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Nitrogen fertilization of Cayman Blend grass (*Urochloa* hybrid cv. GP0423 + GP4467) on the chemical composition and fatty acid profile in milk from grazing cows

Fertilización nitrogenada del pasto Cayman Blend (*Urochloa* híbrida cv. GP0423 + GP4467) sobre la composición química y el perfil de ácidos grasos en leche de vacas en pastoreo

Isabel Cristina Acosta–Balcazar[1], *Yuridia Bautista–Martínez*[1], *Benigno Estrada–Drouaillet*[2], *José Felipe Orzuna–Orzuna*[3], *Miguel Ruíz–Albarran*[1], *Jorge David Guiot–García*[4], *Lorenzo Danilo Granados–Rivera*[5]*

[1]*Universidad Autónoma de Tamaulipas, Facultad de Medicina Veterinaria y Zootecnia. Ciudad Victoria, Tamaulipas, México.*
[2]*Universidad Autónoma de Tamaulipas, Facultad de Ingenierías y Ciencias. Ciudad Victoria, Tamaulipas, México.*
[3]*Universidad Autónoma Chapingo, Departamento de Zootecnia. Chapingo, Estado de México, México.*
[4]*Grupo Papalotla S.A. de C.V. Ciudad de México, México.*
[5]*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental–General Terán, Nuevo León, México.*
**Corresponding author: granados.danilo@inifap.gob.mx*

ABSTRACT

Conjugated linoleic acid (CLA) is a constituent of bovine milk that has been shown to possess protective effects against various diseases, including cancer. Therefore, there is a compelling rationale for increasing the content of CLA in milk. The feeding of cows in pastures has been demonstrated to increase CLA, as pastures typically have higher concentrations of linoleic and α–linolenic acids, which serve as precursors of CLA in the process of ruminal biohydrogenation. The enhancement of linoleic and α–linolenic acids can be achieved through management techniques that promote rapid vegetative growth, such as nitrogen fertilization. An experiment was conducted on a ranch in the state of Tabasco, Mexico, to determine the effect of nitrogen fertilization on the chemical composition and fatty acid profile of grass and bovine milk. The experimental design involved two plots, one of which was fertilized with urea (150 kg·ha^{-1}), while the other served as a control. Each plot was assigned a group of five cows in production, utilizing a randomized complete block design. An intensive rotational grazing system was used, and grass and milk samples were taken on days 14, 21, and 28 of the experimental periods. Nitrogen fertilization of the grass increased ($P≤0.01$) more than 100% forage production and 15.75% the crude protein content in the grass. In milk, nitrogen fertilization of the grass increased ($P≤0.05$) the fat content by 31.68% but did not affect ($P>0.05$) the protein and lactose content or the content of CLA. A positive linear relationship was found ($P≤0.05$) between the concentration of linoleic acid in grass and the concentration of CLA in milk. The nitrogen fertilization of Cayman Blend grass increases forage production, the crude protein content in the grass, and the fat content in milk without affecting the content of conjugated linoleic acid and other fatty acids.

Key words: Conjugated linoleic acid; grazing; forage; lipids; tropics

RESUMEN

El ácido linoleico conjugado (ALC) presente en la leche bovina tiene efectos protectores contra diversas enfermedades incluyendo el cáncer, por lo que es importante incrementar su contenido en leche. La alimentación de las vacas bajo pastoreo incrementa el CLA ya que los pastos tienen mayor concentración de ácidos linoleico y α–linolénico, precursores del ALC en la biohidrogenación ruminal. Tanto linoleico y α–linolénico pueden incrementarse a través de técnicas de manejo que promuevan un rápido crecimiento vegetativo, tal es el caso de la fertilización a base de nitrógeno. En un rancho del estado de Tabasco, México se realizó un experimento con el objetivo de conocer el efecto de la fertilización nitrogenada sobre la composición química y el perfil de ácidos grasos del pasto y de la leche bovina. Se utilizaron dos parcelas con pasto Cayman Blend y sólo una de ellas se fertilizó con urea (150 kg·ha^{-1}) y a cada parcela se le asignó un grupo de cinco vacas en producción mediante un diseño de bloques completos al azar. Se utilizó un pastoreo rotacional intensivo y se tomaron muestras de pasto y leche los días 14, 21 y 28 del periodo experimental. La fertilización nitrogenada aumentó ($P≤0,01$) más del 100 % la producción de forraje y un 15.75 % el contenido de proteína bruta en el pasto. El pasto fertilizado aumentó ($P≤0,05$) el contenido de grasa en un 31.68 % en leche, pero no afectó ($P>0,05$) los contenidos de proteína, lactosa y CLA. Se encontró relación lineal positiva ($P≤0,05$) entre la concentración de ácido linoleico del pasto y la concentración de CLA en leche. La fertilización nitrogenada del pasto Cayman Blend aumenta la producción de forraje, el contenido de proteína bruta en el pasto y el contenido de grasa en leche sin afectar al contenido de CLA y otros ácidos grasos.

Palabras clave: Ácido linoleico conjugado; pastoreo; forraje; lípidos; trópicos

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INTRODUCTION

Milk is a nutrient–rich food that constitutes a primary dietary component, particularly for children [1]. It is a significant source of several essential nutrients, including vitamin D, calcium, protein, and energy. Approximately 57% of the milk consumed globally is cow's milk (*Bos taurus*) [2].

The fat content of milk is approximately 3–4% of its total composition, with 98% of this being triglycerides. The fatty acid profile of milk is comprised of approximately 70% saturated fatty acids (SFA), 25% monounsaturated fatty acids (MUFA), and 5% polyunsaturated fatty acids (PUFA) [3]. Conjugated linoleic acid (CLA) is the end product of the ruminal biohydrogenation process of linoleic and α–linolenic acids [4]. The cis–9, trans–11 CLA isomer is generated endogenously or exogenously [5]. CLA has been associated with enhanced health outcomes and may potentially contribute to the prevention of obesity, arteriosclerosis, diabetes, and certain types of cancer [6].

In the contemporary era, the number of consumers who demand foods that are more natural, healthy, and functional, with a concomitantly low environmental impact, has increased. Consequently, countries such as the United States, Argentina, and Spain have begun to commercially offer milk with a high content of CLA and PUFA, produced using grazing as the primary feeding method [3].

The content and chemical composition of conjugated linoleic acid (CLA) in milk from dairy cows can be modified by a number of factors, including the type of feed provided, the breed, age, health status, and lactation stage of the cow. For example, the primary factor influencing the fat and protein content of milk is the composition of the diet [7]. In particular, milk fat synthesis is influenced by the type of feed consumed by the animal, including grass, green fodder, silage, and supplements with fats or oilseeds [8], as well as the use of vitamin–mineral supplements [9].

The scientific evidence suggests that the concentration of conjugated linoleic acid (CLA) in milk derived from grazing cows is higher than in milk from cows that are confined [10]. This effect is attributable to the fact that 95% of the fat in grasses is comprised of linoleic and α–linolenic acid, with the latter being the predominant form (50 to 75%) [11]. The content of linoleic and α–linolenic fatty acids in grasses can be increased through the implementation of management techniques that promote rapid vegetative growth. These techniques include the application of nitrogen fertilization, which has been observed to cause an increase in the synthesis and accumulation of lipids in forage plants [11, 12]. In nitrogen–fertilized tropical grasses, significant increases of 18, 12, and 40% in palmitic, linoleic, and α–linolenic fatty acids, respectively, as well as a 26% increase in total fatty acid concentration have been reported in comparison to non–nitrogen–fertilized grasses [11, 13]. In consideration of the aforementioned background, the present study's hypothesis postulates that dairy cows fed a nitrogen–fertilized pasture consumed at an early stage of regrowth will produce milk with a higher concentration of CLA. Accordingly, the objective of this study was to assess the impact of nitrogen fertilization on the chemical composition and concentration of fatty acids in milk from grazing cows.

MATERIALS Y METHODS

Location and area of study

The study was conducted on a farm with a dual–purpose cattle production system located in the state of Tabasco, Mexico (Longitude: -98.102778 and Latitude: 22.784167; 20 masl). The region's climate is tropical, with rain all year round, and the average recorded precipitation is 2,452 mm·year^{-1} [11]. Two plots of 7,200 m^2 each were used, which were planted with Cayman Blend grass (*Urochloa* hybrid cv. GP0423 + GP4467; Grupo Papalotla).

Fertilization and pasture management

Fifty-six days (d) before the start of the experiment, a group of five cows (with characteristics similar to those that would be used in the study) was assigned to each of the plots to consume the available grass and ensure that it had a uniform height at the start of the experiment.

Twenty–eight and 10 d before the start of the experimental phase, only one of the plots was fertilized with 150 kg·ha^{-1} of nitrogen (using urea), and the other remained as a control plot, so there were two treatments: 1) fertilized Cayman Blend grass and 2) unfertilized Cayman Blend grass.

Cow management and feeding

Ten ¾ American Brown Swiss x ¼ Zebu cows (500 ± 20 kg live weight) (Torrey, PG 2000, Mexico); 180 ± 20 d in milk, and 2.3 ± 0.8 calving were used, distributed in two groups of five cows each. The groups were randomly assigned to each plot using a randomized complete block design.

Cows had a pre–experimental period of 7 d to adapt to the management and an experimental period of 21 d for sampling. The type of grazing used was intensive rotational grazing, where each plot was divided into 29 sections (approximately 60 m × 4 m) and the occupancy time per section was 24 hours (h). This ensured that the pasture of section 1 had 28 d of rest before starting a new grazing cycle. The cows' diet was supplemented with commercial feed (2 kg DM·cow^{-1}·d^{-1}), which was offered daily at machine milking (6:00).

Sampling

After the pre–experimental period, grass and milk samples were taken every 7 days, i.e. on d 14, 21 and 28 of the grazing cycle. Three sampling points were randomly chosen within the corresponding division of that days to obtain the grass samples using a 0.25 m^2 square. The grass was cut 10 cm from the ground, simulating the animal's bite, and the samples were stored in previously identified paper bags to be dried and analysed. This process was carried out on the three sampling days in both treatments. The milk samples were weighed using an automatic milk weigher (Waikato MK New Zeeland), from which proportional milk samples were obtained from each cow. These samples were stored in sterilized jars and refrigerated (4°C; Torrey, RVSA-23UI, Mexico) until analysis. At the end of the experiment, 9 grass samples and 15 milk samples were collected for each treatment.

Laboratory analysis

The chemical composition of the grass was determined using the procedures described in the following methodologies: AOAC [13] for PC, Van Soest *et al*. [14] for ADF and NDF, Mabjeesh *et al.* [15] for IVDMD. On the other hand, the chemical composition of the milk was determined using a LactiCheck™–01 RapiRead brand ultrasound analyser (P&P Inc. LactiCheck–01, United States).

The fatty acid (FA) profile in grass and milk was determined using gas chromatography, which had three phases: extraction, quantification, and identification of FA. The extraction of FA was performed according to the methodology modified by Granados–Rivera *et al*. [4]. A Hewlett Packard 6890 chromatograph with an automatic injector and a silica capillary column (100 m × 0.25 mm × 0.20 μm thick, Sp–2560, Supelco) was used to quantify FA methyl esters. The identification of FA was done comparing the retention times of each peak obtained from the chromatogram, with a standard of 37 FA methyl ester components (Supelco 37 Component FAME) and a specific standard for isomers C18:1 t11 (Sigma–Aldrich) and cis–9, trans–11 and trans–10, cis–12 (Nu–Check–Prep.).

Measured response variables

On pasture

Total forage production (kg·ha^{-1}), leaf production (kg·ha^{-1}), stem production (kg·ha^{-1}), bromatological composition (crude protein –CP– %) (Buchi, KjelDigester K-446; Switzerland), acid detergent fiber (ADF), neutral detergent fiber (NDF) (ANKOM 2000, United States), *in vitro* dry matter digestibility (IVDMD) (ANKOM, Daisy Incubator, United States), and ether extract (EE) on a dry basis) (Labconco, Goldfisch Fat Extractor, United States) and FA profile by gas chromatography (g·100 g^{-1} of FA) (Hewlett Packard, 6890, United States).

In milk

Daily milk production per cow (kg·d^{-1}), energy–corrected milk production (ECMP; kg·d^{-1}), chemical composition (percentage (%) and yield (g·d^{-1}) of protein, fat, and lactose), and fatty acid profile (g·100 g^{-1} of FA).

Energy–corrected milk production was determined with the following equation:

ECMP = (0.327×milk yield –kg·d^{-1}–) + (12.95×fat yield –kg·d^{-1}–) + (7.65×protein yield –kg·d^{-1}–) [16].

Statistical analysis

The data on chemical composition and fatty acid profile in grass and milk were analyzed using the PROC MIXED procedure through a repeated measures model. For this, the Bayesian information criteria of Schwarz and Akaike were obtained and used to determine the most appropriate covariance structure for each variable. The comparison of means was performed through the Tukey test ($P \leq 0.05$). In addition, a correlation analysis was performed between the concentration of linoleic and α–linolenic acid in the grass and the concentration of conjugated linoleic acid in milk.

RESULTS AND DISCUSSION

Forage production

Total forage production, leaf production, and stem production of the grass increased ($P \leq 0.01$) by more than 100% with nitrogen fertilization (TABLE I). Likewise, the grass's total forage production, leaf production, and stem production increased ($P \leq 0.05$) as the sampling days increased, where the maximum production of total forage and its components was obtained on d 21 of sampling.

TABLE I
Forage production (total, per leaf and stem) and bromatological composition of Cayman Blend grass (*Urochloa* hybrid cv. GP0423 + GP4467) with and without nitrogen fertilization

Variables	Treatments		Sampling days			*P*–Value		
	F[1]	NF[1]	14	21	28	Treatment	Days	T*D
Forage production (kg·ha^{-1})								
Total	16.806.1^{a}	7.866.4^{b}	6.633.4^{c}	16.732.1^{a}	13.553.1^{b}	**	*	NS
By leaf	9.075.3^{a}	4.090.5^{b}	3.449.3^{c}	9.035.3^{a}	7.047.6^{b}	**	*	NS
By steam	7.730.8^{a}	3.775.9^{b}	3.184.0^{c}	7.696.7^{a}	6.505.5^{b}	**	*	NS
Bromatological composition (DM %)								
CP[1]	17.0^{a}	14.3^{b}	15.6	14.6	16.8	**	NS	NS
ADF[1]	34.7^{b}	36.2^{a}	36.6^{a}	36.5^{ab}	33.1^{b}	*	*	NS
NDF[1]	55.2^{b}	56.6^{a}	56.0	57.2	54.5	*	NS	NS
IVDMD[1]	74.4	74.9	74.6	73.1	76.3	NS	NS	NS
EE[1]	2.6^{b}	3.6^{a}	2.9^{b}	2.9^{b}	3.4^{a}	*	*	NS

[1]F: fertilized, NF: unfertilized, T*D treatment*day, DM: dry matter, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber, IVDMD: *in vitro* dry matter digestibility, EE: ether extract. ^{a,b}: Different superscripts in the same row and within each factor (treatments or sampling days) indicate a significant difference (Tukey, $P \leq 0.05$). *$P \leq 0.05$, ** $P \leq 0.01$, NS: non–significant difference, $P > 0.05$

As posited by Acosta–Balcazar *et al*. [3], the forage production and nutritional quality of grasses are subject to the influence of both abiotic and biotic factors. The former encompasses temperature, humidity, solar radiation, soil fertility, and mineral fertilization, while the latter pertains to grass species and crop management. Among the primary elements utilized in mineral fertilization is nitrogen, which plays a pivotal role in the synthesis of the cytokinin hormone, a vital regulator of plant growth. This hormone also initiates the process of cell division and differentiation. Similarly, nitrogen has been observed to elevate foliar nitrogen concentrations, stimulate photosynthesis and internode elongation, and augment the size and number of leaves, as well as the leaf area (+31 to +79%), in grasses [17, 18]. These effects of nitrogen may be responsible for the higher forage production observed in the fertilized Cayman Blend grass in the current study. Benalcázar–Carranza *et al*. [19] asserts that nitrogen is the most crucial nutrient for forage production, as it can facilitate the optimization of biomass production in grasses when administered in appropriate quantities.

Bromatological composition in grass

The CP content of the grass increased ($P \leq 0.01$) by 15.75% with nitrogen fertilization (TABLE I). In contrast, the NDF, ADF, and EE contents in the nitrogen–fertilized grass decreased ($P \leq 0.05$) by 4.26, 2.53, and 36.39%, respectively, compared with the unfertilized grass. However, the IVDMD of the grass was not affected ($P > 0.05$) by nitrogen fertilization.

The nitrogen plays a pivotal role in the synthesis of metabolic compounds in grass, particularly in leaves [11]. The elevated CP content observed in nitrogen–fertilized grass is consistent with expectations, given that nitrogen is the primary component of proteins. The application of nitrogen fertilizers has been demonstrated to enhance CP content in tropical grasses by up to 57% [19].

Likewise, the present study revealed that the contents of ADF (34.72%) and NDF (55.23%) were lower in nitrogen–fertilized Cayman Blend grass than in unfertilized grass. The ADF content is useful for evaluating digestibility in grasses, while NDF is associated with the proportion of structural carbohydrates (lignin, cellulose, and hemicellulose), which can influence the availability of metabolizable energy and limit ingestive capacity in ruminants [20]. A high lignin content in the cell wall of pastures reduces the contact area between ruminal bacteria and forage particles, which has a detrimental impact on the ruminal degradability of the feed and the equilibrium between energy and protein at the ruminal level.

As vegetative development progresses, the cell content declines at an accelerated rate, and the leaves age and lose their photosynthetic capacity. This physiological effect may be associated with the reduced levels of ADF and EE observed on the final sampling day, as reported by Merlo–Maydana *et al*. [20].

Fatty acid profile in grass

In the grass samples, 16 FA were identified. Of these FA, ten belong to the group of saturated FA (SFA; lauric, myristic, pentadecanoic, palmitic, heptadecanoic, stearic, arachidic, behenic, tricosanoic, and lignoceric), two are monounsaturated FA (MUFA; palmitoleic and oleic) and four are polyunsaturated FA (PUFA; linoleadic, linoleic, γ–linolenic and α–linolenic) (TABLE II).

TABLE II
Fatty acid profile (g·100 g^{-1} of FA) of Cayman Blend grass (*Urochloa* hybrid cv. GP0423 + GP4467) with and without nitrogen fertilization

Fatty acids	Treatments				*P*–Value
	Fertilized	SEM	Unfertilized	SEM	
g.100 g^{-1} of fatty acids					
Lauric	0.93	0.045	0.64	0.250	NS
Myristic	0.43	0.037	0.43	0.108	NS
Pentadecanoic	0.18	0.022	0.13	0.039	NS
Palmitic	24.35	3.408	24.10	1.980	NS
Palmitoleic	0.45	0.178	0.41	0.152	NS
Heptadecanoic	0.30	0.009	0.25	0.034	NS
Stearic	2.66	0.248	2.49	0.044	NS
Oleic	2.59	0.316	1.94	0.591	NS
Linoleadic	0.12^{a}	0.020	0.08^{b}	0.020	**
Linoleic	17.74	2.184	17.04	1.389	NS
Arachidic	0.53	0.080	0.46	0.051	NS
γ–Linolenic	0.23	0.017	0.18	0.017	NS
α–Linolenic	37.66	2.232	36.88	0.526	NS
Behenic	0.94	0.277	0.84	0.219	NS
Tricosanoic	0.37^{b}	0.032	0.47^{a}	0.023	**
Lignoceric	1.30	0.365	1.30	0.375	NS
Unidentified	11.10	0.6040	10.14	0.451	NS

SEM: standard error of the mean. ^{a,b}Different letters between treatments indicate a significant difference (Tukey, $P \leq 0.05$). ** $P \leq 0.01$. NS: non–significant difference, $P > 0.05$

With the exception of linoleic and tricosanoic acids, the other fatty acids found were similar in both treatments. Fertilisation increased the content of linoleic acid (LFA) and decreased that of tricosanoic acid (TFA), but their concentrations were not sufficient to make a difference between treatments.

Despite this, the linoleic and α–linolenic contents of Cayman Blend grass with and without fertilization were higher than the FA values reported by Mojica *et al*. [12] in grasses of the same genus (*Urochloa*). In the aforementioned studies [12], the linoleic acid values ranged between 0.32 and 0.99 g.·100 g^{-1} of FA, while the α–linolenic acid values ranged from 0.12 to 1.08 g.·100 g^{-1} of FA in the Toledo, Mulato, and Humidicola grasses.

Morales–Almaráz *et al*. [10] mention that the fat portion of linoleic and α–linolenic is 95%. However, in the present study, linoleic and α–linolenic FA only represented 54.4 and 53.9% of the total FA in fertilized and unfertilized grass. This variation in the percentages of linoleic and α–linolenic FA could be mainly explained by the difference in the forage grasses used, the treatments applied, and the environmental conditions of each experiment [12]. In this regard, Acosta–Balcazar *et al*. [8] pointed out that the content and composition of FA in forage grasses are affected by several factors, such as the species and variety of plants, climate, light intensity, rainfall, fertilization, growth stage, soil fertility, among others.

Chemical composition of milk

Milk production was similar across treatments ($P>0.05$) but was 15.39% lower ($P\leq0.05$) on d 21 compared to d 14 of sampling (TABLE III). Treatments did not affect milk protein and lactose content ($P>0.05$). However, cows consuming fertilized Cayman Blend grass had 31.68% higher ($P\leq0.05$) milk fat content. Across sampling days, milk fat increased ($P\leq0.05$) by 9.85% on d 28 compared to d 14 of sampling. Fat, protein, and lactose yields were not affected ($P>0.05$) by the treatments (TABLE III).

TABLE III
Chemical composition of milk from cows that consumed Cayman Blend grass (*Urochloa* hybrid cv. CIAT BR02/1752 + GP0423) with and without nitrogen fertilization

Variables	Treatments		Sampling days			*P*–Value		
	F	NF	14	21	28	Treatment	Days	T*D
Production (kg·d^{-1})	6.30	6.90	7.21^{a}	6.10^{b}	6.50^{ab}	NS	*	NS
ECMP (kg·d^{-1})	6.11	6.72	6.96^{a}	5.92^{b}	6.37^{ab}	NS	*	NS
Percentage (%)								
Fat	3.99^{a}	3.03^{b}	3.35^{b}	3.50^{ab}	3.68^{a}	*	NS	NS
Protein	3.37	3.41	3.42	3.42	3.33	NS	*	NS
Lactose	4.85	4.92	4.94	4.93	4.79	NS	*	NS
Yield (g·d^{-1})								
Fat	197.96	224.67	222.49^{a}	193.38^{b}	218.08^{ab}	NS	*	NS
Protein	227.71	214.32	244.18^{a}	204.44^{ab}	214.43^{b}	NS	*	NS
Lactose	332.01	305.54	352.58^{a}	294.98^{b}	308.77^{ab}	NS	*	NS

F: fertilized, NF: unfertilized, T*D treatment*day, ECMP: energy–corrected milk production $^{a,b:}$ Different superscripts in the same row and within each factor (treatments or sampling days) indicate a significant difference (Tukey, $P\leq0.05$). *$P\leq0.05$, NS: non–significant difference, $P>0.05$

The differences found in fat, protein and lactose yields per sampling day were due to the amount of milk produced on those days.

The milk production observed among the experimental groups was comparable, with the mean values recorded (6.3 and 6.9 kg·cow^{-1}·d^{-1}) falling within the typical range (3 to 9 kg·cow^{-1}·d^{-1}) for cows under grazing conditions with tropical grasses [21]. However, the results were lower than those reported by Plata *et al*. [22], who observed milk yields of between 14 and 16 kg·d^{-1} in grazing cows. In contrast, the results of the present study were higher than those reported by Mojica *et al*. [12], who observed a daily milk yield of 4.8 kg in lactating cows consuming different grasses. The discrepancy in the observed daily milk production between studies may be attributed to the specific species and variety of grass utilized or the breed of cows under evaluation.

Conversely, milk production in both treatments demonstrated a decline as the experimental period increased. Acosta–Balcazar *et al*. [8] cite the observation that following the peak of milk production, milk–secreting cells in the mammary gland undergo a decline as the lactation period increases. This results in a reduction in milk production by approximately 10% each month. This natural physiological mechanism of the mammary gland provides an explanation for the reduction in milk production at the conclusion of the experimental period.

Fatty acid profile in milk

In the milk of each treatment, 18 FA were identified in total (TABLE IV). Of these FA, 10 are SFA (butyric, caproic, capric, lauric, myristic, pentadecanoic, palmitic, heptadecanoic, stearic, and arachidic), 5 are MUFA (myristoleic, palmitoleic, cis–10–heptadecanoic, elaidic, and oleic), and 3 are PUFA (linoleic, α–linolenic, and the cis–9 trans–11 isomer of conjugated linoleic acid (CLA). The remaining detected FA were grouped as "AGNI" since the quantities found were not considered significant (less than 0.1 g·100 g^{-1} of FA). Of the FA detected in milk, those with the highest concentration were myristic, palmitic, stearic, and oleic, representing between 10 and 33 g·100 g^{-1} of total FA.

The FAs with significant interaction ($P\leq0.05$) between treatments and sampling days were caproic, pentadecanoic, heptadecanoic, cis–10–heptadecanoic, CLA, and AGNI (TABLE V). The highest concentration of caproic and cis–10–heptadecanoic FA was obtained with the NF/28 interaction. Likewise, heptadecanoic and CLA FA showed higher concentrations with the NF/21 interaction than with other interactions. The highest concentration of FA from the AGNI group was obtained with the F/14 and NF/21 interactions.

TABLE VI shows that there was a positive linear relationship (0.053 g·100 g^{-1})($P\leq0.05$) between the linoleic content in grass and CLA in milk, in which when the linoleic content in grass increased by one percentage unit, the CLA content in milk increased by 0.053 g·100 g^{-1} of FA.

The percentage of milk fat was found to be higher in cows that consumed nitrogen–fertilized grass. This effect may be attributed to the increased availability of fiber for consumption by cows in the fertilized plot, resulting from the enhanced total forage production. In ruminants, a high fiber intake has been demonstrated to increase

TABLE IV
Fatty acid profile (g·100 g^{-1} of FA) in milk from cows that consumed Cayman Blend grass (*Urochloa* hybrid cv. CIAT BR02/1752 + GP0423) with and without nitrogen fertilization

Variables	Treatments		Sampling days			*P*–Value		
	F	NF	14	21	28	Treatment	Days	T*D
Butyric	0.84	0.82	0.84	0.64	1.01	NS	NS	NS
Caproic	0.23	0.38	0.20	0.22	0.50	NS	NS	*
Capric	1.39	1.59	1.61^{a}	1.32^{b}	1.53^{ab}	NS	*	NS
Lauric	2.61	2.59	2.76^{ab}	2.22^{b}	2.82^{a}	NS	*	NS
Myristic	10.52	10.55	11.00^{ab}	9.39^{b}	11.20^{a}	NS	*	NS
Myristoleic	0.83	0.80	0.89^{a}	0.72^{b}	0.83^{ab}	NS	*	NS
Pentadecanoic	1.30	1.21	1.24	1.22	1.30	NS	NS	**
Palmitic	32.08	32.70	32.15^{ab}	29.64^{b}	35.39^{a}	NS	*	NS
Palmitoleic	1.52	1.37	1.49	1.59	1.25	NS	NS	NS
Heptadecanoic	0.83	0.87	0.86	0.84	0.84	NS	NS	**
Cis–10–Heptadecanoic	0.83	0.87	0.86	0.84	0.84	NS	NS	**
Stearic	13.27	15.00	13.71	14.31	14.39	NS	NS	NS
Elaidic	3.02	3.28	3.39^{ab}	3.40^{a}	2.66^{b}	NS	*	NS
Oleic	27.12	24.49	25.23^{ab}	29.77^{a}	22.41^{b}	NS	*	NS
Linoleic	0.93	0.92	0.94^{ab}	1.00^{a}	0.84^{b}	NS	*	NS
Arachidic	0.16	0.16	0.17	0.14	0.18	NS	NS	NS
α–Linolenic	0.25	0.23	0.26^{a}	0.26^{ab}	0.20^{b}	NS	*	NS
CLA	0.66	0.67	0.73^{ab}	0.75^{a}	0.50^{b}	NS	*	**
Unidentified	2.21	2.10	2.23	2.17	2.07	NS	NS	**

F: fertilized, NF: unfertilized, T*D treatment*day, CLA: conjugated linoleic acid. $^{a,b:}$ Different superscripts in the same row and within each factor (treatments or sampling days) indicate a significant difference (Tukey, *P*≤0.05). **P*≤0.05, ** *P*≤0.01, NS: non–significant difference, *P*>0.05

TABLE V
Comparison of means of fatty acids in milk (g·100 g^{-1} of FA) that had significant interaction (*P*≤0.01) between treatments and sampling days

Fatty acids	Interactions (treatments*days)					
	F/14	F/21	F/28	NF/14	NF/21	NF/28
Caproic	0.15^{c}	0.27^{b}	0.27^{b}	0.25^{bc}	0.17^{bc}	0.72^{a}
Pentadecanoic	1.34^{ab}	1.16^{b}	1.40^{a}	1.14^{b}	1.28^{ab}	1.20^{ab}
Heptadedcanoic	0.92^{ab}	0.75^{b}	0.81^{ab}	0.80^{ab}	0.93^{a}	0.87^{ab}
Cis–10–Heptadecanoic	0.27^{bc}	0.28^{abc}	0.19^{c}	0.20^{c}	0.28^{ab}	0.35^{a}
CLA	0.78^{ab}	0.66^{ab}	0.54^{ab}	0.68^{ab}	0.85^{a}	0.47^{b}
Unidentified	2.36^{a}	2.02^{ab}	2.26^{ab}	2.10^{ab}	2.32^{a}	1.89^{b}

F: fertilized, NF: unfertilized, CLA: conjugated linoleic acid. ^{a,b,c}: Different letters in the same row indicate significant difference (Tukey, *P*≤0.05)

the ruminal production of acetic and butyric acid, which serve as the primary substrates for synthesizing milk fat in the mammary gland [8].

Bovine milk contains approximately 400 FA, among which the majority are at trace levels, and only 15 are greater than or equal to 1% [4]. However, in the current study, in both treatments, only nine FA had values greater than 1% (capric, lauric, myristic, pentadecanoic, palmitic, palmitoleic, stearic, elaidic, and oleic).

TABLE VI
Estimators of the linear regression model between the linoleic and α–linolenic acid contents of Cayman Blend grass (*Urochloa* hybrid cv. CIAT BR02/1752 + GP0423) with and without nitrogen fertilization and the concentration of conjugated linoleic acid in milk

	Estimator
Intercept/F	0.430
Intercept/NF	0.388
Linoleic	0.053*
α–Linolenic	-0.017

F: fertilized, NF: unfertilized, **P*≤0.05

While the FA profile in milk was comparable across treatments, the concentration of the four most abundant FAs differed from the findings of previous studies. The concentration of palmitic (29.18 to 31.20%), stearic (11.96 to 14.96%), and myristic (9.12 to 9.50%) FA in milk from grazing cows was found to be lower than the values reported in previous studies [23, 24]. However, the range (27.27 to 29.51 g·100 g^{-1} FA) of oleic FA in milk from grazing cows reported by Lahlou *et al*. [23] and Ortega *et al*. [24] was higher than the values of oleic FA observed in the present study. Acosta–Balcazar *et al*. [3] state that the content of unsaturated FA (oleic) in the milk of grazing ruminants typically increases, while the content of saturated FA (e.g., palmitic, stearic, and myristic) typically decreases.

In the present study, the concentration of linoleic and α–linolenic FA in milk was found to exceed the ranges of linoleic (1.40 to 2.37 $g \cdot 100\ g^{-1}$ of FA) and α–linolenic (0.34 to 0.44 $g \cdot 100\ g^{-1}$ of FA) reported in milk from cows raised in production systems similar to the one evaluated in the current study [25, 26]. These discrepancies may be attributed to factors such as grazing duration or the administration of concentrated feed supplements. A study with dairy cows [26] demonstrated that the concentration of PUFA in milk increased by 9.1 $g \cdot kg^{-1}$ and 11.5 $g \cdot kg^{-1}$, respectively, when the animals grazed for half a day and a full day.

As indicated by Prieto *et al*. [26], the concentration of CLA in milk of lactating ruminants increases in correlation with the duration of the lactation period. However, the present study did not find an effect of the treatments on the concentration of CLA in milk, which instead decreased at the end of the experimental period. This effect may be attributed to alterations in lipid metabolism in dairy cows, as evidenced by the findings of Kliem and Shingfield [27], who observed a pronounced mobilization of PUFA from adipose tissue reserves during the initial stages of lactation, which subsequently diminished with the progression of the lactation period.

Morales–Almaráz *et al*. [10] indicate that the concentration of conjugated linoleic acid (CLA) in milk from ruminants that are fed only grass is higher than in milk from ruminants that are supplemented with concentrate or fed total mixed rations. This effect is anticipated, as grasses are known to contain higher concentrations of linoleic and α–linolenic FA, which serve as precursors in the formation of CLA through ruminal biohydrogenation [8]. Similarly, Morales–Almaráz *et al*. [10] indicate that the concentration of α–linolenic FA is higher in grasses than in legumes, due to the fact that lipids are found in leaf chloroplasts, and grasses contain a greater proportion of vegetative material than legumes. It can thus be surmised that the ingestion of grasses comprising a considerable proportion of foliage may result in an augmented intake of α–linolenic FA, consequently leading to an elevated concentration of CLA in milk.

Although α–linolenic acid content is higher in pastures (50–75% of total FA), the current study found that only the concentration of linoleic FA positively influenced milk CLA concentration. It can thus be concluded that management strategies designed to increase the concentration of linoleic acid in pasture may result in a higher concentration of CLA in the milk of dairy cows.

CONCLUSION

The application of nitrogen fertilizers has been demonstrated to enhance the production of leaves, stems, and total forage of Cayman Blend grass. Similarly, nitrogen fertilization enhances the bromatological composition of the grass by elevating the protein concentration and reducing the concentration of neutral detergent fiber, acid detergent fiber, and ether extract. The ingestion of fertilized Cayman Blend grass has been demonstrated to enhance milk fat concentration without influencing the fatty acid profile of the milk. Nevertheless, a positive linear relationship exists between the concentration of linoleic acid in grass and the proportion of conjugated linoleic acid in milk.

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Conflict of interest

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