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DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v29n10e284291 ORIGINAL ARTICLE

Fermentation and nutritional characteristics of silage composed of proportions of *Tithonia diversifolia* and *Sorghum bicolor*[1]

Fermentação e características nutricionais da silagem composta por proporções de *Tithonia diversifolia* e *Sorghum bicolor*

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HIGHLIGHTS:

Mexican sunflower exclusive silage increases protein content.
The inclusion of 0, 25, 50, 75, or 100% Mexican sunflower improves the fermentation process.
Mexican sunflower was efficient in replacing sorghum for forage.

ABSTRACT: The determination of the ideal proportion of Mexican sunflower in sorghum silage is essential for evaluating the nutritional value and quality of the feed, ensuring a balanced diet, better utilization by animals, and greater efficiency in livestock production. This experiment aimed to evaluate the replacement of sorghum with Mexican sunflower regarding fermentation parameters, microbial population, and chemical composition of the silage. The experimental design was a randomized block design with five treatments and five repetitions. A total of 25 experimental silos made of polyvinyl chloride tubes with a density of 600 kg m^{-3} were used. The silos were opened 365 days after ensiling. Mexican sunflower efficiently replaced sorghum and improved the fermentation process quality, reducing losses in both dry matter (85%) and ethanol fermentation (86%). Mexican sunflower silage increased the protein content of the ensiled mass by 50% compared to sorghum silage, which was associated with low losses of ammoniacal nitrogen. The use of Mexican sunflower in all evaluated proportions improved fermentation variables and the final quality of sorghum silages.

Key words: alternative foods, anaerobic fermentation, forage conservation, Mexican sunflower, sustainability

RESUMO: A determinação da proporção ideal de girassol-Mexicano na silagem de sorgo é essencial para avaliar o valor nutricional e a qualidade do alimento, garantindo uma dieta equilibrada, com melhor aproveitamento pelos animais e maior eficiência na produção pecuária. Este experimento teve como objetivo avaliar a substituição do sorgo pelo girassol-Mexicano quanto aos parâmetros de fermentação, população microbiana e composição química da silagem. O delineamento experimental foi de blocos ao acaso com cinco tratamentos e cinco repetições. No total, foram utilizados 25 silos experimentais feitos de tubos de cloreto de polivinila com densidade de 600 kg m^{-3}. Os silos foram abertos 365 dias após a ensilagem. O girassol-Mexicano substituiu eficientemente o sorgo e melhorou a qualidade do processo de fermentação, reduzindo as perdas tanto na matéria seca (85%) quanto na fermentação etanólica (86%). A silagem de girassol-Mexicano aumentou em 50% o teor de proteína da massa ensilada em comparação com a silagem de sorgo, o que foi associado a baixas perdas de nitrogênio amoniacal. O uso de girassol-Mexicano em todas as proporções avaliadas melhorou as variáveis de fermentação e a qualidade final das silagens de sorgo.

Palavras-chave: alimentos alternativos, fermentação anaeróbica, conservação de forragem, girassol-Mexicano, sustentabilidade

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Introduction

Corn and sorghum are among the most commonly used crops for silage, with sorghum being the most used because it presents less risk than other crops, such as lower sensitivity to water deficit and less vulnerability to pests and diseases. Additionally, it has high productivity and good nutritional quality, yielding results similar to corn in livestock feed (Fernandes, 2020).

Sorghum bicolor (L) Moench meets the values set out in the literature for good silage fermentation, with a dry matter content of around 30%, soluble carbohydrates of 15% and a pH close to 4 (Chattha et al., 2020). However, variations may occur according to the physiological stage and age of the ensiled plants (Wang et al., 2021).

Silages produced with mixtures of sorghum and protein-rich plants can have positive associative effects in the ideal balance between fermentation quality, nutritional value, aerobic stability, and animal performance (Wang et al., 2021). Using forage species that are well adapted to a given region can be an interesting alternative to take advantage of available forage resources and overcome forage seasonality problems throughout the year (Valenzuela-Que et al., 2022). Among these options, *Tithonia diversifolia* (Hemsl.) A. Gray, known as Mexican sunflower, has great potential as forage, not only due to its adaptation to different soil and climate conditions and fertilization levels, but also due to its high nutritional value. The species is rich in crude protein and minerals, making it a viable alternative to improve the quality of animal feed and increase production efficiency. (Silva et al., 2021). In fact, it has been documented that shrub plants can replace up to 35% of commercial concentrate feed for livestock production farming (Cardona et al., 2022).

The Mexican sunflower is a perennial shrub native to Central America and widely distributed throughout the tropics and subtropics. It has a low demand for inputs and is easy to propagate, which makes Mexican sunflower an attractive option for animal breeders as it can be easily included in livestock systems (Letty et al., 2021). Its root system is quite voluminous, has good nutrient absorption from the soil, and accumulates more than 33% protein in its leaves (Dlamini et al., 2020). However, there is a gap in knowledge regarding the potential use of Mexican sunflower in agriculture (Rai et. al, 2023). Furthermore, there is little information about the fermentation of this forage when ensiled (Chattha et al., 2020), as well as its potential to improve the conservation and quality of sorghum silage.

Given this, this experiment aimed to evaluate the substitution of sorghum for Mexican sunflower on fermentation parameters, microbial population, and chemical composition the silage.

Material and Methods

The experiment was conducted at the Universidade Federal dos Vales do Jequitinhonha e Mucuri at the Juscelino Kubitschek Campus, Diamantina, MG, Brazil. Sowing, plant harvesting, and silo filling were done at Fazenda Experimental do Moura in Curvelo, MG, Brazil. The geographical coordinates of Diamantina and Curvelo are 18º 14' 58" S, 43º 36' 44" W and 18º 45' 40" S, 44º 25' 01" W, respectively, with altitudes of 1,288 m and 672 m, respectively. The climate of the Diamantina region is classified as Cwb (high-altitude subtropical climate, with dry winters and mild summers), and Curvelo is classified as Aw, with rainy summers and dry winters according to the classification of Köppen-Geiger's (Alvares et al., 2013).

The experimental design was completely randomized blocks with five treatments and five replicates. The treatments consisted of sorghum silages substituted by 0, 25, 50, 75, or 100% Mexican sunflower based on fresh matter. In total, 25 experimental silos made of polyvinyl chloride tubes with a density of 600 kg m^{-3} were used.

Samples of soil were collected at randomly distributed sites throughout the area, and the soil was analyzed for physical and chemical attributes in the 0-20 and 20-40 cm layers. Soil acidity and sorghum fertilization were corrected according to the soil's chemical and physical analysis, adopting the base saturation method (Alvarez & Ribeiro, 1999). The soil was classified as an Oxisol soil (United States, 2022) that corresponds to a Latossolo Vermelho-Amarelo in the Brazilian Soil Classification System (EMBRAPA, 2018), presenting the characteristics described in Table 1. The fertilization was 400 kg ha^{-1} of formulated NPK 08-28-16 (32 kg of N, 112 kg of P, and 64 kg of K), and top dressing with 100 kg ha^{-1} of urea, according to the soil analyses.

The sorghum cultivar BRS655 was sown with 0.90 m spacing between rows and a density of 122,223 plants ha^{-1}, and the soil was previously plowed and harrowed. Sorghum was harvested manually at 0.10 m height from the soil when the plants reached the pasty-grain stage 110 days after sowing, with 27.4% dry matter (DM).

The soil corrections and Mexican sunflower fertilization were carried out following the recommendations for a sunflower cultivar (Alvarez & Ribeiro, 1999), as the Mexican sunflower does not yet have agronomic recommendations.

Table 1. Chemical and physical attributes of dystrophic Red Latosol (LVd) and Quartz Arenite Neosol (NQ) before correcting soil acidity and fertilization

Attributes	Unity	LVd	NQ
pH	-	5.90	6.60
MO	dag kg^{-1}	3.92	0.99
P	mg dm^{-3}	1.03	4.53
K	mg dm^{-3}	146.33	31.20
Ca	cmolc dm^{-3}	4.75	1.02
Mg	cmolc dm^{-3}	0.76	0.54
Al	cmolc dm^{-3}	0.10	0.04
H+Al	cmolc dm^{-3}	4.23	1.95
SB	cmolc dm^{-3}	3.97	1.64
CEC (t)	cmolc dm^{-3}	4.07	1.68
CEC (T)	cmolc dm^{-3}	8.26	3.60
V	%	47.67	45.7
m	%	4.00	2.38
P-rem	mg L^{-1}	13.00	11.00
Zn	mg dm^{-3}	0.67	2.52
Fe	mg dm^{-3}	39.30	130.70
Mn	mg dm^{-3}	59.56	1.64
Cu	mg dm^{-3}	1.30	0.24
B	mg dm^{-3}	0.10	0.20

MO - Soil organic matter; SB - Sum of bases; CEC (t) - Effective cation exchange capacity; CEC (T) - Cation exchange capacity at pH 7.0; V - Base saturation; m - Aluminum saturation; and P-rem - Remaining phosphorus

The soil was classified as with an Oxisol soil (United States, 2022) that corresponds to a Latossolo Vermelho-Amarelo in the Brazilian Soil Classification System (EMBRAPA, 2018), presenting the characteristics described in Table 1. Before sowing, the soil was fertilized according to chemical analysis, with 70 kg ha^{-1} of P_2O_5, using single superphosphate, 774 kg ha^{-1} of dolomitic limestone, and 33 t of organic compost applied in the planting furrows and incorporated into the soil. The organic compost was from a mixture of vegetable waste (grass), quail litter, restaurant, and feed waste, with the following chemical characteristics: pH (H_2O) = 6.22; CO = 22.5%; N = 2.30%; P = 0.95%; K = 1.36%; Ca = 2.68%; Mg = 0.59%; S = 0.71%; Na = 0.11%; Zn = 254 ppm; Fe = 5915 ppm; Mn = 220 ppm; Cu = 98 ppm; B = 25.1 ppm.

The Mexican sunflower planting was carried out using stems with 70 days of regrowth, distributed in furrows after plowing and harrowing at a 0.1 m depth. Spacing of 1 m between furrows and 0.5 m between seedlings was used, totaling 50,000 plants ha^{-1}. Whole Mexican sunflower plants were manually cut at 0.30 m height from the soil, on the same day as the sorghum harvest, 140 days after planting. The Mexican sunflower has on average, 15% DM, 21% CP, and 63% NDF (Verdecia et al., 2018); the plant composition evaluated in this study is shown in Table 2.

After harvesting, sorghum and Mexican sunflower were chopped separately into 1 to 2 cm pieces using a conventional forage chopper Nogueira brand, Model EN-6600. The polyvinyl chloride (PVC) silos were 100 mm diameter × 45 cm high, with a Bunsen valve to exhaust gases. The mass to be ensiled was homogenized to the sorghum before each silo was filled. Before ensiling, mass samples (original mass) were taken to analyze the chemical composition of the silage in each treatment (Table 2).

Compaction was carried out using a wooden bat until a 600 kg m^{-3} density was achieved. The silos were closed and sealed with silicone and adhesive tape. Then, they were weighed and stored at room temperature (19.3 °C) in a protected place until the opening (365 days after ensiling). Silos kept closed for a long time may undergo favorable or unfavorable changes in the stable phase of the ensiling process (Silva et al., 2021). Therefore, the silos were only opened after 365 days to allow a study of the feasibility of using silages stored for long periods to adapt more production strategies to different regions.

A sample of 300 g was taken from each silo. That sample was partially dried in a forced air oven for 72 hours at 55 ºC and ground in 1 mm peace. All samples and the original mass were determined for dry matter (DM) (Silva & Queiroz, 2002); ash (ASH) (AOAC, 2006, Method 923.03); neutral detergent fiber (NDF) (Method 6; Ankom Technology, by Van Soest et al., 1994); acid detergent fiber (ADF) and lignin (LIG) (Ankom Technology Method 9 – AOAC, 2006). Hemicellulose (HEM) was calculated by subtracting NDF from ADF, and cellulose (CEL) content was calculated by subtracting ADF from LIG (Silva & Queiroz, 2002). Alcohol-soluble carbohydrates (CHOs) were analyzed using the method by Bailey (1967). Crude protein (CP) (Method 990.03; AOAC International, 2006), acid detergent insoluble protein (ADIP), and neutral detergent insoluble protein (NDIP) were evaluated using the Elemental Analysis apparatus LECO® CHNS/O (model TruSpec® Micro; Leco Corporation, St Joseph, MI, USA); this model provides an output of N, in which the CP is calculated as N x 6.25. The data obtained from the plant mass before ensiling are presented in Table 2.

Losses, expressed in dry matter, were calculated as the difference between the initial weight and dry matter (105 °C) of the original mass prior to ensiling and the final weight and dry matter (105 °C) of the silage, using the following Eq. 1 (Jobim et al., 2007).

$$\text{Losses}(\%) = \frac{\text{MSi} - \text{MSf}}{\text{MSi}} \times 100 \quad (1)$$

where:

MSi - the initial weight of the silo (kg) plus the dry matter of the original mass; and,

MSf - the final silo (kg) weight plus the silage's dry matter.

An aliquot of 25 g silage was also taken at the opening for immediate analysis of the microbial population. Silage samples were prepared for microbiological analysis by dilution in sterile saline solution with 25 g silage (fresh matter) in 225 mL phosphate buffer solution. Successive dilutions were performed using screw cap tubes of 15 mL capacity, with 9 mL of sterile water to which 1 mL of the prepared 10^{-1} dilution solution was added, obtaining the 10^{-2} dilution, and so on, in order to obtain dilutions from 10^{-1} to 10^{-10}. Subsequently, the plates were

Table 2. Chemical composition of sorghum, Mexican sunflower, and their mixtures before ensiling

Characteristics	Proportions of Mexican sunflower (%)				
	0	25	50	75	100
Dry matter, g kg^{-1} DM	274	258	269	267	266
Crude protein (CP), g kg^{-1} DM	78.6	92.4	116	132	151
Neutral detergent insoluble protein (NDIP), g kg^{-1} CP	42.5	44.2	47.6	47.6	69.9
Acid detergent insoluble protein (ADIP), g kg^{-1} CP	16	16.5	16.9	17.6	17.7
Neutral detergent fiber (NDF), g kg^{-1} DM	565	531	528	506	486
Acid detergent fiber (ADF), g kg^{-1}DM	313	289	287	273	258
Cellulose (CEL), g kg^{-1} DM	235	209	203	185	169
Hemicellulose (HEM), g kg^{-1} DM	253	242	241	233	229
Lignin (LIG), g kg^{-1} DM	77.8	79.4	83.7	87.8	89.1
Soluble carbohydrates (CHOs), g kg^{-1} DM	118	94.3	65.7	37.8	14.6
Ash (ASH), g kg^{-1} DM	44.6	56.2	63.8	71.7	82.3
Calcium (Ca), g kg^{-1} DM	1.9	3.5	4.1	4.7	5.6
Phosphorus (P), g kg^{-1} DM	1.9	1.8	1.8	1.8	1.8

DM - Dry matter; CP - Crude protein

prepared with a specific medium for each microorganism type studied (Silva et al., 2018). Lactic acid bacteria (LAB), yeast fungi (FL), filamentous fungi (FF), and enterobacteria (ENT) were evaluated using culture media selected for each microbial group, with MRS agar for LAB, potato dextrose agar for FF and FL, and Violet Red Bile for ENT. The incubator was set to 35 °C for LAB, 28 °C for FL and 28 °C for FF (Chaves et al., 2013). After inoculation, Petri dishes were sealed with film paper for LAB and with aluminum foil for the other microbe types. Dishes were stored in an inverted position in an incubator for 96 h (BAL and FL) or 120 h (FF) in order to count the colony-forming units (cfu) for each type of microorganism (Kung et al., 2018).

Samples were processed in a hydraulic press to obtain the liquid extracted from the silage, and the volume of the liquid was measured. Then, pH was determined immediately after extraction using a potentiometer with an expanded scale (Tecnopon mPA 210®). The N-NH_3 concentration in relation to total nitrogen was determined by distillation with magnesium oxide and calcium chloride, using a boric acid receptor solution and titration with 0.1 N hydrochloric acid.

The liquid extract obtained by pressing the silage was cold centrifuged at 10,000 rpm for 10 min and used to determine lactic, acetic, butyric, and propionic organic acid contents and ethanol production in the ensiled mass. The organic acids and ethanol in silage extracts were determined by high-performance liquid chromatography (HPLC) on Shimadzu Prominence FPLC 20 A system as performed by Jesus et al. (2021). Injections of 5 μL samples were done by using a programmed automatic injector on the Rezex ROA-Shodex® column (300 × 7.8 mm) at 60 °C. The analytes were eluted with 0.0025 mol L^{-1} H_2SO_4 at a flow rate of 0.6 mL min^{-1}. All examined organic acids were analyzed at 210 nm using a UV-Vis detector (Model SPD-10AV, Shimadzu®). Ethanol was analyzed using a refractive index detector (Model RID-20A, Shimadzu®). Compound identification and quantification were performed using external standards.

All data were analyzed using the Statistical Analysis System (SAS 9.1; SAS Institute, 2002) statistical software program. The data were subjected to analysis of variance at $p \leq 0.05$, and linear and quadratic regressions, also $p \leq 0.05$ The equation with the best fit was chosen according to the significance of the regression coefficients, and the biological responses of each characteristic.

Results and Discussion

Dry matter (DM) contents were not influenced by Mexican sunflower replacement ($p > 0.05$) (Table 3). The DM values found in the produced silages may be due to the DM content of both sorghum and Mexican sunflower at the time of ensiling. However, it did not affect any qualitative or fermentation variable. According to Kung Jr. et al. (2018), tropical forages below 25% DM can present increased losses, impairing the nutritional value of the silage. That is an important point because in the sorghum silage without Mexican sunflower and with 25% inclusion, the lowest DM contents were 262 and 258 g kg^{-1}, respectively, showing higher dry matter losses than other treatments.

The increasing proportions of Mexican sunflower in sorghum silage linearly increased ($p \leq 0.001$) the crude protein (CP) content. The highest CP was observed in the silage with 100% Mexican sunflower, 139 g kg^{-1}. These values represent a 99% increase in crude protein compared to the sorghum silage (Table 3). This increase was already expected, considering that Mexican sunflower had, at ensiling, 72.4 g kg^{-1} more CP than sorghum (Table 2). Sorghum silage had adequate CP content, close to 7%. However, Mexican sunflower silages showed 50% higher CP. This high crude protein content was also observed by Rivera et. al (2021) evaluating the biomass production and nutritional quality of seven Mexican sunflower genotypes. Generally, the protein contribution is always positive when a product with CP higher than the ensiled grass is added. Studies have shown that the CP requirement for finishing cattle is 12.5%, based on dry matter (Valadares et al., 2016). It suggests that Mexican sunflower in proportions higher than 75% in sorghum silage or Mexican sunflower silage may be viable methods of meeting the protein demand of these animals, reducing expenses with protein supplementation. In addition, Mexican sunflower silage can reduce feed costs, as it can partially substitute soybean meal to compose the total

Table 3. Regression equations for the chemical composition of sorghum and Mexican sunflower silages and the proportions of substitution of Mexican sunflower for sorghum

Characteristics	Proportions of Mexican sunflower (%)					p-value[1]	CV (%)
	0	25	50	75	100		
Dry matter (DM), g kg^{-1}as-fed	262	258	269	268	265	0.1361	2.7
[2]Crude protein (CP), g kg^{-1} DM	69.8	83.8	102	122	139	<.0001	25.3
[3]Neutral detergent insoluble protein (NDIP), g kg^{-1}CP	25.6	28.6	34.5	36.9	37.8	<.0001	14.5
Acid detergent insoluble protein (ADIP), g kg^{-1} CP	12.6	16.1	16.4	17	17.1	0.3219	24.1
[4]Neutral detergent fiber (NDF), g kg^{-1} DM	516	491	485	478	458	0.0017	5.8
Acid detergent fiber (ADF), g kg^{-1} DM	280	274	272	267	255	0.0866	8.0
[5]Cellulose (CEL), g kg^{-1} DM	201	193	191	182	168	0.004	12.0
Hemicellulose (HEM), g kg^{-1} DM	236	217	213	210	203	0.1279	14.5
Lignin (LIG), g kg^{-1} DM	77	80.7	80.7	85.6	87.7	0.3006	11.1
[6]Soluble carbohydrates (CHOs), g kg^{-1} DM	0.1	0.4	0.9	1.2	1.7	<.0001	67.8
[7]Ash (ASH), g kg^{-1} DM	44.4	54.9	64.6	73.2	80	<.0001	22.4
[8]Calcium (Ca), g kg^{-1} DM	1.9	3.1	4.1	4.6	5.1	<.0001	41.8
Phosphorus (P), g kg^{-1} DM	1.6	1.7	1.7	1.7	1.7	0.8887	22.6

TN - Total nitrogen; [1] Probability of significant effect due to treatment effect; CV - Coefficient of variation; ns - Not significant; * - Significant at $p \leq 0.05$ by F test; R^2 - Coefficient of determination; [2]$Y = 5.027 + 1.775^{*}x$ (R^2= 0.99); [3]$Y = 2.287 + 0.327^{*}x$ (R^2 = 0.94); [4]$Y = -52.389 - 1.277^{*}x$ (R^2 = 0.94); [5]$Y = 21.02 - 0.776^{*}x$ (R^2 = 0.93); [6]$Y= 0.034 - 0.04^{*}x$ (R^2 = 0.99); [7]$Y = 3.657 + 0.895^{*}x$ (R^2 = 0.99). [8]$Y = 0.127 + 0.085^{*}x$ (R^2 = 0.98) diet (Silva et al., 2018). Takahashi et al. (2023), evaluating the incorporation of Mexican sunflower in sugarcane silage, concluded that this can be considered a source of high-quality forage and an economical alternative that efficiently increases the supply of crude protein in diets, while reducing methane emissions.

The neutral detergent insoluble protein increased linearly as the proportion of Mexican sunflower in sorghum silage increased ($p < 0.001$). The acid detergent insoluble protein was not influenced by Mexican sunflower ($p > 0.05$), with an average of 15.2 g kg^{-1} for all treatments (Table 3). The neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP) in the mass after ensiling were lower than in the original mass (Table 2). The higher the percentages of NDIP and ADIP in feed, the lower or slower the degradation of proteins (Daniel et al., 2017). In our study, a positive correlation of NDIP was observed, which may have occurred due to the composition of the original mass, as Mexican sunflower had higher NDIP than sorghum, with contents of 69.9 and 42.5 g kg^{-1}, respectively (Table 2). However, in the case of *T. diversifolia*, there are several investigations that associate factors such as the age of plant harvest with the bromatological composition (Navas-Panadero & Montaña, 2019; Guatusmal-Gelpud et al., 2020; Paniagua-Hernández et al., 2020; Arias-Gamboa et al., 2023). There were no significant differences in the percentages of ADIP after ensiling, with an average level of 15.2 g kg^{-1}. This level does not impair the availability of CP in the silages, which can be proven by the CP levels observed in the silages and the low N-NH3 content, indicating a slight loss in CP (Chattha et al., 2020).

The Mexican sunflower linearly increased ($p \leq 0.001$) ammoniacal nitrogen (N-NH3/NT), with the highest content observed in Mexican sunflower silage (19.4 g kg^{-1}), and the lowest content was in sorghum silage (13 g kg^{-1}) (Table 4). The low N-NH3 in the silage (Table 4) indicates that the fermentation process did not result in an excessive breakdown of ammoniacal protein (Chattha et al., 2020). Silages with $N\text{-}NH_3$ levels higher than 15% of the total nitrogen demonstrate considerable protein breakdown, and such silages may be less preferred by animals, resulting in low intake (Van Soest, 1994). In the present study, the sorghum silages had lower levels of $N\text{-}NH_3$/NT (13 g kg^{-1}). However, in Mexican sunflower silages, the values did not exceed 19.4 g kg^{-1}, leading to lower protein losses from the mass (Table 3) and indicating an adequate fermentation in the ensiled mass (Table 4).

The Mexican sunflower linearly decreased ($p < 0.001$) the neutral detergent fiber of the silages, i.e., the lowest NDF content was observed in the silage with 100% Mexican sunflower (458 g kg^{-1}) and the highest in silage with 100% sorghum (516 g kg^{-1}) (Table 3). The acid detergent fiber was not influenced by Mexican sunflower ($p > 0.05$) (Table 3). In this work, all the silages showed average NDF contents below 60%, which according to Van Soest (1994), NDF contents above 60% are one of the limiting factors for dry matter intake. This reduction in NDF content is directly related to the lower NDF in Mexican sunflower (506 g kg^{-1}) compared to sorghum (565 g kg^{-1}) (Table 2). Morais et al. (2023) also found a relatively low NDF content in Mexican sunflower plants when compared to tropical forages typically used to feed ruminants. All evaluated Mexican sunflower proportions showed lower LEK levels than sorghum silage. This fact is related to the characteristics of the mass before ensiling, as the sorghum had a CEL of 235 g kg^{-1}, and the Mexican sunflower had 169 g kg^{-1} CEL (Table 2). Cellulose showed a decreasing linear effect ($p < 0.001$); the highest levels of this fraction were observed in the sorghum silage (201 g kg^{-1}), in which the addition of 25% Mexican sunflower during ensiling provided a 3.98% decrease. As for the hemicellulose, there was no effect of including Mexican sunflower, with an average of 215.8 g kg^{-1}. The variation in cellulose and hemicellulose contents may be related to the determination of NDF and ADF fractions, as they are obtained by the difference between these fractions. With the inclusion of Mexican sunflower, which was high in lignin (Table 2), there was a decrease in cellulose due to dilution effect. Also, NDF decreased, but the FDA did not show any significant effect, ranging from 280 g kg^{-1} with 0% and 255g kg^{-1} with 100% Mexican sunflower. Rivera et. al (2023) evaluating wild materials of Mexican sunflower in Colombia and Mexico, with the aim of delineating exceptional genotypes for animal production found low levels of FDA in all cases analyzed in this study. The NDF and ADF indicate the quantity and quality of forage fiber, and high NDF limits the intake of forage DM

Table 4. Regression equations for composition for fermentation variables of sorghum and Mexican sunflower silages and substitution proportions of sorghum for Mexican sunflower

Characteristics	Proportions of Mexican sunflower (%)					p-value[1]	CV
	0	25	50	75	100	L	(%)
[2]Hydrogenionic potential (pH)	3.73	3.78	3.84	3.96	4.02	0.0115	3.79
[3]Ammoniacal nitrogen (N-NH3), g kg^{-1} TN	13	16.3	17.7	18.7	19.4	<.0001	13.5
[4]Losses, g kg^{-1} DM	39.1	27.3	16.6	9.3	5.7	<.0001	63.2
[5]Propionic acid, g kg^{-1} DM	16.1	11.6	8.3	4	1.5	<.0001	68.3
Lactic acid, g kg^{-1} DM	52.7	52.3	61.6	54.6	55.4	0.2305	14.4
Acetic acid, g kg^{-1}DM	14.8	13.6	14	12.6	12.5	0.4694	15.1
Butyric acid, g kg^{-1} DM	ND	ND	ND	ND	ND	-	-
[6]Ethanol (ET), g kg^{-1} DM	50.9	48.1	25.6	22.6	7.1	0.2441	17.7
Lactic acid bacteria (LAB), log10 ufc g^{-1}	4.05	4.48	4.46	4.18	4.06	0.6993	12.5
Yeast fungi (FL), log10 ufc g^{-1}	5.53	5.61	5.94	5.68	5.79	0.6964	22.7
Filamentous fungi (FF), log10 ufc g^{-1}	6.34	6.34	6.34	6.33	6.04	0.7804	17.7
Enterobacteria (ENT), log10 ufc g^{-1}	ND	ND	ND	ND	ND	-	-

DM - Dry matter; ufc - Colony-forming unit; [1] Probability of significant effect due to treatment effect. SEM - Standard error of the mean; CV - Coefficient of variation; ns - Not significant; * - Significant at $p \leq 0.05$ by F test; R^2 - Coefficient of determination; ND - Not detected. <2 log10 cfu/g of natural matter. [2]$Y = 4,0948 - 0,076^{*}x$ (R^2= 0.98); [3]$Y = 1.246 + 0.152^{*}x$ (R^2 = 0.90); [4]$Y= 4.504 - 0.848^{*}x$ (R^2 = 0.96); [5]$Y = 1.934 - 0.368^{*}x$ (R^2 = 0.99) by ruminants due to low degradation rates (Verdecia, 2024). Cediel-Devia et al (2020) mentioned that one of the main reasons for choosing Mexican sunflower for feeding ruminants is its acceptable concentrations of NDF and ADF, compared to other commonly used grasses, which corresponds with the results obtained in this research. The ADF determines the cell wall's quality, as it indicates the insoluble and less digestible fractions of the cell wall (cellulose and lignin - insoluble or remaining in acid detergent) (Van Soest, 1994). I.e., the higher the ADF of feed, the lower the digestibility. On the other hand, HEM is a fibrous carbohydrate that undergoes more significant degradation during the fermentation process due to the hydrolysis of its structure. This breakdown forms acetic and/or lactic acid, essential in preserving silage (McDonald et al., 1991), and it generally occurs in the absence of CHOs, the primary substrates used by microorganisms to produce organic acids. In the present study, HEM ($p > 0.05$) and LIG ($p > 0.05$) contents were not influenced by Mexican sunflower ($p > 0.05$) in all treatments (Table 3), proves the utilization efficiency of CHOs by the microorganisms in producing organic acids (Table 4). The CHOs increased linearly ($p < 0.001$) as increasing proportions of Mexican sunflower were added to sorghum silage, with contents of 0.1 and 1.7 g kg^{-1} for sorghum silage and Mexican sunflower silage, respectively (Table 3). The CHOs content of the sorghum silages decreased after ensiling for all Mexican sunflower proportions, showing the consumption of CHOs by the microorganisms during the fermentation of the ensiled mass (Tables 2 and 3). Adding Mexican sunflower to sorghum silages also produced low residual levels of CHOs (Table 3), which helps prevent undesirable fermentation after the development of lactic acid bacteria (Silva et al., 2018).

The inclusion of Mexican sunflower linearly increased (ASH) ($p \leq 0.001$) and calcium ($p \leq 0.001$), with the highest levels of both observed in silage with 100% Mexican sunflower (Table 3). The phosphorus of the evaluated silages was not influenced by the Mexican sunflower (Table 3). However, the ASH did not vary so much between the silages and the pre-ensiled mass (Tables 2 and 3), indicating the correct preservation of nutrients during ensiling (Table 4). Calcium (Ca) is the second most abundant mineral element in animal organisms, followed by phosphorus (P), and is closely associated with animal metabolism. Therefore, diets must be formulated to meet the animals' minimum Ca and P requirements so that the ratio between these minerals is supplied and balanced according to the requirements of each animal category (Wild et al., 2021). Including Mexican sunflower in the sorghum silage resulted in a 140% increase in Ca between the silages with the lowest and highest proportion of Mexican sunflower, reducing costs with mineral supplementation for the animals. However, Mexican sunflower proportions did not significantly affect P concentrations (Table 3). Uu-Espens et. al, (2023) Mexican reports that Mexican sunflower can play a very important role in the recycling of labile soil nutrients that would otherwise be lost through leaching. In the case of phosphorus, the association with mycorrhizae can play an important role in its mobilization (Oros-Ortega et al., 2020). The chemical composition of Mexican sunflower biomass in general can vary depending on several factors, such as the phenological state of the plant, the higher it is, the lower the concentration of nutrients (Senarathne et al., 2019).

The Mexican sunflower linearly increased the silage pH ($p < 0.001$), with the lowest value observed for silage with 100% sorghum (3.73) and the highest value in silage of 100% Mexican sunflower (4.02); Silages with 50% Mexican sunflower reached pH value of 3.84 (Table 4). Mexican sunflower is an Asteraceae with a crude protein content of 151 g kg^{-1} DM (Table 2). This protein content can act as a buffer during fermentation (McDonald et al.,1991) and prevent the pH of the ensiled mass from dropping to 3.8 to 4.2, which is desirable in the ensiled mass (Kung Jr. et al., 2018). In this study, even with 100% Mexican sunflower in the sorghum silage, the pH remained constant (4.2) (Table 4) and within the recommended range for adequate conservation of the mass in the silo. There were reduced silage losses with the inclusion of Mexican sunflower, which indicates good conditions for conserving the ensiled mass. With the inclusion of Mexican sunflower in sorghum silages, DM losses decreased linearly ($p < 0.001$), and the highest losses were observed in 100% sorghum silage (39.1 g kg^{-1}) (Table 4). The dry matter decrease of the silages may also have contributed to these higher pH values with the inclusion of Mexican sunflower since a high humidity in the silage stimulates the growth of undesirable microorganisms (e.g., enterobacteria and bacteria of the genus Clostridium), which are responsible for the high production of organic acids and consequent increase in pH values (McDonald et al., 1991 and Kung et al., 2018) that can influence the preservation of ensiled mass.

Propionic acid decreased linearly ($p \leq 0.001$) by up to 91%, with increasing proportions of Mexican sunflower, compared to sorghum silage, which did not affect the fermentation process (Table 4). The levels of lactic and acetic acids were not influenced by Mexican sunflower. Regarding the butyric acid concentration, it was not detected any production of this acid in any evaluated silage (Table 4). In this work, the adopted silage methods promoted a satisfactory drop in pH values in all evaluated silages, contributing to the absence of enterobacteria (ENT) populations during silage opening. Muck (1996) reported that ENT grows intensively during the first days of ensiling and decreases rapidly as the medium is acidified, i.e., when the silages reach low pH values. The losses that occur during fermentation and the growth of undesirable bacteria are inhibited by a high rate of lactic acid production, which is also responsible for the rapid drop in pH, as lactic acid is stronger than the other produced acids (McDonald et al., 1991). The present study found no significant effects of Mexican sunflower inclusion on lactic acid concentrations (Table 4). However, lactic acid concentrations remained close to the recommended (50 g kg^{-1}) for good-quality silage (Filya et al., 2010). Butyric acid was not detected in any of the evaluated silages, and the quantification of Clostridium bacteria was not performed in the present study. Propionic and acetic acids have been proven to effectively inhibit the growth of yeasts and filamentous fungi, with propionic acid being the most efficient (Kung et al., 2018). No significant differences in acetic acid concentrations were found (Table 4), but the concentrations of these acids remained at the recommended average of 0.8% (McDonald et al., 1991). Propionic bacteria can convert lactic acid and glucose into propionic acid. In addition, propionic acid can be converted to propionyl-CoA, which inhibits pyruvate dehydrogenase and, thus, glucose metabolism by molds and yeasts (Jesus et al., 2021). Therefore, as all the silages evaluated had concentrations of these acids, it is possible that these acids may have improved the conservation of the ensiled mass by preventing further growth of undesirable microorganisms due to their inhibitory effects.

However, the ethanol decreased linearly ($p < 0.001$) with the Mexican sunflower in the sorghum silage. The highest concentrations of ET were observed in the sorghum silage (50.9 g kg^{-1}) and the lowest in the Mexican sunflower silage (7.1 g kg^{-1}; Table 4), a decrease of 86%. Low ethanol content is desirable, as ethanol production indicates losses in the ensiled material (Supong et al., 2020). In Table 4, we can see that high CHOs concentrations in the mass to be ensiled lead to greater ET production and higher losses, which allows us to infer that these higher concentrations of CHOs were used for ET production in sorghum silage. The opposite effect was observed in silages with high proportions of Mexican sunflower, for which there were low concentrations of CHOs in the mass to be ensiled, low ET production, and fewer losses were recorded.

The population count of lactic acid bacteria ($p > 0.05$), yeasts ($p > 0.05$), and filamentous fungi ($p > 0.05$) were not influenced by the Mexican sunflower in the silage. Regarding enterobacteria ($p > 0.05$), the population growth of these microorganisms was not detected. Therefore, Mexican sunflower and sorghum silages enabled desirable growth parameters for the populations of the main silage microorganisms (Kung Jr. et al., 2018). The proportions of Mexican sunflower inclusion did not influence the growth of microorganisms in the evaluated silages. This fact allowed effective fermentation without affecting the fermentation parameters, evidenced by the production of organic acids through these microorganisms.

Conclusions

1. Mexican sunflower efficiently substituted sorghum and improved the fermentation process's quality, as it reduced losses in both dry matter (85%) and ethanolic fermentation (86%).
2. Mexican sunflower silage increased by 50% the protein content of the ensiled mass compared to sorghum silage, which was associated with low losses of ammoniacal nitrogen.

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