Revista de Ciências Agroveterinárias
Universidade do Estado de Santa Catarina (UDESC)

Research Article

Fermentation characteristics of silages made with different proportions of sunflower and *Gliricidia sepium* added with doses of citric acid

Características fermentativas de silagens confeccionadas com diferentes proporções de girassol e Gliricidia sepium aditivadas com doses de ácido crítico

Barbara Venâncio Barbosa (ORCID 0009-0009-0252-0038), **Eric Haydt Castello Branco Van Cleef** (ORCID0000-0003-1656-2502), **Vanessa Cury Galati** *(ORCID0000-0001-9014-550X), **Flávio Hiroshi Kaneko** (ORCID 0000-0002-4287-4529)

Federal University of Triângulo Mineiro, Uberaba, MG, Brazil. *Corresponding author: vanessa.galati@uftm.edu.br

Submission: November 21, 2024 | Acceptance: June 22, 2025

ABSTRACT

The aim on this study was to assess the fermentative characteristics of silages produced with different proportions of sunflower and *Gliricidia sepium*, supplemented with doses of citric acid. The ensiled material was composed of two crops, sunflower and gliricidia. The experimental design was completely randomized in a 5 × 4 factorial scheme, with five proportions of sunflower and gliricidia (100% sunflower; 75% sunflower and 25% gliricidia; 50% sunflower and 50% gliricidia; 25% sunflower and 75% gliricidia; and 100% gliricidia) and four concentrations of citric acid (0; 0.25; 0.50; 0.75%), with three replications, totaling 60 experimental silos. It was observed that treatments with higher proportions of Gliricidia resulted in lower fermentation capacity and reduced dry matter levels. The pH decreased in treatments with higher proportions of Gliricidia and acid. When analyzing soluble carbohydrate values, higher Gliricidia inclusion demonstrated higher percentages of carbohydrates. The addition of acid showed a linear effect on dry matter losses. Gas production exhibited a quadratic effect for Gliricidia, with the treatment with 75% inclusion showing the lowest production. There was a quadratic effect for acid addition, with the 0.25% acid treatment presenting the highest gas production. Gliricidia inclusion linearly increased gas losses, and the same occurred for citric acid. For ammoniacal nitrogen analysis, a significant quadratic effect (P<0,05) was observed for acid addition, with the highest mean observed in treatments with 0.50% acid. The final pH of silage was linearly influenced by Gliricidia addition (P<0,0001) and linearly for citric acid (P<0,0001). When analyzing CP, no significant differences were observed (P>0,05). It is concluded that the inclusion of *Gliricidia sepium* in the silage reduces fermentative capacity and dry matter content, while the addition of citric acid linearly influences gas losses and final pH, demonstrating that both factors significantly affect the fermentative characteristics of the silages.

KEYWORDS: Ensilage. Forage conservation. *Helianthus annuus*. Legume tree.

RESUMO

O objetivo neste estudo foi avaliar as características fermentativas de silagens produzidas com diferentes proporções de girassol e *Gliricidia sepium* aditivadas com doses de ácido cítrico. O material ensilado foi composto por duas culturas, girassol e gliricídia. O delineamento experimental foi inteiramente casualizado em esquema fatorial 5 × 4, sendo cinco proporções de girassol e gliricidia (100% girassol; 75% girassol e 25% gliricidia; 50% girassol e 50% gliricídia; 25% girassol e 75% gliricídia; e 100% gliricídia) e quatro concentrações de ácido cítrico (0; 0,25; 0,50; 0,75%), com três repetições. Observou-se que os tratamentos com maiores proporções de gliricídia resultaram em menor capacidade de fermentação e em redução dos teores de matéria seca. O pH diminuiu em tratamentos com maiores proporções de gliricídia e ácido cítrico. Ao analisar os valores de carboidratos solúveis, a maior inclusão de gliricídia demonstrou maiores percentuais de carboidratos. A adição de ácido mostrou efeito linear nas perdas de matéria seca. A produção de gás apresentou efeito quadratico para a gliricídia, o tratamento com 75% de inclusão, apresentou a menor produção. Houve efeito quadrático para adição de ácido, em que o tratamento com 0,25% de ácido apresentou a maior produção de gás. A inclusão de gliricídia, na silagem, aumentou linearmente as perdas por gases e para o ácido cítrico. Para análise de nitrogênio amoniacal, observou-se efeito quadrático significativo (P<0,05) para a adição de ácido, com maior média observada nos tratamentos com 0,50% de ácido. O pH final das silagens foi influenciado linearmente pela adição de gliricídia e para o ácido cítrico (P<0,0001). Conclui-se que a inclusão de *Gliricidia sepium* na silagem reduz a capacidade fermentativa e os teores de matéria seca, enquanto a adição de ácido cítrico influencia as perdas por gases e o pH final, demonstrando que os fatores afetam significativamente as características fermentativas das silagens.

PALAVRAS-CHAVE: Conservação de forragem. Ensilagem. *Helianthus annuus.* Leguminosa arbórea.

INTRODUCTION

The Brazilian cattle herd consists of approximately 234 million animals, occupying a prominent position in the national economy and being responsible for supplying both the domestic and international markets. The production system is based on grazing, with approximately 154 million hectares dedicated to this activity (ABIEC 2023).

Brazil is considered one of the world's leading exporters of beef: around 2.29 million tons exported, supplying 157 countries on all continents. This data highlights the importance of Brazilian livestock farming on a global scale, according to information from the Brazilian Association of Meat Exporting Industries (ABIEC 2024).

The high productivity of Brazilian livestock farming is directly related to the quality of the pastures. Well-managed and high-quality pastures provide adequate nutrition for the animals, resulting in better productive performance.

Thus, the strategic planning of a livestock farmer who seeks to maximize the efficiency of their property should include the conservation of forage produced during the rainy season for use during periods of seasonal forage production shortages. One of the possibilities for preservation is the ensiling process, which is based on the anaerobic fermentation of the ensiled mass and the consequent maintenance or enhancement of the nutritional value of the original material. In this sense, there is a variety of grasses and legumes that have potential for ensiling, whose fermentative quality depends mainly on the dry matter content, the concentration of soluble carbohydrates, and the buffering capacity of the material (CÂNDIDO & FURTADO 2020)

One of the crops with potential for use in animal feed, due to its agronomic and nutritional characteristics, is the sunflower (*Helianthus annuus*). Sunflower silage has higher protein and ether extract content, and can be used to balance rations at a low cost (RODRIGUES et al. 2001).

However, one of the limitations to using sunflower in the form of silage is related to its high buffering capacity. Therefore, it is necessary to use additives to reduce the pH of the ensiled mass and decrease the action of undesirable microorganisms, reducing cellular respiration and protein degradation, resulting in lower losses and greater aerobic stability of the silage (JIANG et al. 2020).

Among the main additives used in animal feed, organic and inorganic acids stand out, playing a fundamental role in reducing pH and facilitating the fermentation process. These additives create a more favorable environment for microbial activity, promoting more efficient fermentation and improving the digestibility of nutrients

(MASSARO JÚNIOR et al. 2020). Citric acid, which is easy to find and relatively safe for farmers to handle, can therefore be considered an additive option to be used in the ensiling process.

Another limiting factor in sunflower silage is its protein content, of around 10% (SANTOS et al. 2020), falling below the nutritional requirements of most ruminant animals. This condition limits its viability as an exclusive food source for supplementation purposes. To overcome this limitation, it becomes feasible to add leguminous forage plants, which have high levels of crude protein in their composition, such as gliricidia (*Gliricidia sepium*), which can contain 22 to 30% dry matter and 20 to 30% crude protein (SANTANA et al. 2019).

Gliricidia sepium (Jacq.) Steud., belongs to the Fabaceae family, and is a drought-resistant tree legume that is being considered a forage crop of commercial and economic interest, in addition to having potential for silage production (OLIVEIRA et al. 2018).

It is hypothesized that the inclusion of Gliricidia in sunflower silage may increase the amount of protein in the silage, while the addition of citric acid may improve the overall quality of the ensiling process. These changes are expected to result in silage that is more nutritious and has better preservation over time. Therefore, the objective of this study was to evaluate the fermentation characteristics of silages produced with different proportions of sunflower and *Gliricidia sepium* supplemented with different doses of citric acid.

MATERIALS AND METHODS

Description of the location and agronomic details

The experiment was conducted at the "Alípio Soares Barbosa" School Farm, belonging to the Federal University of Triângulo Mineiro, located in the municipality of Iturama, Minas Gerais, Brazil. The farm is geographically located at latitude 19°43'52"S, longitude 50°14'06"W and an altitude of approximately 453 meters. According to the Köppen-Geiger climate classification, the region enjoys an AW type climate, characterized as tropical, with the highest incidence of precipitation between the months of October and March and the lowest between April and September, resulting in an average annual rainfall of 1266 mm. The average annual temperature in the region is 23.5 °C (REBOITA et al. 2015).

The ensiled material consisted of two crops, sunflower and gliricidia. The gliricidia used was established in the area in 2019. The trees were subjected to three annual cuttings, pruned to a height of 0.5 m. This study used green branches and leaves (edible part) from the third regrowth of the trees.

Before the start of the experimental period, soil samples were collected to assess the fertility level, and at the time of sunflower sowing, 250 kg/ha of the 8-28-16 N:P:K formulation was applied. After 60 days, topdressing fertilization was carried out with 60 kg/ha of N, using the 20-0-20 N:P:K formulation.

The sunflower crop was established in January 2021. The chosen cultivar was CATISSOL 01, developed by the Coordination of Integral Technical Assistance – CATI, and harvested 90 days after sowing, with an average of 27% dry matter. The sowing was done manually, maintaining a spacing of 0.50 m between rows, resulting in a population of 100,000 plants per hectare. Thinning was then necessary to achieve a final population of 40,000 plants per hectare.

The experimental design was completely randomized in a 5 × 4 factorial scheme, with five proportions of sunflower and gliricidia (100% sunflower; 75% sunflower and 25% gliricidia; 50% sunflower and 50% gliricidia; 25% sunflower and 75% gliricidia; and 100% gliricidia) and four concentrations of citric acid (0; 0.25; 0.50; 0.75%), with three replications, totaling 60 experimental silos.

Process of harvesting and ensiling plants

When they reached harvest point, with a yellow-brown coloration and more than 50% of the grains mature, the sunflower plants were harvested manually and, with the aid of a stationary electric forage crusher, the material was processed to obtain uniform particle size (approximately 2 cm). The gliricidia plant was harvested, observing the edible leaves and stems (discarding the woody/lignified parts), and processed as described for the sunflower (EMBRAPA 2025).

Each of the materials was weighed separately and mixed manually, according to the proportion of each treatment (on a wet basis). The citric acid was diluted in deionized water and applied to the mass using a 50 mL plastic syringe. In the treatment with 0% citric acid, the same amount of water only was applied. At the time of ensiling, aliquots of the original material were sampled in plastic bags and stored in a freezer (-15 ºC) for later analysis of soluble carbohydrates, reducing carbohydrates, total carbohydrates, and buffering capacity.

Plastic bags (25 × 15 cm), suitable for storing food under vacuum, were used as experimental silos. An absorbent device was placed at the bottom of each bag to absorb effluent. Approximately 300 g of each treatment were placed in the bags, which were then vacuum-sealed using a vacuum packaging machine. Each experimental silo was weighed before storage, and the material was packed in cardboard boxes and stored vertically in the laboratory, remaining in this condition for a period of 180 days.

Evaluations of the original materials and the silages

The production of gases resulting from fermentation was measured weekly using a graduated beaker with a capacity of 4 L, filled to 2⁄3 of its capacity with deionized water. The bags were immersed and the water displacement was noted. The volume of gas produced was obtained by difference. After the procedure, the bags were dried and subjected to vacuum again. The evaluation was carried out until gas production stabilized. The data presented represent the total amount of gas accumulated during the ensiling period.

To evaluate the fermentative quality of the silages, methodologies described by Jobim et al. (2007) were used. At the time the silos were opened, the final sample was weighed and separated from the absorbent device. The samples were weighed, and the variables gas loss (GL; Equation 1), total dry matter loss (TDML; Equation 2), dry matter recovery (DMR; Equation 3), and effluent loss (EL; Equation 4) were estimated.

$$\text{Gas loss (GL)} = ([(WSe - WSo)] / DMf) \qquad \text{Eq. 1}$$

Where:

WSe: Weight of the silo at the time of ensiling;

WSo: Weight of the silo at the time of opening;

DMf: Dry matter content of the forage at the time of ensiling

Total dry matter loss (TDM) = ([(DMi – DMf)] / DMi) x 100 Eq. 2

Where:

DMi: Initial dry matter quantity. Silo weight after filling – weight of the empty silo assembly, without the forage, before filling x dry matter content of the forage during ensiling.

DMf: Final DM quantity. Weight of the full silo before opening – weight of the empty silo assembly, without the forage, after opening the silos x MS content of the forage at the time of opening.

Dry matter recovery (DMR) = (MFop x DMop)/ (FMcl x DMcl)*100 Eq. 3

Where:

MFop: forage mass at the opening;

DMop: DM content at the opening;

FMcl: forage mass at closing;

DMcl: DM content of the forage at closing.

Effluent loss (EL) = (Ew x 1000) / GFe Eq. 4

Where:

Ew: effluent weight (Weight of the empty container after opening – weight of the empty container before filling);

GFe: quantity of green forage mass ensiled

Water-soluble carbohydrates (WSC) were evaluated using the phenol-sulfuric method described by (DUBOIS et al. 1956). The extract was prepared using a 250 mL Erlenmeyer flask, where approximately 5 g of the sample was weighed. This sample was immersed in 70 mL of distilled water and the material was heated in a water bath for 30 min. It was then filtered using filter paper in a 100 mL flask and the volume was completed. Two milliliters of the diluted extract were reserved and mixed with 1 mL of phenol solution and 5 mL of sulfuric acid. After a 10-minute resting period at room temperature and cooling in distilled water, the absorbance of the developed color was measured in a spectrophotometer (model 800 XI, Femto, São Paulo, SP, Brazil), using a wavelength of 490 nm.

The extract prepared to determine the soluble carbohydrate content was used for the determination of reducing carbohydrates (RC). For this purpose, 2 mL of the properly diluted extract were transferred to test tubes, to which 1 mL of the 3,5-dinitrosalicylic acid (DNS) solution was added. The tubes were then placed in boiling water for 5 minutes and subsequently cooled for three minutes in ice. After this process, 7.5 mL of distilled water was added. The absorbance was measured in a spectrophotometer at 540 nm.

To determine total carbohydrates (TC), the filter paper, previously weighed and containing the residues resulting from the filtration of the extract used in the determination of soluble carbohydrate content, was dried in a forced-air oven at a temperature of 65 ºC until a constant weight was reached. Approximately 5 mg of the residue was removed and added to 25 mL of distilled water and 5 mL of hydrochloric acid. This material, placed in a 250 mL Erlenmeyer flask, was heated to 60-70 ºC for one hour. Subsequently, it was filtered into a 100 mL volumetric flask, and the volume was made up with distilled water. A solution containing 40 µg of glucose per mL was used as the reference standard.

To determine the buffering capacity (CAPT) of the forage, a methodology adapted from PLAYNE & MCDONALD (1966) was used. Briefly, approximately 15 g of fresh material were weighed, macerated in a mortar, and added to beakers containing 250 mL of deionized water. The containers were placed on a shaker and the initial pH was measured using a digital benchtop pH meter (model PHS-3E, Ionlab, Araucária, PR, Brazil). The solution was acidified with 0.1 N HCl, and then the pH was raised from 3 to 4 using 0.1 N NaOH. Then, the pH was raised to 6, and the amount of hydrochloric acid used to raise the pH was recorded.

After opening the silos, 25 g of sample were collected and diluted in 225 mL of deionized water, using a blender, for about one minute (KUNG JR et al. 1996). The material was filtered using quantitative filter paper and the pH was measured immediately. In this extract, the concentration of ammoniacal nitrogen was evaluated using the method proposed by BOLSEN et al. (1992). For this, 25g of the sample were weighed into a beaker, and then 200 ml of sulfuric acid (0.2 N) were added. After this step, the beaker was hermetically sealed with plastic film and kept refrigerated for a period of 48 hours.

The resulting preparation was filtered and transferred to a plastic bottle, and subsequently stored in the freezer until a few hours before the distillation process. In the distillation procedure, 4 ml of the filtrate were introduced into the micro-Kjeldahl apparatus. Additionally, 10 ml of potassium hydroxide (2 N) were added, and the volume was made up to 20 ml with distilled water. A beaker containing 10 ml of 2% boric acid was prepared to receive the resulting distillate. The distillation was carried out until a volume of 100 ml was reached. Subsequently, the distillate was titrated with 0.1N hydrochloric acid until the desired color change was observed. The percentage of $N\text{-}NH_3$ in the total DM was then calculated.

The ensilability (CF; Equation 5) of the material was determined according to WEISSBACH & HONIG (1996), as follows:

$$\text{Ensilability (CF)} = DM + 8 * SC/BC \qquad \text{Eq. 5}$$

Where:

DM: Dry Matter;

SC: Soluble carbohydrates;

BC: Buffering Capacity - equivalent mg of HCl / 100g of dry matter.

Statistical analysis

The experimental design was completely randomized in a 5 × 4 factorial scheme, with five proportions of sunflower and gliricidia (100% sunflower; 75% sunflower and 25% gliricidia; 50% sunflower and 50% gliricidia; 25% sunflower and 75% gliricidia; and 100% gliricidia) and four concentrations of citric acid (0; 0.25; 0.50; 0.75%), with three replications, totaling 60 experimental silos.

The data were tested for normality and homoscedasticity, and analysis of variance was performed. The linear and quadratic effects of both the addition of gliricidia to sunflower and the increasing addition of citric acid were studied using SAS software (Version 9.4), and significance was declared at 5%.

RESULTS

Upon analyzing the original material, it was observed that treatments with higher proportions of gliricidia exhibited lower fermentative capacity and lower dry matter content. On the other hand, treatments with 0% gliricidia showed the highest values for dry matter and fermentative capacity. For pH analysis, it was observed that treatments with higher proportions of gliricidia and citric acid had lower pH values, while higher concentrations of sunflower and lower concentrations of acid resulted in an increase in pH (Table 1).

Table 1. Contents of water-soluble carbohydrates (WSC), reducing carbohydrates (RC), total carbohydrates (TC), dry matter (DM), pH, buffering capacity (BC), and fermentation capacity (FC) as affected by the addition of gliricidia (GL) and citric acid (CA).

GL (%)	CA (%)	WSC (%)	RC (%)	TC (%)	DM (%)	pH	BC	FC
0	0	1.34	4.24	5.83	29.06	6.16	39.69	29
25	0	1.50	3.07	5.98	26.56	5.93	27.61	27
50	0	1.08	3.03	6.47	25.37	5.78	34.68	26
75	0	1.64	5.37	8.20	24.79	5.55	33.61	25
100	0	2.18	5.90	7.11	21.78	5.75	28.47	22
0	0.25	1.05	1.84	4.50	32.35	6.10	30.71	33
25	0.25	1.26	3.53	5.93	27.90	6.54	32.02	28
50	0.25	1.27	3.46	7.27	25.40	5.33	28.87	26
75	0.25	1.65	5.04	8.03	24.88	5.32	32.16	25
100	0.25	1.40	5.40	8.30	24.34	5.09	35.61	31
0	0.5	1.29	2.96	5.23	30.86	5.29	36.51	31
25	0.5	0.72	1.61	5.93	29.71	5.24	37.70	30
50	0.5	1.47	3.96	8.27	25.72	5.21	34.47	26
75	0.5	1.28	4.27	7.24	24.99	5.07	35.75	25
100	0.5	2.23	5.76	7.40	23.67	4.73	36.90	24
0	0.75	0.69	1.02	6.42	31.38	5.14	32.93	32
25	0.75	1.42	3.68	6.03	27.41	5.00	39.64	28
50	0.75	0.50	1.44	4.04	26.02	4.93	37.41	26
75	0.75	1.84	4.43	7.02	25.10	4.82	37.97	25
100	0.75	1.04	2.65	4.66	24.08	4.54	39.03	24

The analysis of WSC values (Table 1) showed that the treatments with a higher inclusion of gliricidia had the highest percentages of carbohydrates. No interactions were observed between the factors evaluated in this experiment, so the analysis was conducted separately for each factor (percentage of gliricidia inclusion and percentage of citric acid addition). For the silage analyses, a linear effect of acid addition on TDML was observed, Equation 2 ($P<0.0001$), as well as a linearly increasing effect of gliricidia inclusion. Gas production showed a quadratic effect for gliricidia, with the treatment containing 75% inclusion resulting in the lowest production. Furthermore, a quadratic effect was observed for acid addition, with the 0.25% treatment showing the highest gas production. In the DMR analysis, Equation 3 ($P<0.0001$), a linear effect was observed for both citric acid and gliricidia (Table 2).

The inclusion of gliricidia increased gas losses linearly, Equation 1 ($P=0.005$), an effect that was also observed for citric acid ($P<0.05$). In the analysis of ammoniacal nitrogen, a significant quadratic effect was observed ($P<0.05$) for the addition of acid, with the highest mean recorded in the treatments with 0.50% acid. The final pH of the silages was linearly influenced by both the addition of gliricidia ($P<0.0001$) and citric acid (P<0.0001). On the other hand, in the EL analysis, Equation IV, no significant differences were observed (P>0.05).

Table 2. Fermentation losses and quality of silages with different proportions of sunflower and gliricidia supplemented with up to 0.75% citric acid.

		Acid						P-value*						
Gliricidia	Sunflower	0.0	0.25	0.50	0.75	Average	EPM	GL	CA	GL×CA	L_{GL}	Q_{GL}	L_{CA}	Q_{CA}
Dry matter loss (%)														
0	100	0.58	9.55	9.57	16.49	9.05								
25	75	0.79	7.99	13.66	10.77	8.31								
50	50	3.69	8.24	12.34	17.30	10.39	0.66	0.06	<0.0001	0.12	0.01	0.99	<0.0001	0.12
75	25	6.28	9.57	12.08	13.76	10.42								
100	0	0.0	14.63	12.49	15.72	10.71								
Average		2.27	9.99	12.03	14.81									
EPM			0.59											
Dry matter recovery (%)														
0	100	99.42	90.45	90.42	83.51	90.95								
25	75	99.21	92.00	86.34	89.23	91.70								
50	50	96.30	91.76	87.66	82.70	89.61	0,66	0.06	<0.0001	0.12	0.01	0.99	<0.001	0.12
75	25	93.72	90.43	87.92	86.24	89.58								
100	0	100.00	85.37	87.51	84.28	89.29								
Average		97.73	90.00	87.97	85.19									
EPM			0.59											
Effluent loss (kg/t MV)														
0	100	20.00	20.00	20.43	19.98	19.70								
25	75	22.66	25.24	18.80	14.03	20.18								
50	50	20.61	22.80	19.97	18.80	20.25	2.16	0.94	0.03	0.06	0.55	0.67	0.07	0.62
75	25	24.86	17.39	24.92	18.83	21.49								
100	0	25.79	11.19	28.30	16.61	20.47								
Average		22.63	19.16	22.49	17.65									
EPM			2.04											
Gas loss (%)														
0	100	0.23	0.23	0.22	0.26	0.23								
25	75	0.25	0.25	0.28	0.53	0.33								
50	50	0.29	0.28	0.30	0.36	0.31								
75	25	0.26	0.30	0.29	0.33	0.30	0.03	0.008	0.0007	0.31	0.005	0.55	0.0007	0.06
100	0	0.33	0.35	0.29	0.46	0.36								
Average		0.27	0.28	0.28	0.39									
EPM			0.03											
Gas production (mL)														
0	100	2023	2120	1753	1854	1938								
25	75	1818	1943	2081	2025	1967								
50	50	1618	1784	1815	1755	1743	41.47	<0.0001	0.04	0.15	<0.0001	0.69	0.23	0.01
75	25	1273	1513	1703	1581	1517								
100	0	1575	1718	1528	1494	1579								
Average		1662	1816	1776	1742									
EPM			37.09											
Ammoniacal nitrogen (%MS)														
0	100	0.85	0.68	0.60	1.02	0.79								
25	75	0.72	0.80	1.11	0.83	0.87								
50	50	0.58	0.77	0.68	0.55	0.64	0.06	0.005	0.0012	0.10	0.29	0.78	0.04	0.0008
75	25	0.48	0.81	1.49	0.71	0.87								
100	0	0.62	0.92	0.66	0.61	0.70								
Average		0.65	0.80	0.91	0.75									
EPM			0.05											
final pH														
0	100	4.53	4.56	4.52	4.50	4.53								
25	75	4.51	4.52	4.50	4.53	4.51								
50	50	4.50	4.48	4.45	4.43	4.46	0.01	<0.0001	0.0001	0.12	<0.0001	0.06	<0.0001	0.49
75	25	4.38	4.43	4.38	4.34	4.38								
100	0	4.43	4.39	4.24	4.24	4.33								
Average		4.47	4.47	4.41	4.41									
EPM			0.01											

*GL = Gliricidia, CA = citric acid, GL×CA = interaction between proportions of Gliricidia and concentrations of citric acid, LGL = linear effect of Gliricidia addition, QGL = quadratic effect of Gliricidia addition, LCA = linear effect of citric acid addition, QCA = quadratic effect of citric acid addition.

DISCUSSION

The lowest value found in ensilability, associated with the reduced DM content in the treatments with higher proportions of gliricidia, corroborates the conclusions of RÊGO et al. (2010), who suggest that materials with a low percentage of dry matter usually result in less efficient silage. This happens because they offer a smaller amount of fermentable substrate, which can hinder the fermentation process. Furthermore, the high moisture content in these materials can increase losses during fermentation, making proper preservation of the product more difficult.

Forage ensiling is a common practice in ruminant feeding systems that is based on the fermentation of soluble carbohydrates present in the material into organic acids, leading to a reduction in pH and the preservation of the nutritional quality of the ensiled material (BERNARDI et al. 2019). However, it is important to emphasize that a low final pH does not, in itself, guarantee the stability of the silage. The determining factor for the adequate preservation of the material is the speed of the pH drop, which is primarily responsible for inhibiting undesirable anaerobic microorganisms during the fermentation process.

As highlighted by MUCK et al. (2018), the association between pH values and dry matter content is a fundamental criterion for evaluating silage quality. In materials with a higher dry matter content, pH becomes less critical for fermentation stability, allowing for the production of good quality silage even with relatively higher pH values (Table 1). In the present study, the increasing trend in pH in treatments with higher sunflower concentrations may be associated with the higher protein levels observed, which reduces the ratio between soluble carbohydrates and nitrogenous compounds, directly influencing the acidification of the silage (EVANGELISTA et al. 2001).

During ensiling, soluble carbohydrates represent the main substrate to ensure efficient fermentation. According to ZHANG et al. (2010), the content of these carbohydrates is a crucial indicator of forage quality for ensiling, with a minimum concentration of 3.0% in dry matter being necessary for adequate fermentation. However, there is an inverse relationship between the need for soluble carbohydrates and the dry matter content of the material, as reported in the meta-analysis conducted by RIDLA et al. (2024). These authors observed that forages with low dry matter content require a higher content of soluble carbohydrates to ensure efficient fermentation, while materials with a higher dry matter content may have a lower requirement for soluble carbohydrates without compromising silage quality. This relationship was confirmed in the present study (Table 1), in which the treatments with lower WSC values showed the highest DM content.

The addition of acid to the silage linearly influenced dry matter losses (Table 2), corroborating the findings of OLIVEIRA et al. (2009), who reported that these losses may be associated with increased gas production resulting from fermentation by CO_2-producing microorganisms. This phenomenon was also observed in this study, where the greater addition of citric acid led to an increase in gaseous losses.

It was expected that the inclusion of acid would result in better quality silage, since the initial acidification could favor a more efficient fermentation. However, after the silos were opened, a reduction in pH was observed in all treatments, ranging from 4.24 to 4.56, with an average of 4.44. According to PENG et al. (2021), the observed pH is higher than the range considered ideal for silages (3.8–4.2), indicating that the initial acidification was not sufficient to promote a highly efficient fermentation.

The quadratic effect observed for $N\text{-}NH_3$ suggests protein degradation during the ensiling process, which can compromise the quality of the silage. The compounds resulting from this degradation, in addition to reducing palatability and intake by animals, exhibit low efficiency in nitrogen utilization by ruminants. Furthermore, the greater release of nitrogenous compounds can delay the drop in pH, as observed in the treatment with 0.50% acid, negatively impacting the fermentative stability of the silage.

CONCLUSION

Incorporating gliricidia into sunflower silage reduces the pH but increases losses due to gases and dry matter. Similarly, the addition of citric acid lowers the pH and reduces gas losses, but increases dry matter losses. Since the effects of these factors proved to be independent, the proper balancing of each one is essential to optimize silage quality, considering nutritional objectives and production conditions. For the producer, this implies the need to evaluate the cost-benefit of using these additives, since excessive losses of dry matter directly impact the efficiency and profitability of the production system.

AUTHOR'S CONTRIBUTIONS

Conceptualization, methodology and formal analysis, Bárbara Venâncio Barbosa and Eric Haydt Castello Branco van Cleef; software and validation; investigation, Bárbara Venâncio Barbosa and Eric Haydt Castello Branco van Cleef; resources and data curation, Bárbara Venâncio Barbosa and Eric Haydt Castello Branco van Cleef; writing - original draft preparation, Vanessa Cury Galati; writing - review and editing, Vanessa Cury Galati and Flávio Hiroshi Kaneko; visualization, Bárbara Venâncio Barbosa; supervision, Eric Haydt Castello Branco van Cleef; Vanessa Cury Galati and Flávio Hiroshi Kaneko, project administration, Bárbara Venâncio Barbosa and Eric Haydt Castello Branco van Cleef; funding acquisition, Eric Haydt Castello Branco van Cleef. All authors have read and agreed to the published version of the manuscript.

FINANCING

This work was supported by PIBIC/CNPq, call for proposals no. 11/2022/PROPPG/UFTM.

STATEMENT OF THE INSTITUTIONAL REVIEW BOARD

Not applicable to studies that do not involve humans or animals.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available upon request.

ACKNOWLEDGEMENTS

We thank CNPq for the scholarship granted to student Bárbara Venâncio Barbosa.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

ABIEC. 2023. Associação Brasileira das Indústrias Exportadoras de Carne. Beef Report. Disponível em: < https://www.abiec.com.br/>. Acesso em 10 set. 2024

ABIEC. 2024. Associação Brasileira das Indústrias Exportadoras de Carne. Censo Agropecuário. Disponível em: < https://www.abiec.com.br/>. Acesso em 08 abril. 2024

BERNARDI A et al. 2019. A meta-analysis examining lactic acid bacteria inoculants for maize silage: effects on fermentation, aerobic stability, nutritive value and livestock production. Grass and Forage Science 74: 596–612.

BOLSEN KK et al. 1992. Effect of silage additives on the microbial succession and fermentation process of alfalfa and corn silages. Journal of Dairy Science 75: 3066-3083.

CÂNDIDO MJDD & FURTADO RN. 2020. Estoque de forragem para a seca: produção e utilização da silagem. E-book. Fortaleza: Imprensa universitária.

DUBOIS M et al. 1956. Colorimetric method for determination of sugars and related substances. Analytical chemistry 28: 350-356.

EMBRAPA. 2025. EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. Colhei-ta. Disponível em: <http://cnpso.embrapa.br/producaogirassol/colheita.htm>. Acesso em: 10 fev. 2025.

EVANGELISTA AR et al. 2001. Aditivo na ensilagem de Coast cross (Cynodon dactylon Pers). Farelo de trigo e pré-secagem. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 38., 2001, Piracicaba. Anais... Piracicaba: Sociedade Brasileira de Zootecnia, 71-72.

JIANG F et al. 2020. Treatment of whole-plant corn silage with lactic acid bacteria and organic acid enhances quality by elevating acid content, reducing pH, and inhibiting undesirable microorganisms. Front. Microbiol. 11: 1-10.

JOBIM CC et al. 2007. Avanços metodológicos na avaliação da qualidade da forragem conservada. Revista Brasileira de Zootecnia 36: 101-119.

KUNG JR L. 1996. Preparation of silage water extracts for chemical analyses. Standard operating procedure. 6.ed. Newark: University of Delaware.

MASSARO JUNIOR FL et al. 2020. Effect of storage time and use of additives on the quality of grape pomace silages. Journal Food Processing and Preservation 44: e14373.

MUCK RE et al. 2018. Silage review: Recent advances and future uses of silage additives. Journal of Dairy Science 101: 3980–4000.

OLIVEIRA APD et al. 2018. Effect of ensiling gliricidia with cassava on silage quality, growth performance, digestibility, ingestive behavior and carcass traits in lambs. Animal Feed Science and Technology 241: 198-209.

OLIVEIRA HC et al. 2009. Perdas e valor nutritivo da silagem de capim-tanzânia amonizado com uréia. Archivos de Zootecnia 58: 195-202.

PENG C et al. 2021. Isolation, identification and utilization of lactic acid bacteria from silage in a warm and humid climate area. Scientific Reports 11: 12586.

PLAYNE MJ & MCDONALD P. 1966. The buffering constituents of herbage and of silage. Journal Science Food and Agriculture 17: 264-268.

REBOITA MS et al. 2015. Aspectos climáticos do estado de Minas Gerais. Revista Brasileira de Climatologia 17: 206- 225.

RÊGO MMT et al. 2010. Chemical and bromatological characteristics of elephant grass silages with the addition of dried cashew stalk. Revista Brasileira de Zootecnia 39: 255-261.

RIDLA M et al. 2024. Effects of wilting on silage quality: A meta-analysis. Animal Bioscience 37: 1185–1195.

RODRIGUES PHM et al. 2001. Efeito do inoculantes microbianos sobre a composição química e fermentativa da silagem de girassol produzidas em silos experimentais. Revista Brasileira de Zootecnia 30: 169-175.

SANTANA JSC et al. 2019. Características fermentativas, composição química e fracionamento da proteína da silagem de gliricídia submetida a diferentes períodos de fermentação. Boletim de Indústria Animal 76: 1-9.

SANTOS CBD et al. 2020. Production and quality of sunflower and Paiaguas palisadegrass silage in monocropped and intercropping in different forage systems. Acta Scientiarum. Animal Sciences 42: e48304.

WEISSBACH F & HONING H. 1996. Über die Vorhersage und Steuerung des Gärungsverlaufs bei der Silierung von Grünfutter aus extensivem Anbau. Landbauforschung Völkenrode 46: 10-7.

ZHANG J et al. 2010. Relationships between the addition rates of cellulase or glucose and silage fermentation at different temperatures. Animal Science Journal 81: 325–330.