CHILEAN JOURNAL of AGRICULTURAL RESEARCH

CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

RESEARCH ARTICLE

Nitrogen and phosphorus fertilization and regrowth age on the fatty acid profile in tropical grasses during the dry and rainy seasons

Isabel Cristina Acosta-Balcazar[1], Yuridia Bautista-Martínez[1], Benigno Estrada-Drouaillet[2], and Lorenzo Danilo Granados-Rivera[3*]

[1]Universidad Autónoma de Tamaulipas, Facultad de Medicina Veterinaria y Zootecnia, 87274, Ciudad Victoria, Tamaulipas, México.
[2]Universidad Autónoma de Tamaulipas, Facultad de Ingenierías y Ciencias, 87149, Ciudad Victoria, Tamaulipas, México.
[3]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental-General Terán, Nuevo León, México.
[*]Corresponding author (granados.danilo@inifap.gob.mx).
Received: 15 November 2023; Accepted: 10 January 2024, doi:10.4067/S0718-58392024000200291

ABSTRACT

The concentration of linoleic and α-linolenic fatty acids (FA) in grasses can be increased through agronomic techniques; however, there is little information in this regard for tropical grasses. Therefore, the objective of the study was to evaluate the effect of N fertilization and regrowth age management on the FA profile of three tropical grasses. To do this, three plots of 100 m^2 each were used for the species: *Urochloa decumbens* (Stapf) R.D. Webster, *U. humidicola* (Rendle) Morrone & Zuloaga, and *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs. The study was conducted during dry and rainy seasons. In each period, they were divided into two subplots and only one of them was fertilized. Grass sampling was carried out on days 20, 25, and 30 after fertilization. Five samples were taken from each subplot and an agronomic evaluation was made before each cut. The chemical composition and lipid profile were then determined. An ANOVA of a randomized complete block design with split plot arrangement was performed. The concentration of linoleic and α-linolenic acid was higher in *M. maximus* during drought (48.5 g 100 g^{-1} FA) and in *U. humidicola* during the rainy season (55.1 g 100 g^{-1} FA). This proportion was higher on day 20 (50.5 g 100 g^{-1} FA) compared to that recorded on days 25 and 30 (50.1 and 45.7 g 100 g^{-1} FA, respectively). The fertilized grasses had a higher concentration of linoleic and α-linolenic acid compared to the unfertilized ones (53.4 *vs*. 44.3 g 100 g^{-1} FA). It is concluded that fertilization with 50-20-00 (N-P-K) increases the proportion of linoleic and α-linolenic acids in grasses; however, such concentration decreases with increasing plant maturity.

Key words: Conjugated linoleic acid, forage production, lipid metabolism, ruminant nutrition.

INTRODUCTION

In the world, the area dedicated to forage production is 33% of the total arable land (FAO, 2023). Annually, livestock consume about 3 billion tons DM derived from grasses. Animal production systems, particularly ruminants, are highly dependent on forage resources, since 90% of the nutrients they require are satisfied through pastures and forages (Herrera et al., 2014). These are the most abundant and economical sustenance to produce meat and milk (Livas Calderón, 2015).

Regarding dairy cattle, the chemical composition of milk, fat in particular, increases with grazing; consequently, the content of polyunsaturated fatty acids also increases, thus improving its nutritional quality (Rivera Herrera et al., 2012).

Cow milk contains fatty acids such as *trans*-vaccenic acid (TVA), docosahexaenoic acid (DHA), and conjugated linoleic acid (CLA), which are beneficial for human health due to their atherogenic and hypercholesterolemic effects. Of these, CLA is found in a greater proportion (Prieto-Manrique et al., 2016). Its content in milk can be increased through the supply of pastures, silage, and supplements based on vegetable oils or oilseeds (Kęsek et al., 2014). In the latter there is a higher content of linoleic acid, while flaxseed and fresh forages have a higher concentration of α-linolenic acid; both fatty acids are the main precursors of CLA synthesis in the rumen (Roca-Fernández et al., 2014). It should be noted that milk from grazed cows contains a higher concentration of CLA compared to milk from cows fed with supplements or concentrates (Morales-Almaráz et al., 2018). The concentration of linoleic and α-linolenic acids in grasses depends on factors such as: Species, variety, climate, light intensity, and management techniques such as N fertilization and age of regrowth (Elizondo-Salazar, 2017).

In this regard, the lipid fraction in grasses ranges between 30 and 100 g kg^{-1} DM, much of it contributed by lipids in chloroplasts (Elgersma et al., 2005). Therefore, the highest concentration of fat in grasses is found in the leaves. Therefore, management strategies that increase the number of leaves in relation to the stem could increase FA intake in grazing ruminants. Among the most successful strategies to increase the number of leaves in the grassland are N fertilization and early regrowth ages. In this sense, Boufaïed et al. (2003) applied 120 *vs*. 0 kg N ha^{-1} in Timothy grass (*Phleum pratense* L.), which increased the concentrations of C16:0 (18%), C18:2 (12%) and C18:3 (40%) in the grass, with an overall increase of 26% in the FA concentration of the grass. Likewise, Lopes et al. (2021; 2022) observed that the FA profile was modified in elephant grass (*Pennisetum purpureum* Schumach), due to the effect of the age of regrowth, with an inversely proportional relationship between the age of the grass and the concentration of FA C18:2 and C18:3.

More information has been produced on this topic in temperate environments with legume species (Acosta Balcazar et al., 2022). The information for tropical regions is scarce, even though the tropics have great potential to produce milk and meat from ruminants, with high nutritional content and higher concentrations of CLA, because the nutritional base is grazing, especially, forage grasses. This leads to greater consumption of α-linolenic acid in animals (Plata-Pérez et al., 2022). For this reason, the present research aimed to evaluate the effect of agronomic management (N fertilization and age of regrowth) on the fatty acid profile of three species of tropical grasses.

MATERIALS AND METHODS

Study location and area

The study was carried out during the dry season (March 15 to June 15) and rainy season (June 16 to October 15) of the year 2022, on a private farm located in the state of Tabasco, Mexico (21°52'00" N,102°55'00" W; 20 m a.s.l.) The climate of the region is tropical with rainfall all year round, Af according to the Koppen classification, where the average annual rainfall recorded was 2452 mm yr^{-1}, of which 10.13% corresponded to the dry season (248.5 mm) and 41.53% to rain (1018.3 mm).

Three plots of 100 m^2 were established in 2005, one for each forage species to be evaluated; *Urochloa decumbens* 'CIAT 606', *Urochloa humidicola* 'CIAT 679' and *Megathyrsus maximus* 'CIAT 6962'.

Experimental design

A randomized complete block experimental design with split plot structure was used. The main plot was the forage species (*U. decumbens*, *U. humidicola* and *M. maximus*) and the subplot was the combination of fertilization levels (F = 50N-20P-0K and NF = 0N-0P-0K; Garza et al., 1972) and the regrowth ages (20, 25 and 30 d), with five replicates each (90 samples in total per season).

Fertilization was broadcast using urea and calcium triple superphosphate as a source of N and P, respectively.

Sampling

On day 1 of the sampling period, a uniformity cut was made 10 cm from the ground and fertilization was applied. Samples were taken on days 20, 25 and 30 after fertilization using a 0.25 m^2 square. With the table, five points were chosen at random in each subplot and, before cutting, an agronomic evaluation of the pasture was carried out to record the following variables; height (cm; using a 1 m ruler), number of plants, number of shoots, leaf/stem ratio (counting was done manually), stem diameter (mm; using a vernier) and coverage (%; respect to the 0.25 m^2 square). After cutting the samples were weighed to estimate forage production (t ha^{-1}), performing the necessary operations. This same procedure was followed in both periods.

Laboratory analysis

In each of the seasons, a composite sample was made with the grass from the five replicates of each treatment (combinations between species, fertilization, and age), 300 g were taken from each composite sample to determine the chemical composition and fatty acid (FA) profile. The samples were dried in a forced air oven at 60 °C for 48 h and ground in a Wiley mill (Thomas Scientific, Swedesboro, New Jersey, USA) using a 1 mm mesh.

The chemical composition of the grass was determined following the methodologies AOAC (1990) for crude protein (CP), Van Soest et al. (1991) for acid detergent fiber (ADF) and neutral detergent fiber (NDF), Goering and Van Soest (1970) for in vitro digestibility of DM (DIVMS) and Soxhlet (1879) for fat. The forage samples were inoculated with ruminal fluid, which was obtained from a cow with fistula fed with grass of the genus *Urochloa* spp.

The extraction of FA in the grass was carried out according to the methodology modified by Granados-Rivera et al. (2017). For the quantification of FA methyl esters, an autosampler chromatograph (Hewlett-Packard 6890, Hewlett-Packard, Wilmington, Delaware, USA) with a silica capillary column (100 m × 0.25 mm × 0.20 μm thickness, Sp-2560, Supelco, Bellefonte, Pennsylvania, USA) was used. The identification of FA was carried out by comparing the retention times of each peak obtained in the chromatogram, using a standard of 37 components of methyl esters of FA (Sigma-Aldrich, St. Louis, Missouri, USA).

Statistical analysis

The ANOVA of the data obtained in the agronomic evaluation, chemical composition and FA profile of the pastures was carried out using a randomized complete block design with divided plot structure. The main plot was the forage species (*U. decumbens*, *U. humidicola* and *M. maximus*) and the subplot was the different management conditions that were evaluated (F-20 d, F-25 d, F-30 d, NF- 20 d, NF-25 d and NF-30 d), with five replicates each (45 samples per treatment making a total of 90 samples per season). In the factors that were significant ($p \leq 0.05$), the comparison of means was carried out through the Tukey test ($P = 0.05$). Statistical analyses were performed with the GLM procedure of the SAS 9.0 (2002) statistical program (SAS Institute, Cary, North Carolina, USA).

RESULTS

Agronomic evaluation and chemical composition

In the dry season, with respect to the species, forage production was the only variable that did not show a significant difference. According to the level of fertilization, height, forage production, and cover showed significance ($P = 0.05$), while the variables number of shoots and cover presented differences ($P = 0.05$) with respect to age of regrowth (Table 1). In the rainy season, height and forage production showed differences ($P = 0.05$) with respect to the species and fertilization level. According to the age of regrowth, forage production, number of leaves, and coverage showed nonsignificant differences (Table 1). Both height and forage production were higher in the rainy season with respect to the three factors evaluated (species, fertilization, and age).

Chemical composition in both seasons was different ($P = 0.05$) in the variables CP, NDF, ADF, and DIVMS according to the level of fertilization and age of regrowth, and with respect to the species, CP, fat and ADF did not present significant differences (Table 2).

Table 1. Agronomic evaluation of three forage species with two treatments and at three regrowth ages in the dry and rainy seasons in the tropics. *U. humidicola*: *Urochloa humidicola*; *U. decumbens*: *Urochloa decumbens*; *M. maximus*: *Megathyrsus maximus*; SEM: standard error of the mean; F: fertilized; NF: unfertilized; Ep: species; Tr: treatment; Ag: age. Different letters indicate significant difference (P = 0.05) of variables by species, treatments, and ages.

	Species				Treatments			Ages (d)				P-Values			
Variables	*U. humidicola*	*U. decumbens*	*M. maximus*	SEM	F	NF	SEM	20	25	30	SEM	Ep×Tr	Ep×Ag	Tr×Ag	Ep×Tr×Ag
							Dry season								
Height, cm	20.63^{b}	26.03^{a}	27.63^{a}	0.88	26.22^{a}	23.31^{b}	0.88	21.53^{b}	25.30^{ab}	27.46^{a}	0.88	P≤0.05	P>0.05	P>0.05	P>0.05
Plants	6.76^{a}	5.46$_{b}$	5.70^{b}	0.12	6.04^{a}	5.91^{a}	0.12	6.26^{a}	6.00^{ab}	5.66^{b}	0.12	P>0.05	P≤0.05	P≤0.05	P>0.05
Shoots	17.26^{a}	11.58^{b}	13.94^{b}	0.50	14.75^{a}	13.76^{a}	0.50	13.80^{a}	13.84^{a}	15.13^{a}	0.50	P>0.05	P>0.05	P≤0.05	P>0.05
Leaves/stem	5.54^{a}	4.26^{b}	3.12^{c}	0.15	4.31^{a}	4.30^{a}	0.15	4.52^{a}	3.96^{b}	4.43^{ab}	0.15	P>0.05	P≤0.05	P>0.05	P>0.05
Stem diameter, mm	0.34^{a}	0.33^{a}	0.30^{b}	0.00	0.32^{a}	0.32^{a}	0.00	0.35^{a}	0.33^{ab}	0.30^{b}	0.00	P>0.05	P≤0.05	P>0.05	P>0.05
Cover, %	60.66^{a}	51.66^{b}	63.00^{a}	1.17	62.55^{a}	54.33^{b}	1.17	59.00^{a}	57.33^{a}	59.00^{a}	1.17	P>0.05	P>0.05	P>0.05	P>0.05
							Rainy season								
Height, cm	33.10^{a}	33.20^{a}	28.86^{b}	0.93	33.11^{a}	30.33^{b}	0.93	28.40^{b}	29.70^{b}	37.06^{a}	0.93	P≤0.05	P≤0.05	P>0.05	P≤0.05
Plants	6.26^{a}	6.96^{a}	7.26^{a}	0.21	6.80^{a}	6.86^{a}	0.21	7.26^{a}	6.70^{a}	6.53^{a}	0.21	P≤0.05	P>0.05	P>0.05	P>0.05
Shoots	11.83^{a}	12.93^{a}	11.02^{a}	0.40	12.58^{a}	11.27^{a}	0.40	10.68^{b}	13.16^{a}	11.95^{ab}	0.40	P>0.05	P>0.05	P>0.05	P>0.05
Leaves/stem	4.24^{a}	4.05^{a}	3.82^{a}	0.08	4.11^{a}	3.96^{a}	0.08	4.18^{a}	3.86^{a}	4.07^{a}	0.08	P≤0.05	P>0.05	P>0.05	P>0.05
Stem diameter, mm	1.12^{a}	1.21^{a}	1.22^{a}	0.04	1.16^{a}	1.21^{a}	0.04	1.07^{b}	1.09^{b}	1.39^{a}	0.04	P≤0.05	P>0.05	P>0.05	P≤0.05
Cover, %	61.16^{ab}	66.00^{a}	58.50^{b}	1.15	62.66^{a}	61.11^{a}	1.15	62.00^{a}	63.00^{a}	60.66^{a}	1.15	P≤0.05	P>0.05	P>0.05	P>0.05

Table 2. Chemical composition of three forage species with two treatments and at three regrowth ages in the dry and rainy seasons in the tropics. SEM: Standard error of the mean; CP: crude protein; F: fat; ADF: acid detergent fiber; NDF: neutral detergent fiber; DIVMS: In vitro DM digestibility; *U. humidicola*: *Urochloa humidicola*; *U. decumbens*: *Urochloa decumbens*; *M. maximus*: *Megathyrsus maximus*. Different letters in the same column indicate significant difference.

	Ages	Fertilized					Unfertilized				
Species	(d)	CP	F	ADF	NDF	DIVMS	CP	F	ADF	NDF	DIVMS
						Dry season					
U. humidicola	20	8.6^{a}	2.3^{a}	42.0^{c}	72.1^{b}	70.8^{a}	11.7^{a}	2.5^{a}	43.1^{b}	73.7^{b}	70.4^{a}
	25	4.8^{c}	2.5^{a}	45.2^{b}	76.4^{a}	63.1^{b}	7.2^{b}	1.4^{a}	44.0^{c}	75.8^{b}	60.7^{b}
	30	3.7^{c}	2.3^{a}	49.5^{a}	77.3^{a}	53.8^{c}	5.1^{c}	2.1^{a}	47.5^{c}	77.2^{a}	43.0^{c}
U. decumbens	20	8.9^{a}	2.0^{a}	42.8^{c}	71.1^{c}	69.3^{a}	12.3^{a}	2.6^{a}	39.3^{a}	68.4^{c}	70.8^{a}
	25	6.5^{b}	2.7^{a}	45.9^{b}	73.6^{b}	57.4^{b}	9.2^{b}	3.0^{a}	40.8^{b}	70.4^{b}	66.3^{b}
	30	4.3^{c}	3.0^{a}	48.3^{a}	75.1^{a}	53.6^{c}	6.7^{b}	2.3^{a}	45.7^{c}	75.8^{a}	61.2^{b}
M. maximus	20	8.6^{a}	2.0^{a}	43.1^{b}	73.7^{b}	70.4^{a}	12.2^{a}	2.2^{a}	41.8^{a}	69.8^{c}	68.2^{a}
	25	6.4^{b}	2.7^{a}	45.7^{b}	76.6^{a}	59.1^{b}	9.7^{b}	2.2^{a}	45.0^{b}	71.0^{b}	57.9^{c}
	30	4.5^{c}	2.0^{a}	48.0^{a}	78.7^{a}	53.0^{c}	6.9^{c}	2.3^{a}	47.9^{c}	75.8^{b}	53.8^{c}
SEM		0.168	0.100	0.584	0.795	0.627	0.168	0.100	0.584	0.795	0.627
							P-Values				
Species		P>0.05	P>0.05	P>0.05	P≤0.05	P≤0.05	P>0.05	P>0.05	P>0.05	P≤0.05	P≤0.05
Treatments		P≤0.05	P>0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P>0.05	P≤0.05	P≤0.05	P≤0.05
Age		P≤0.05	P>0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P>0.05	P≤0.05	P≤0.05	P≤0.05
Spe×Treat×Ages		P≤0.05	P>0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P>0.05	P≤0.05	P≤0.05	P≤0.05
						Rainy season					
U. humidicola	20	11.2^{a}	1.5^{a}	41.9^{b}	67.6^{c}	68.7^{b}	14.3^{a}	2.1^{a}	37.8^{c}	68.2^{c}	70.3^{a}
	25	8.1^{b}	1.1^{a}	42.8^{b}	73.4^{a}	54.7^{c}	10.4^{b}	1.3^{a}	38.0^{c}	69.7^{b}	66.2^{b}
	30	5.3^{c}	1.6^{a}	44.6^{a}	75.7^{a}	50.2^{c}	7.0^{b}	1.7^{a}	40.6^{b}	72.4^{a}	55.0^{c}
U. decumbens	20	10.2^{b}	2.2^{a}	40.8^{b}	66.4^{c}	72.4^{a}	14.7^{a}	1.4^{a}	34.5^{c}	67.5^{c}	75.1^{a}
	25	7.4^{b}	1.7^{a}	41.6^{b}	76.8^{a}	56.6^{c}	8.4^{b}	1.8^{a}	36.0^{c}	70.4^{b}	65.0^{b}
	30	6.8^{c}	3.4^{a}	43.9^{a}	79.5^{a}	47.5^{c}	5.5^{c}	2.0^{a}	41.5^{b}	76.0^{a}	56.7^{c}
M. maximus	20	9.8^{b}	1.6^{a}	41.6^{b}	68.2^{c}	66.9^{b}	12.6^{a}	0.8^{a}	34.4^{c}	66.6^{c}	70.1^{a}
	25	6.2^{c}	1.2^{a}	43.5^{b}	72.0^{a}	57.0^{c}	9.8^{b}	1.2^{a}	38.7^{c}	70.2^{b}	67.6^{b}
	30	4.1^{c}	1.9^{a}	46.9^{a}	76.4^{a}	52.6^{c}	5.3^{c}	2.9^{a}	40.8^{b}	76.9^{a}	58.9^{c}
SEM		0.152	0.100	0.438	0.589	0.602	0.099	0.100	0.476	0.681	0.615
							P-Values				
Species		P>0.05	P>0.05	P>0.05	P≤0.05	P>0.05	P>0.05	P>0.05	P>0.05	P≤0.05	P>0.05
Treatments		P≤0.05	P>0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P>0.05	P≤0.05	P≤0.05	P≤0.05
Age		P≤0.05	P>0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P>0.05	P≤0.05	P≤0.05	P≤0.05
Spe×Treat×Ages		P≤0.05	P>0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P>0.05	P≤0.05	P≤0.05	P≤0.05

Fatty acid profile

The profile was composed of 10 FAs, in particular, lauric (C12:0), myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), heptadecanoic (C17:0), stearic (C18: 0), oleic (C18:1n9c), linoleic (C18:2n6c), α-linolenic (C18:3n3), and arachidic (C20:0) acids, of which C16:0, C18:2n6c, and C18:3n3 comprise more than 80% of the acids found (Table 3).

Table 3. Fatty acid profile of three forage species with two treatments and at three regrowth ages in the dry and rainy seasons in the tropics. *U. humidicola*: *Urochloa humidicola*; *U. decumbens*: *Urochloa decumbens*; *M. maximus*: *Megathyrsus maximus*; SEM: standard error of the mean; F: fertilized; NF: unfertilized; Ep: species; Tr: treatment; Ed: age. Different letters indicate significant difference (P = 0.05) of the variables by species, treatments, and age.

	Species			Treatments				Ages (d)				P-Values			
Variables	*U. humidicola*	*U. decumbens*	*M. maximus*	SEM	F	NF	SEM	20	25	30	SEM	Ep×Tr	Ep×Ag	Tr×Ag	Ep×Tr×Ag
Dry season															
C12:0	3.4^{b}	4.3^{a}	3.2^{b}	0.041	3.2^{b}	4.6^{a}	0.035	3.6^{b}	3.3^{b}	4.1^{a}	0.062	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C14:0	2.3^{a}	2.6^{a}	2.4^{a}	0.063	1.8^{b}	2.7^{a}	0.092	2.3^{a}	2.2^{a}	2.5^{a}	0.097	P≤0.05	P>0.05	P>0.05	P>0.05
C16:0	39.7^{a}	39.8^{a}	34.2^{a}	0.706	34.7^{b}	37.5^{a}	0.628	36.2^{b}	36.7^{b}	39.0^{a}	0.493	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C16:1	1.5^{a}	1.2^{a}	1.3^{a}	0.012	1.2^{a}	1.4^{a}	0.001	1.2^{a}	1.3^{a}	1.4^{a}	0.087	P>0.05	P>0.05	P>0.05	P>0.05
C17:0	0.8^{b}	1.6^{a}	1.4^{a}	0.011	0.9^{b}	2.1^{a}	0.073	1.3^{a}	1.0^{a}	1.5^{a}	0.002	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C18:0	3.3^{a}	2.4^{b}	2.6^{b}	0.091	2.8^{a}	2.3^{a}	0.081	2.6^{a}	2.9^{a}	2.7^{a}	0.007	P>0.05	P>0.05	P>0.05	P>0.05
C18:1n9c	3.2^{b}	2.5^{c}	4.7^{a}	0.043	3.3^{b}	5.6^{a}	0.001	3.5^{a}	3.7^{a}	3.8^{a}	0.015	P≤0.05	P≤0.05	P>0.05	P≤0.05
C18:2n6c	26.7^{a}	16.3^{c}	20.3^{b}	0.157	22.7^{a}	19.9^{b}	0.191	19.8^{b}	23.2^{a}	21.0^{a}	0.001	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C20:0	1.4^{a}	1.1^{a}	1.1^{a}	0.001	1.0^{a}	1.4^{a}	0.003	1.1^{a}	1.2^{a}	1.3^{a}	0.071	P>0.05	P>0.05	P>0.05	P>0.05
C18:3n3	18.2^{b}	26.3^{a}	28.2^{a}	0.163	28.1^{a}	22.9^{b}	0.103	27.5^{a}	24.8^{b}	22.5^{b}	0.133	P≤0.05	P≤0.05	P≤0.05	P≤0.05
Rainy season															
C12:0	1.6^{c}	2.9^{b}	4.9^{a}	0.014	3.1^{b}	3.3^{a}	0.022	2.5^{b}	2.8^{b}	3.7^{a}	0.004	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C14:0	1.3^{c}	1.9^{b}	3.0^{a}	0.002	1.9^{b}	2.2^{a}	0.043	1.7^{b}	1.9^{b}	2.4^{a}	0.001	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C16:0	32.5^{b}	34.8^{a}	32.1^{b}	0.612	30.3^{b}	36.0^{a}	0.483	32.5^{a}	33.4^{a}	34.3^{a}	0.001	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C16:1	1.7^{a}	0.6^{b}	0.8^{b}	0.007	0.7^{b}	1.3^{a}	0.063	1.0^{a}	1.1^{a}	0.9^{a}	0.001	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C17:0	0.5^{b}	0.7^{b}	1.7^{a}	0.001	0.5^{b}	1.4^{a}	0.014	0.6^{b}	0.8^{b}	1.3^{a}	0.007	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C18:0	2.7^{a}	2.8^{a}	2.9^{a}	0.027	2.7^{a}	3.0^{a}	0.002	2.7^{a}	2.8^{a}	2.9^{a}	0.179	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C18:1n9c	1.9^{c}	2.9^{b}	5.1^{a}	0.010	3.2^{a}	3.3^{a}	0.009	2.7^{b}	3.0^{b}	3.8^{a}	0.031	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C18:2n6c	21.0^{a}	17.8^{b}	21.0^{a}	0.314	19.1^{a}	20.8^{a}	0.146	19.3^{a}	20.1^{a}	19.9^{a}	0.017	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C20:0	1.1^{a}	1.2^{a}	0.9^{a}	0.002	0.7^{a}	1.3^{a}	0.012	1.0^{a}	1.0^{a}	1.1^{a}	0.071	P≤0.05	P≤0.05	P≤0.05	P≤0.05
C18:3n3	34.1^{a}	32.3^{a}	26.8^{b}	0.237	37.0^{a}	25.1^{b}	0.103	34.5^{a}	32.1^{a}	28.1^{b}	0.031	P≤0.05	P≤0.05	P≤0.05	P≤0.05

In both times of year, differences were found (P = 0.05) according to the species, with the exception of C20:0. Regarding the treatment, there were also differences for almost all FAs, except for C18:0 and C20:0 and for the age of regrowth, the FAs that showed nonsignificance were C16:1, C18:0, and C20:0.

In the dry season, of the three FAs with the highest proportion (C16:0, C18:2n6c, and C18:3n3), the grass *U. humidicola* had the highest proportion of C18:2n6c, *U. decumbens* presented the highest concentration of C16:0, meanwhile, *M. maximus* presented the highest amount of C18:3n3. According to the treatments, fertilized grasses had a higher concentration of C18:2n6c and C18:3n3 and C16:0 was present in a higher proportion in the unfertilized grasses. Regarding regrowth ages, an increase was recorded in the proportion of C16:0 and C18:2n6c as age advanced, while C18:3n3 decreased (Table 3). On the other hand, in the rainy season, of the three FAs with the highest proportion (C16:0, C18:2n6c, and C18:3n3), the grass *U. humidicola* recorded the highest proportion of C18:2n6c and C18:3n3, while *U. decumbens* presented the highest concentration of C16:0. According to the treatments, the fertilized grasses had a higher concentration of C18:3n3, while C16:0 and C18:3n3 were present in a higher proportion in the unfertilized grasses. With respect to regrowth ages, an increase in the proportion of C16:0 and C18:2n6c was also recorded as age advanced, while C18:3n3 decreased.

DISCUSSION

Agronomic evaluation

Species with tillering growth, such as *U. humidicola* and *M. maximus*, may be taller than those with erect or creeping growth (Rojas-Hernández et al., 2011). However, the rains caused the *U. humidicola* grass to develop better than the other species, as it was taller. This could be due to the greater use of soil moisture. In tropical regions, grass stems tend to grow better with temperatures between 25 and 35 °C, and with an average monthly precipitation of 150 mm (Cruz López et al., 2011), conditions that were met in the present study. On the other hand, N and P fertilization caused a greater production of green forage compared to the unfertilized grasses. Nitrogen participates in the production of the hormone cytokinin, which is responsible for plant growth and activates the process of cell division and differentiation, which produces changes in the structural and morphological characteristics of grasses, that is, it increases the size and number of leaves, and stimulates plant growth through the elongation of the internodes (De Andrade et al., 2017). In addition, N and P are elements involved in physiological processes such as photosynthesis, respiration and energy storage and transfer. They also promote rapid root formation and growth. Aspects related to the growth and development of leaves in pastures (Aydin and Uzun, 2005).

Furthermore, N and P fertilization together with precipitation caused the number of plants, shoots, coverage and leaf/stem ratio to increase in the rainy season. This indicates that N was used efficiently. Likewise, the climatic conditions at this time favored photosynthesis (De Dios-León et al., 2022). However, this accelerated growth caused the diameter of the stem to increase, consequently, its structural components increased, decreasing digestibility and nutritional value (Calzada-Marín et al., 2019).

Finally, the low forage yields that occurred in the dry season could be due to the little precipitation recorded in that season, so the biochemical process of photosynthesis is negatively affected (De Dios-León et al., 2022).

Chemical composition

Although there were no differences in nutritional content between the evaluated species, it is pertinent to indicate that the CP percentages obtained in our study in fertilized grasses were higher than those reported by other authors. In this regard, Rincón et al. (2018) found averages between 8.4% and 9.9% for *U. humidicola*; Zambrano and Obando (2013) reported a maximum content of 10.5% for the grass *U. decumbens*, and Suárez et al. (2011) found ranges between 8.5% and 9.5% for the species *M. maximus*. Furthermore, the DIVMS percentages were within the averages reported by Meléndez (2012), 68%, 71%, and 68% for *U. humidicola, U. decumbens,* and *M. maximus*, respectively.

In the rainy season, the decrease in CP was greater, since at this time the growth and maturation of the grass occurs more quickly because the conditions of sunlight, temperature, and humidity are favorable. Therefore, a process called "protein dilution" is accelerated (Fernández et al., 2012). As in CP, there is a negative relationship between the age of the grass and the percentage of DIVMS, since this decreases as the grass matures, due to the increase in the concentration of structural components of the cell wall such as cellulose, hemicellulose, and lignin, decreasing the nutritional quality of the grasses (De Dios-León et al., 2022).

With N fertilization, the grasses increased the CP content in the leaves. Which is attributed to the fact that N increases the synthesis of metabolic compounds in the grass and greater biomass production is obtained (Acosta Balcazar et al., 2022). In addition, it is essential for the synthesis of chlorophyll, so it is also involved in the photosynthesis process (De Andrade et al., 2017).

The content of ADF and NDF was different from that of the CP and the DIVMS, since these increased with the advance of the age of the pastures. The ADF content of grasses helps us evaluate their digestibility, while NDF is a component that can limit the ingestive capacity of animals (Merlo-Maydana et al., 2017).

The differences in DIVMS, ADF, and NDF found between seasons could be due to the fact that precipitation helped the fertilized grasses to have an increased cellular content, while, in the unfertilized ones, it accelerated their growth and maturation process, making them more fibrous and less digestible.

On the other hand, the percentages of fat found in this research in both seasons remained constant with respect to the age of regrowth and showed no difference due to the type of treatment; however, they were

higher than the percentages reported by Canchila Asencio (2014), who found between 1.3% and 2.3% fat in different varieties of *U. humidicola* grass. Mahecha-Ledesma et al. (2017) recorded 1.49% fat in *U. decumbens*, while Sosa-Montes et al. (2022) reported an average of 1.20% fat for *M. maximus*. In the rainy season, fat contents were negatively affected due to precipitation and humidity (Zambrano and Obando, 2013).

Fatty acid profile

About 75% CLA in bovine milk originates from the consumption of linoleic and α-linolenic acid contained in dietary ingredients (Mojica-Rodríguez et al., 2017). In this regard, grasses are the main source of PUFA for ruminants, because they consume large amounts of forage, causing a greater intake of PUFA (Khan et al., 2015).

In the present study, 10 FA were found, of which linoleic (C18:2n6c) and α-linolenic (C18:3n3) contributed between 45% and 52% to the total FA. These results differ from the study by Morales-Almaráz et al. (2018) who found that the fat portion of C18:2n6c and C18:3n3, in grasses, is 95%, and of this, between 50% and 75% belongs to C18:3n3. The variations in concentrations were mainly due to the forage species, as well as the treatments applied and the environmental conditions that occurred during the sampling periods (Glasser et al., 2013). In this regard, Elizondo-Salazar (2017) mentions that the concentration of linoleic and α-linolenic acids in grasses will depend on factors such as: Species, variety, climate, light intensity, and management techniques such as N fertilization and age. of regrowth.

In the dry season, C16:0 was the FA most present in the three species, and in the rainy season it was for *U. decumbens* and *M. maximus*. High temperatures positively influence the production of C16:0, because an adaptation mechanism occurs that reduces the fluidity of membranes in plant cells and evapotranspiration is decreased in this type of environment, directly affecting C18:3n3 (Toyes-Vargas et al., 2013). Our results differed with the data obtained by Mojica-Rodríguez et al. (2017), who also used cultivars of the species *M. maximus* (Mombasa and Tanzania) and compared them with species of *Urochloa* (*humidicola* and *brizantha*), with those of the species *M. maximus* having a higher proportion of C18:2n6c and C18:3n3 (1.58 and 2.93 *vs*. 1.01 and 1.46 g kg^{-1} DM). On the contrary, the rains favored the production of C18:2n6c and C18:3n3, whose portion was higher in the *Urochloa* (55.10 and 50.10 *vs*. 47.80 g 100 g^{-1} FA). This result could be because leaves of the *Urochloa* are larger, causing greater coverage and forage production.

The FAs are present in the lipids of leaf membranes, mainly in thylakoid membranes of the chloroplasts, which contain on average 20% lipids, so there is a positive correlation between chlorophyll content and total content of FA (Khan et al., 2015). It is worth mentioning that, regarding the type of grass, Plata-Pérez et al. (2022) state that the concentration of C18:3n3 is higher in grasses, because they are characterized for having a greater amount of vegetative material.

On the other hand, FA concentrations, especially C18:2n6c and C18:3n3, will increase according to management techniques that induce rapid vegetative growth such as N fertilization, or techniques that determine the appropriate age for the use of grass as management of regrowth age (Acosta Balcazar et al., 2022); both techniques were used in this research.

In both the dry and rainy seasons, grasses were favored by fertilization since their PFA content increased. In this regard, Elgersma et al. (2005) recorded significant increases of 18%, 12%, and 40% of the acids C16:0, C18:2n6c, and C18:3n3, when pastures were fertilized. In the present study, the greatest increase was in C18:3n3 (22.70% and 47.41% for dry and rainy season, respectively), decreasing the proportion of C18:0 and C18:2n6c.

Nitrogen is essential for the synthesis of chlorophyll, so it is also involved in the photosynthesis process (De Andrade et al., 2017); in addition to intervening in the photosynthetic activity of the plant, participates in the expansion of leaf area (increases the number of leaves) and the production of new shoots. Furthermore, it influences the morphogenetic characteristics of the grass and participates in the synthesis of proteins and FA, causing greater accumulation of lipids in the plant and increasing the amount of FA within the cellular content (León et al., 2011; González, 2014; Rincón et al., 2018). Regarding the age of regrowth, Mojica (2017) states that leaves have a higher FA content than stems, and young plants have a higher FA concentration than older plants. Therefore, pasture management is important in the FA composition of pastures. In the present study, some of the FAs decreased as the age of the grass advanced, specifically

C18:3n3, which decreased around 18% in both seasons. The decrease in FA with respect to the age of the grass may be due to three factors: Decrease in leaf/stem ratio, leaf maturation, or the beginning of flowering and senescence (Khan et al., 2015). With plant maturity, the concentrations of other metabolites such as cellulose, hemicellulose, and lignin increase (Jarquin Almanza et al., 2013). As the grass grows, DM production increases, but nutritional quality is lost. A negative relationship can be seen between the increase in percentage of ADF and the decrease in the amount of C18:3n3, since as the age of the grass increases, metabolites such as proteins, minerals and lipids are lost and the plant becomes more fibrous, so grass is less digestible.

CONCLUSIONS

Under the experimental conditions of the study, it is concluded that N and P fertilization increased forage production and the nutritional quality of the pastures. The fat content was not affected by the fertilization. While the concentration of linoleic and α-linolenic acids, independently of the season, increased with N and P fertilization. Which was highest on day 20 and decreased with the increase in plant maturity.

Author contribution

Conceptualization: L.D. Methodology: I.C., L.D., Y.B. Validation: L.D., Y.B. Formal analysis: I.C., B.E. Investigation: I.C. Resources: L.D. Data curation: B.E., Y.B. Writing-original draft: I.C. Writing-review & editing: L.D., Y.B., B.E. Visualization: L.D. Supervision: L.D. Project administration: L.D. Funding acquisition: L.D. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgements

The authors would like to express their gratitude to the Consejo Nacional de Ciencia y Tecnología (CONACYT) for providing the scholarship (CVU:737742) to the first author.

References

Acosta Balcazar, I.C., Granados Rivera, L.D., Salinas Chavira, J., Estrada Drouaillet, B., Ruíz Albarrán, M., Bautista Martínez, Y. 2022. Relationship between the composition of lipids in forages and the concentration of conjugated linoleic acid in cow's milk: A review. Animals 12:1621. doi:10.3390/ani12131621.

AOAC. 1990. Official methods of analysis. 17th ed. Association of Analytical Chemists (AOAC), Gaithersburg, Maryland, USA.

Aydin, I., Uzun, F. 2005. Nitrogen and phosphorus fertilization of rangelands affects yield, forage quality and the botanical composition. European Journal of Agronomy 23(1):8-14.

Boufaïed, H., Chouinard P.Y., Tremblay G.F., Petit H.V., Michaud R. Bélanger G. 2003. Fatty acids in forages. I. Factors affecting concentrations. Canadian Journal of Animal Science 83:501-511.

Calzada-Marín, J.M., Enríquez-Quiroz, J.F., Ortega-Jiménez, E., Alfonso, H.G., Vaquera-Huerta, H., Escalante-Estrada, J.A., et al. 2019. Growth analysis of Toledo grass *Urochloa brizantha* (Hochst. Ex A. Rich.) R.D. Webster in sub-humid warm climate. Agroproductividad 12:3-9. doi:10.32854/agrop.v0i0.1443.

Canchila Asencio, E.R. 2014. Evaluación de accesiones de *Brachiaria humidicola* (Rendle) Schweickerdt en suelos de baja fertilidad en Barrancabermeja, Colombia. 141 p. PhD thesis. Universidad de Matanzas, Camilo Cienfuegos, Estación Experimental de Pastos y Forrajes, Matanzas, Cuba.

Cruz López, P.I., Hernández Garay, A., Enríquez Quiroz, J.F., Mendoza Pedroza, S.I., Quero Carrillo, A.R., Joaquín-Torres, B.M. 2011. Desempeño agronómico de genotipos de *Brachiaria humidicola* (Rendle) Schweickt en el trópico húmedo de México. Revista Fitotecnia Mexicana 34:123-131.

De Andrade, F.M.G., Zavarez, H.B., Gerdes, L., Giacomini, A.A., Batista, K., Teixeira, W., et al. 2017. The utilization of tropical legumes to provide nitrogen to pastures: A review. African Journal of Agricultural Research 12:85-92. doi:10.5897/AJAR2016.11893.

De Dios-León, G.E., Ramos-Juárez, J.A., Izquierdo-Reyes, F., Joaquín-Torres, B.M., Meléndez-Nava, F. 2022. Comportamiento productivo y valor nutricional del pasto *Pennisetum purpureum* cv Cuba CT-115, a diferente edad de rebrote. Revista Mexicana de Ciencias Pecuarias 13:1055-1066. doi:10.22319/rmcp.v13i4.5217.

Elgersma, A., Maudet, P., Witkowska, I.M., Wever, A.C. 2005. Effects of nitrogen fertilisation and regrowth period on fatty acid concentrations in perennial ryegrass (*Lolium perenne* L.) Annals of Applied Biology 147:145-152. doi:10.1111/j.1744-7348.2005.00020.x.

Elizondo-Salazar, J.A. 2017. Producción de biomasa y calidad nutricional de tres forrajes cosechados a dos alturas. Agronomía Mesoamericana 28:329-340. doi:10.15517/ma.v28i2.23418.
FAO. 2023. FAO's role in animal production. FAO, Rome, Italy. Available at https://www.fao.org/animal-production/en (accessed February 2023).
Fernández, J.L., Gómez Angulo, I., Cordoví Castillo, E. 2012. Efecto de la edad de rebrote en el rendimiento y contenido proteico del pasto *Brachiaria humidicola* cv CIAT-609 en un suelo vertisol. Revista de Producción Animal 24:1471.
Garza, T.R., Arroyo, D., Monroy, J. 1972. Potencial del zacate Pangola bajo pastoreo rotacional en el trópico húmedo Am. 1. Técnica Pecuaria en México 20:15-22.
Glasser, F., Doreau, M., Maxin, G., Baumont, R. 2013. Fat and fatty acid content and composition of forages: A meta-analysis. Animal Feed Science and Technology 185:19-34. doi:10.1016/j.anifeedsci.2013.06.010.
Goering, H.K., Van Soest, P.J. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agriculture Handbook N°379. USDA Agricultural Research Service, Beltsville, Maryland, USA.
González, P.J.C. 2014. Manejo efectivo de la simbiosis micorrízica arbuscular vía inoculación y la fertilización mineral en pastos del género *Brachiaria*. 167p. PhD tesis. Instituto Nacional de Ciencias Agrícolas, Departamento de Biofertilizantes y Nutrición de las Plantas, Mayabeque, Cuba.
Granados-Rivera, L.D., Hernández-Mendo, O., González-Muñoz, S.S., Burgueño-Ferreira, J.A., Mendoza-Martínez, G.D., Arriaga-Jordán, C.M. 2017. Effect of palmitic acid on the mitigation of milk fat depression syndrome caused by *trans*-10, *cis*-12-conjugated linoleic acid in grazing dairy cows. Archives of Animal Nutrition 71:428-440.
Herrera, J.A., García, A., Suárez, J. 2014. Análisis prospectivo de la base alimentaria en una lechería tropical con programación lineal. Pastos y Forrajes 37:435-441.
Jarquin Almanza, J.A., Rocha Espinoza, J.D., Rocha, L., Reyes-Sánchez, N., Mendieta-Araica, B. 2013. Degradabilidad ruminal del follaje de *Moringa oleifera* a tres diferentes edades de rebrote. La Calera 13:76-81.
Kęsek, M., Szulc, T., Zielak-Steciwko, A. 2014. Genetic, physiological and nutritive factors affecting the fatty acid profile in cows' milk–A review. Animal Science Paper and Reports 32:95-105.
Khan, N.A., Farooq, M.W., Ali, M., Suleman, M., Ahmad, N., Sulaiman, S.M., et al. 2015. Effect of species and harvest maturity on the fatty acids profile of tropical forages. Journal of Animal & Plant Sciences 25:739-746.
León, J.M., Pavón, M.L., Carulla, J.E. 2011. Pasture traits and conjugated linoleic acid (CLA) content in milk. Revista Colombiana de Ciencias Pecuarias 24:63-73.
Livas Calderón, F. 2015. Estrategias de alimentación para ganado bovino en las regiones tropicales. Puebla, México. Available at https://www.ganaderia.com/destacado/Estrategias-de-alimentaci%C3%B3n-para-ganado-bovino-en-las-regiones-tropicales (accessed February 2023).
Lopes, F.C.F., Bedeschi, G.V., Morenz, M.J.F., da Silva Lédo, F.J., Paciullo, D.S.C., de Miranda Gomide, C.A., et al. 2022. Chemical composition and fatty acid profile of a seed-propagated elephant grass genotype, ensiled at different regrowth ages. Semina: Ciências Agrárias 43(5):2003-2030.
Lopes, F.C.F., Morenz, M.J.F., Lédo, F.J.daS., Carneiro, J.daC., Paciullo, D.S.C., Andrade, P.J.M., et al. 2021. Chemical composition and fatty acid profile of BRS Capiaçu ensiled at different regrowth ages. Semina: Ciências Agrárias 42(3):1981-2004. doi:10.5433/1679-03 59.2021v42n3Supl1p1981.
Mahecha-Ledesma, L., Angulo-Arizala, J., Barragán-Hernández, W.A. 2017. Calidad nutricional, dinámica fermentativa y producción de metano de arreglos silvopastoriles. Agronomía Mesoamericana 28:371-387. doi:10.15517/ma.v28i2.22750.
Meléndez, F.N. 2012. Principales forrajes para el trópico. Secretaría de Desarrollo Agropecuario, Forestal y Pesca y Universidad Popular de la Chontalpa, Cárdenas, Tabasco, México.
Merlo-Maydana, F.E., Ramírez-Avilés, L., Ayala-Burgos, A.J., Ku-Vera, J.C. 2017. Efecto de la edad de corte y la época del año sobre el rendimiento y calidad de *Brachiaria brizantha* (A. Rich.) Staff en Yucatán, México. Journal of the Selva Andina Animal Science 4:116-127.
Mojica, J.E.R. 2017. Evaluación de la alimentación con forrajes tropicales sobre la producción y calidad composicional de la leche en vacas doble propósito del trópico seco colombiano. PhD tesis. Universidad Nacional de Colombia, Facultad de Medicina Veterinaria y Zootecnia, Bogotá, Colombia.
Mojica-Rodríguez, J.E., Castro-Rincón, E., Carulla-Fornaguera, J., Lascano-Aguilar, C.E. 2017. Efecto de la edad de rebrote sobre el perfil de ácidos grasos en gramíneas tropicales. Ciencia y Tecnología Agropecuaria 18:217-232. doi:10.21930/rcta.vol18_num2_art:623.
Morales-Almaráz, E., Domínguez-Vara, I.A., Mejía-Uribe, L.A., Cruz-Monterrosa, R.G., Jiménez-Guzmán, J., Vieyra-Alberto, R. 2018. Effect of the diet type on the composition of fatty acids in cow milk. Agroproductividad 11:21-26. doi:10.32854/agrop.v11i11.1278.

Plata-Pérez, G., Angeles-Hernandez, J.C., Morales-Almaráz, E., Del Razo-Rodríguez, O.E., López-González, F., Peláez-Acero, A., et al. 2022. Oilseed supplementation improves milk composition and fatty acid profile of cow milk: A meta-analysis and meta-regression. Animals 12:1642. doi:10.3390/ani12131642.

Prieto-Manrique, E., Mahecha-Ledesma, L., Angulo-Arizala, J., Vargas-Sánchez, J.E. 2016. Efecto de la suplementación lipídica sobre ácidos grasos en leche de vaca, énfasis en ácido ruménico. Agronomía Mesoamericana 27:421-437. doi:10.15517/am.v27i2.22022.

Rincón, A., Flórez, H., Ballesteros, H., León, L.M. 2018. Efectos de la fertilización en la productividad de una pastura de *Brachiaria humidicola* cv. Llanero en el Piedemonte de los Llanos Orientales de Colombia. Tropical Grasslands 6:158-168. doi:10.17138/tgft(6)158-168.

Rivera Herrera, J.E., Cuartas, C.A., Naranjo, J.F., Tafur, O., Arenas, F., Chará, J.D., et al. 2012. Calidad y cantidad de leche bovina producida bajo sistemas silvopastoriles intensivos (SSPI) en Colombia. Fundación Centro para la Investigación en Sistemas Sostenibles de Producción Agropecuaria (CIPAV), Cali, Colombia.

Roca-Fernández, A.I., González, A., Vázquez, O.P., López, M.E. 2014. Efecto de la fuente de forraje y del tipo de concentrado sobre la producción y el perfil de ácidos grasos de la leche de vaca. Pastos y PAC. 53ª Reunión científica de la Sociedad Española para el Estudio de los Pastos (SEEP), Potes, Cantabria. 9-12 Junio. SEEP, Gijón, España.

Rojas-Hernández, S., Olivares-Pérez, J., Jiménez-Guillén, R., Gutiérrez-Segura, I., Avilés-Nova, F. 2011. Producción de materia seca y componentes morfológicos de cuatro cultivares de *Brachiaria* en el trópico. Avances en Investigación Agropecuaria 15:3-8.

Sosa-Montes, E., Sánchez-Sánchez, Z.S., Mendoza-Pedroza, S.I., Ramírez-Bribiesca, E., González-Cerón, F., Huerta, H.V. 2022. Composición química de cuatro gramíneas forrajeras del estado de Jalisco. Revista Mexicana de Ciencias Agrícolas 13:1315-1322. doi:10.29312/remexca.v13i7.3333.

Soxhlet, F.V. 1879. The weight analysis of milk fat. Polytechnic Journal 232:461-465.

Suárez, E., Reza, S., García, F., Pastrana, I., Díaz, E. 2011. Comportamiento ingestivo diurno de bovinos de ceba en praderas del pasto Guinea (*Panicum maximum* cv. Mombasa). Ciencia y Tecnología Agropecuaria 12:167-174.

Toyes-Vargas, E.A., Murillo-Amador, B., Espinoza-Villavicencio, J.L., Carreón-Palau, L., Palacios-Espinosa, A. 2013. Composición química y precursores de ácidos vaccénico y ruménico en especies forrajeras en Baja California Sur, México. Revista Mexicana de Ciencias Pecuarias 4:373-386.

Van Soest, P.V., Robertson, J.B., Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Zambrano, H., Obando, V. 2013. Determinación de los factores edafoclimáticos que afectan la productividad y la calidad nutritiva del pasto kikuyo (*Pennisetum clandestinum*) en el municipio de la Florida, departamento de Nariño. PhD tesis. Universidad de Nariño, Facultad de Ciencias Pecuarias, Programa de Zootecnia, Nariño, Colombia.