}	46.7^{b}	

SEM= standard error of the mean; TC= total carbohydrates; NFC=non-fibrous carbohydrates; FC=fibrous carbohydrates; LIG=lignin; NDF=neutral detergent fiber.

[abcd] Different letters in the same row indicate significant differences (P<0.05).

Non-fibrous carbohydrates (NFC). NFCs decrease during the “north wind” season when sunlight is less intense (Table 6). Oelberg[30] has already identified that sunlight affects the amount of glucose formed during photosynthesis. High solar radiation, such as in summer (rains) and spring (drought), increases the photosynthetic rate, promoting NFC production. The lower concentration of NFC in pastures with FI *vs*. without FI in the rainy and dry seasons is due to the fact that FI favors cell growth, which stimulates stem elongation by using NFCs synthesized in the formation of other biochemical compounds, including soluble proteins[19]. Since the availability of NFC to rumen microbes is almost complete, their contribution to the energy supply to the ruminant will depend on their concentration. In the dry season, FI negatively impacts TC, NFC, and digestible NDF. In the “north wind” season, FI does not affect any of the carbohydrate fractions. In summary, the effect of FI on carbohydrate fractions is climate-dependent, as also found by Rhaony *et al*[22] when evaluating carbohydrate and protein fractions in nitrogen-fertilized Marandu palisade grass.

Conclusions and implications

Unfertilized grasses are exposed to and dependent on soil and climate conditions in sub-humid tropical environment type Aw_1 to express DMY, even in the rainy season. The relevant nutritional deficiency in these pastures is that they have less CP, with the RSTP being the most affected protein fraction. With nitrogen fertilization and irrigation practices, DMY and the contents and availability of the different carbohydrate and protein fractions of tropical grasses respond dynamically and sensitively to the seasonal interactions of the tropical environment. The lower concentration of carbohydrates in the "north wind" and dry seasons is aggravated by FI, as NFCs and digestible NDF decrease. In contrast, protein fractions, which are the first nutritional limitations, particularly the RSTP fraction, are favored in these same periods. Low-nitrogen tropical soils necessarily require nitrogen fertilization. Forage production is not sufficient to cover the requirements of voluntary dry matter consumption of genetically productive cattle, so it is essential to supplement with forage throughout the year. Since protein is the first limiting nutrient, it is recommended to supplement with protein agro-industrial by-products rich in soluble protein or to associate pastures with legumes to provide nitrogen to both cattle and soil. Fertigation saves forage supplementation throughout the year. Nevertheless, it is almost mandatory to associate with legumes to reduce the dose of fertilizer and to provide cattle with rumen-soluble true protein, but that is balanced with non-fibrous carbohydrates that provide energy to maximize microbial protein synthesis and make cattle production of calves and milk more efficient and sustainable.

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Conflict of interest

The authors declare that there is no conflict of interest.

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