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Fermentative characteristics, agronomic evaluation, and microbial populations of sorghum silage with additives

Abstract – The objective of this work was to evaluate the effects of the use of additives on the ensiling of AGRI 002E sorghum (*Sorghum bicolor*), focusing on the chemical composition, loss, microbial profile, and fermentative stability of the fodder. Sorghum was harvested 111 days after sowing, chopped, and ensiled in PVC silos under different treatments, as follows: without additives (SS), with inoculant (SSI), with urea (SSU), and with urea and inoculant (SSUI). The silos were opened and analyzed at 1, 3, 7, 14, 28, and 56 days after ensiling. The inclusion of urea and bacterial inoculant did not significantly affect dry matter content, but influenced dry matter recovery and microbial population, reducing the presence of clostridia and fungi. Dry matter and gas losses were minimal, indicating an adequate fermentation. The additives contribute to a more stable fermentation and a better preservation of the ensiled material, particularly by reducing undesirable microbial populations.

Index terms: buffering capacity, fungi, lactic acid bacteria, losses, pH.

Características fermentativas, avaliação agronômica e populações microbianas de silagem do sorgo-forrageiro com aditivos

Resumo – O objetivo deste trabalho foi avaliar os efeitos do uso de aditivo na ensilagem do sorgo-forrageiro (*Sorghum bicolor*) AGRI 002E, com foco na composição química, na perda, no perfil microbiano e na estabilidade fermentativa da forragem. O sorgo-forrageiro foi colhido 111 dias após a semeadura, picado e ensilado em silos de PVC, sob diferentes tratamentos, conforme a seguir: sem aditivos (SS), com inoculante (SSI), com ureia (SSU), e com ureia e inoculante (SSUI). Os silos foram abertos e analisados aos 1, 3, 7, 14, 28 e 56 dias após a ensilagem. As inclusões de ureia e inoculante bacteriano não afetaram significativamente o teor de matéria seca, mas influenciaram a recuperação de matéria seca e a população microbiana, tendo reduzido a presença de clostrídios e fungos. As perdas de matéria seca e de gases foram mínimas, o que indica uma fermentação adequada. Os aditivos contribuem para uma fermentação mais estável e uma melhor preservação do material ensilado, especialmente pela redução das populações indesejáveis de microrganismos.

Termos para indexação: capacidade tampão, fungo, bactéria ácido-láctica, perda, pH.

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Introduction

Pasture production reduces during dry season because of the seasonality, requiring alternative sources of feed to meet the animal demand, highlighting the importance of conservation processes (Cattani et al., 2017).

Silage production is a usual conservation method. The process involves preserving the material through anaerobic fermentation, that is, removing the oxygen from the ensiled material, which allows of the growing of lactic acid bacteria (LAB) (Arriola et al., 2021).

Forage sorghum (*Sorghum bicolor*) has a high nutritional value, low buffering capacity, high content of soluble carbohydrates, and an adequate concentration of dry matter (DM), which are the characteristics necessary for the ensiling process (Perazzo et al., 2017). However, the high content of soluble carbohydrates (Neumann et al., 2010) can be a problem during fermentation, since it provides favorable conditions for yeasts, molds, and enterobacteria to grow, which results in a high residual content of soluble carbohydrates and increased DM losses during fermentation (Mohd-Setapar et al., 2012).

The increase of these agents in silage promotes the degradation of carbohydrates and proteins, which causes low aerobic stability and undesirable fermentation, reducing the nutritional value of the silage and releasing toxins, endotoxins, and mycotoxins that are harmful to animal health (Weiss et al., 2016).

The inoculation with LAB is commonly performed to increase its population to inhibit harmful microorganisms, and to preserve the nutritional quality of the material to be ensiled (Arriola et al., 2021).

The use of urea as a chemical additive is a good alternative, due to its low cost and ease of handling (Araújo et al., 2023). Urea has an antifungal action with a toxic effect on molds and yeasts, thereby restricting their activity in highly acidic silages (Araújo et al., 2023). In conditions of high soluble carbohydrate concentration, such as in forage sorghum, urea acts as a buffer, preventing a sharp drop of pH (pH<3.0), which restricts the growth of undesirable microorganisms, increasing the matter recovery, maintaining the aerobic stability (Araki et al., 2017). Additionally, urea can be classified as a nutrient additive because it improves the nutritional value of the silage (McDonald et al., 1991).

The objective of this work was to evaluate the effects of the use of additives on the ensiling of AGRI 002E sorghum, focusing on the chemical composition, loss, microbial profile, and fermentative stability of the fodder.

Materials and Methods

The experiment was carried out in field conditions, in 0.62 ha located at 24°32'49.7"S, 54°01'46.4"W, at 392 m altitude.

AGRI 002E forage sorghum harvest took place 111 days after sowing, following technical recommendations. The harvest was conducted using a forage chopper attached to a tractor; forage was cut at 10 cm height above the ground and chopped into particles of 1 cm to 2 cm.

The chopped material was placed in plastic sheets to incorporate additives, to ensure a homogeneous mixture. Subsequently, this material was compacted (using wooden sticks) and put into PVC experimental silos of 10 cm diameter and 50 cm length. The silos were sealed with PVC lids equipped with Bunsen-type valves for the free release of gases (gas losses). The sand used in the experiment was finely sieved, washed, kept at 105°C for 20 min (autoclaved), and dried. A layer of 5 cm of this sand was placed at the bottom of the silo, separated by a layer of cotton fabric for effluent drainage (effluent losses), and sealed with adhesive tape to prevent air exchange with the environment.

The experimental treatments were the following: sorghum silage without additives (SS); sorghum silage enriched with 100.000 CFU (colony-forming units) per gram of fresh matter of the inoculants *Lactobacillus plantarum* and *Pediococcus acidilactici* (SSI); sorghum silage enriched with 0.5% urea on a DM base (SSU); and sorghum silage enriched with 0.5% urea on DM base and with 100.000 CFU g^{-1} of fresh matter of *L. plantarum* and *P. acidilactici* (SSUI).

The experimental silos (silo + autoclaved sand + cotton fabric + nylon cord) were weighed empty and after sealing. The difference between sealed weight and empty weight was the ensiled green mass. At the time of opening, the silos were weighed to check the weight difference in comparison with the initial mass. The difference represented the gas evacuation (GE), the effluent losses (EL), and the DM recovery (DMR), which defines the fermentative quality of

silage (Schmidt et al., 2007). The silos were opened for analysis at 1, 3, 7, 14, 28, and 56 days after ensiling.

At the opening day, silos were weighed, temperature was taken, pH was measured, and samples were collected. Immediately after sample collection, the silos were sealed again. Then, silos were weighed to evaluate the fermentative profile. Ambient temperature and silage temperature were measured using a skewer-type thermometer (23 cm). The pH of the silage was measured using a digital pH meter (Tec 2-mp, Tecnal Scientific Equipment). For the pH measurement, 100 mL of distilled water was added to 10 g of the sample, allowing it to rest for one hour before taking the reading.

A sample of 20 g fresh matter was added to 250 mL distilled water and left to rest for 30 min, to determine the buffering capacity (BC) expressed in milliequivalents per 100 g DM (meq 100 g^{-1} DM).

Microbial populations were determined using culture techniques. Initially, 25 g of the samples were mixed with 225 mL of distilled water under agitation. Then, successive dilutions from 10^{-1} to 10^{-9} were prepared by pipetting 1 mL of the mixture into test tubes containing 9 mL of distilled water. Finally, 0.1 mL of the diluted extracts was spread onto potato dextrose agar (PDA) plates.

The isolation of fungi and yeasts was carried out by inducing mycelial growth in a culture medium. This was achieved by spreading spores on the surface of a PDA medium and letting it incubate at room temperature for seven days. After incubation, the isolated microorganisms were counted, and slides were prepared for microscopic identification of the colonies by genus.

Samples for the enumeration of enterobacteria were streaked on violet red bile agar (VRB) deep plates and incubated at 35°C for 24 hours. For the enumeration of *Clostridium*, plates with reinforced clostridial agar were used and incubated in an anaerobic chamber. Carbon dioxide (CO_2) was injected into the chamber and left to incubate at 35°C for 24 hours. LAB were streaked on the surface of Man, Rogosa, and Sharpe agar (MRS), and then incubated in an oven at 30°C for 48 hours.

After the incubation, a manual colony counting was performed. Plates with up to 300 CFU were counted, and the results were expressed as log CFU g^{-1} of silage.

A completely randomized experimental design was carried out with repeated measures over time. The experimental unit was represented by one silo. Silos were randomly assigned to treatments, and each one was subjected to six opening times. There were four treatments with four replicates each. The Shapiro-Wilk's test was used to assess data normality. The Durbin-Watson's test was used to check the assumption of independence of residuals. The Barlett's test was used to check homoscedasticity. The Mauchly's test was used to check sphericity. The assumptions of normality, independence of residuals, and homoscedasticity were met; however, the assumption of sphericity was not. The analysis of variance was performed at 5% significance, using the Tukey's test for mean comparisons.

The p-values for linear and quadratic effects, when used, were analyzed through regression using orthogonal polynomial contrasts, using the REG procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC).

Results and Discussion

The levels of DM in AGRI 002E sorghum silage was not influenced ($p>0.05$) by the inclusion of additives (Table 1). However, there was an effect observed ($p\leq0.05$) for the silage opening days. There was no interaction ($p>0.05$) between treatments and opening days. The DM content in sorghum silage was higher than that of the original material (133.45 g kg^{-1}), and higher levels were observed on day 1 (172.56 g kg^{-1}) and day 3 (171.08 g kg^{-1}) opening days, followed by a reduction in the subsequent openings, stabilizing on day 56 (167.29 g kg^{-1}).

The addition of urea and bacterial inoculant showed no effect on the DM content. After the opening of the experimental silos, the increase of DM content of sorghum silage was a result of the ensiled material dehydration and water evaporation. However, a slight DM content reduction was observed, due to the prolonged opening days. The associated causes may be the oxidation of soluble substrates, deterioration by microorganisms, and losses in the form of gases, the so-called "metabolism water".

The DM content of sorghum silage was below the recommendations for an adequate fermentation, which are concentrations above 250 g kg^{-1} DM, according to Nussio et al. (2011). However, due to some forage

characteristics, such as high content of soluble carbohydrates and low BC, the fermentation remained stable.

Urea can modify the exposure of cellulose and hemicellulose complexed in the ensiled material, altering the type of microorganisms responsible for fermentation and their development, resulting in an increase of DM content, as observed in the present study, and reducing losses (Araújo et al., 2023).

An important factor in fermentation quality is to observe the DM of the ensiled material. At high DM conditions and its increase, observed during the silo opening, there can be a depression in the LAB development, which reduces the rate and extent of fermentation (Kung Junior et al., 2018).

At high DM content, the increasing effluent flow and the removal of ionic compounds from silage lead to a reduction of the lactic acid content. This reduction requires inoculation with homofermentative and heterofermentative bacteria, to regulate pH and aerobic stability. This may partially explain the lack of effect on the evaluated sorghum silage, considering the low DM content of the ensiled material and the high concentration of soluble carbohydrates, which results in increased LAB concentration.

Oliveira et al. (2017) conducted a meta-analysis on the inoculation of homofermentative and heterofermentative bacteria in various forage species for ensiling. They observed that inoculation had little or no effect on the DM content of sorghum and maize silages; however, positive results were observed in tropical and temperate forages with high moisture content.

The organic matter (OM) variable did not differ among treatments. However, there was an effect on the opening days ($p \leq 0.05$), with an interaction between treatment and time (Table 2). In all tested treatments, OM of the original material differed significantly ($p \leq 0.05$) from the opening days, showing lower concentrations. All evaluated silage treatments had higher OM content on day 28 of the ensiling, followed by a reduction on day 56, except for the treatment SSUI.

OM changed during the fermentation because of the consumption of soluble carbohydrates and the production of organic acids, in the early days after ensiling, until material stabilization. There was an OM reduction up to 14 days of ensiling that increased again on day 28 and day 56, caused by the increase of the mineral fraction of the silage.

The BC did not differ statistically ($p > 0.05$) among silages treated with additives and showed the average of 55.25 meq 100 g^{-1} DM (Table 1).

Sorghum silages with or without additives had a low BC, as evidenced by the rapid decrease of pH due to the high production of organic acids during fermentation

Table 1. Mean values, standard error (SE), and analysis of variation results of sorghum (*Sorghum bicolor*) silage characteristics, as a result of the treatments (T) and days of opening (DO).

Variable	Treatment (T)[1]				SE	p-value		
	SS	SSI	SSU	SSUI		T	DO	T x DO[2]
Dry matter (g kg^{-1})	165.33	164.48	163.70	163.56	0.70	0.27	<0.01*	0.67
Organic matter (g kg^{-1} DM)	923.24	923.28	924.83	924.32	0.53	0.14	<0.01*	<0.01*
Buffering capacity (meq 100g^{-1} DM)	54.32	52.80	57.13	56.75	1.40	0.15	n/a	n/a
Dry matter recovery (%)	92.84	94.17	88.95	87.64	0.76	<0.01*	<0.01*	0.67
Effluents losses (kg Mg^{-1})	45.08	43.12	44.53	42.26	1.72	0.65	<0.01*	0.41
Gas losses (%)	0.90	0.75	0.98	0.82	0.07	0.18	<0.01*	0.60
pH	3.89	3.90	4.13	4.11	0.01	<0.01*	<0.01*	<0.01*
Temperature (ºC)	26.92	27.34	27.11	27.38	0.07	<0.01*	<0.01*	<0.01*
Lactic acidi bacteria (log CFU g^{-1})	5.89	5.77	5.93	5.63	0.16	0.58	<0.01*	0.59
Enterobacteria (log CFU g^{-1})	2.53	2.35	2.58	2.18	0.21	0.52	<0.01*	0.64
Yeasts (log CFU g^{-1})	1.16	1.22	1.12	0.97	0.14	0.62	<0.01*	0.12
Clostridium (log CFU g^{-1})	6.46	6.59	6.27	6.22	0.09	0.05	n/a	0.02

[1]Treatments: SS, sorghum silage without additives; SSI, sorghum silage enriched with inoculant; SSU, sorghum silage enriched with urea; and SSUI, sorghum silage enriched with urea and inoculant. [2]T x DO: interaction between T and DO. *Significant at 1% probability. n/a, not applicable.

(Siqueira et al., 2007). The BC of final product is related to protein content, inorganic composition (Ca, K, Na), combination of organic acids produced, and salts present in the plant. Along with the DM content and the concentration of soluble carbohydrates, BC is one of the most important factors for fermentation quality.

Plants with high BC exhibit a slow decrease of pH, leading to secondary fermentations, and subsequent losses of silage quality. This fact was not observed in the present study, which can be explained by the rapid reduction of pH and the similar results observed for silages with or without additives.

There was an influence of the use of additives and opening days ($p \leq 0.05$) on silage DM recovery (Table 1); however, no interaction was observed between treatments and days ($p > 0.05$). Higher DMR ($p \leq 0.05$) was observed in inoculated treatments and the control than in the treatment of silages with urea. Concerning the opening days, there was a linear increasing effect ($p \leq 0.05$), as indicated by the equation (DMR = 89.401 + 0.0822x), with the highest recovery on day 56.

The high DMR values obtained in the present study are related to proper compaction, high density (Jobim et al., 2007), and low losses, mainly effluents and gases. A high production of carbon dioxide (CO_2) occurs mainly in cases of secondary fermentations; such gas can account for up to 98% of all losses in silos.

The addition of bacterial inoculant to sorghum silage does not have a noticeable contribution. Noninoculated silage (treatment control) contains a high population of lactic acid bacteria, good fermentation pattern, low pH, adequate aerobic stability, and reduced DM losses (Schmidt et al., 2007).

The improvement of DMR in silages due to the addition of bacterial inoculant are limited. When evaluating the inoculation of various forage crops used for ensiling, Oliveira et al. (2017) reported improvements of up to 2.8% of DMR in cool-season and tropical rice grass silages. However, in maize and sorghum silages, there was no difference between materials with or without inoculation. The same authors stated that homofermentative bacterial inoculants can reduce fermentative losses, but it is difficult to measure which losses are due to fermentation and which ones can be attributed to DM losses.

According to Araki et al. (2017), urea contributes to the aerobic stability of sorghum silages, to antimicrobial action, and to the ability to maintain the medium in osmotic conditions. Santos et al. (2018) observed a DMR increase, loss reduction, and an improvement of the nutritional value of forage sorghum treated with 0.5% and 1.0% urea. These effects are due to the toxic effect of urea on yeasts, which are largely responsible for DM losses.

In the present study, urea showed lower DMR than the control and the treatment of silage with inoculant addition. This result is probably related to the high content of soluble carbohydrates. Yeast can use the excess carbohydrate for production of ethanol, resulting in DM losses (Schmidt et al., 2007).

Along the opening days, the effluents caused a linear increasing loss in the ensiled material ($p \leq 0.05$), varying from 37.72 kg Mg^{-1}, at day 1, to 45.56 kg Mg^{-1} at day 56. Gases also caused a linear increasing loss ($p \leq 0.05$) along the openings, from 0.68%, at day 1, to more than 1% at day 28 and day 56.

Effluent losses assess the number of soluble components of plant cellular contents that are lost

Table 2. Means and results of organic matter (g kg^{-1} DM) content in sorghum (*Sorghum bicolor*) silage, on each day of opening, for each treatment[1].

Treatment[2]	Day of opening						
	0	1	3	7	14	28	56
SS	911.50e	925.40bc	927.58ab	922.68cd	920.03d	930.65a	924.88bc
SSI	913.20d	923.58bc	925.20b	925.45b	921.10c	929.00a	925.45b
SSU	909.33c	929.05a	929.25[a]	929.00a	923.53b	930.00a	923.68b
SSUI	909.98c	927.05a	929.35[a]	927.65b	925.78a	927.50a	928.93a

[1]Means followed by different lowercase letters, in the same row, differ by the Tukey's test, at 5% probability. [2]Treatments: SS, sorghum silage without additives; SSI, sorghum silage enriched with inoculant; SSU, sorghum silage enriched with urea; and SSUI, sorghum silage enriched with urea and inoculant.

during the fermentation process through leaching (Ribeiro et al., 2007). Part of the losses are organic compounds, such as sugars, acids, proteins, and minerals (Nussio et al., 2011). Such components have high digestibility and are essential for the development of microorganisms responsible for fermentation and, consequently, for the preservation of silage quality.

Certain additives, particularly chemical ones, can disrupt the cellular structure of plants and their water-holding capacity, increasing the extravasated cellular content, according to Balieiro Neto et al. (2007). When determining losses in sugarcane silage with chemical additives (urea) and bacterial additives (inoculant), Siqueira et al. (2007) reported losses of 58.15 kg Mg^{-1} DM, values that are higher than those observed in the present study.

In this study, the EL measured were below the levels mentioned by Pupo (2002), who found losses up to 150 kg Mg^{-1} of fresh matter in materials of high moisture content. The relative low losses of sorghum silage indicate that the high moisture content in the evaluated sorghum did not have a negative impact on fermentation and quality of the final product (McDonald et al., 1991).

Gas loss (GL) in the present work increased along the opening days, which is possibly due to the intense initial respiratory activity of plants and the development of a population of microorganisms responsible for the fermentation. The highest GL was 1.08% on day 56. França et al. (2011) reported GL of 1.9% of DM in four forage sorghum cultivars. Pupo (2002) reported values from 2% to 5% GL of the initial DM. These losses are expected to gradually decrease, as the microbial and cellular activities of the forage stabilize (Borreani et al., 2018).

The SSU treatment showed the highest pH values ($p \leq 0.05$) in all opening days, in comparison with SS and SSI (Table 1). The pH decreased ($p \leq 0.05$) over time as fermentation progressed in all treatments, stabilizing between day 28 and day 56.

A pH reduction of silage is essential for maintaining quality and inhibiting undesirable fermentations. The ideal fermentation of forage sorghum silage shows minimal pH values seven days after ensiling, remaining stable for the storage period in anaerobic conditions (Fazaeli et al., 2008). Such behavior was observed in the present study.

The silage pH stability depends on the DM concentration, BC, concentration of soluble carbohydrates, LAB population, lactic acid production, and anaerobic conditions in the environment (Borba et al., 2012). However, caution should be taken regarding the content of soluble carbohydrates, since elevated levels of these substances can lead to extremely low pH ($pH < 3.0$), providing favorable conditions for the growth of yeasts, which are responsible for alcoholic fermentation. Ribeiro et al. (2007) assessed the fermentative pattern of silage from five forage sorghum genotypes and observed pH values between 3.69 and 4.58.

The use of bacterial inoculants based on LAB contributed to rapid bacterial growth and pH reduction, primarily due to the predominance of LAB in the fermentation. The use of homofermentative inoculants in sorghum silages reduced the pH and increased the lactic acid production in a large number of studies, according to a review by Oliveira et al. (2017). In the present study, pH did not differ from the control silage, which is possibly due to the high content of soluble carbohydrates.

The addition of urea exerted better control over pH, with values slightly higher than those of the SSI and SS treated silages and had a positive impact on the crude protein concentrations in the silage. Urea has a buffering action; when added to the silage, urea reacts with water becoming ammonia, in the form of ammonium hydroxide (NH_4OH), releasing ammonium ions in the ensiled material. Urea level added to silage should be regulated, since high levels of this substance increase pH and $N\text{-}NH_3/NT$ ratios, allowing of the development of microorganisms responsible for undesirable fermentations (Kung Junior et al., 2018).

Differences for temperature were observed among the treatments. SSI temperature was higher ($p \leq 0.05$) than those of SS and SSU. On day 1, SS temperature was lower than silages treated with additives. At day 3, the treatments exhibited a similar behavior ($p > 0.05$) for temperature (Figure 1).

The evaluated silages consistently exhibited interior temperatures in the silo higher than the ambient temperature, on all opening days, within ± 2°C recommended range. However, in the original material, the silages with the addition of bacterial inoculant showed temperatures up to 2.5°C higher than the ambient temperature.

The heating up to 12°C higher than the ambient temperature of freshly cut silage, during ensiling, is a normal activity conditioned by biological fermentation (Muck et al., 2018), especially in silages with a high population of homolactic bacteria, as observed in the original material.

After the fermentation period, there is a stabilization of temperature, that gradually decreases as it is influenced by ambient temperature conditions, silo dimensions (Borreani et al., 2018), and the DM content of the harvested material.

Under intense aerobic conditions, a temperature increase and moisture of the ensiled material can occur, leading to intense undesirable fermentations (Maillard reaction) caused by fungi, yeasts, and aerobic bacteria, resulting in DM and nutritional value losses of the silage (Santos et al., 2018).

According to Driehuis et al. (2001), the ideal temperature for the development and activity of LAB in silage is between 20 ºC and 40 ºC. This a range was achieved in all periods and treatments evaluated in the present study. Treatments with inoculant showed a higher initial temperature in the silage, which may be related to the increased DM content of the material. More heat is required to raise the temperature in materials with higher moisture content, in comparison with drier forages (Zhou et al., 2016).

Another important point to highlight is the ambient temperature because, under high-temperature conditions during storage, it contributes to the increased development of acetic acid bacteria and acetic acid production (Feng & Wang, 2020). However, under low-temperature conditions (<10°C), the initiation of the fermentation process is delayed, and the pH decline is slowed (Zhou et al., 2016).

Regarding the presence of microorganisms in the silage, the populations of LAB, enterobacteria, and yeasts differed ($p \leq 0.05$) along the silage opening days. LAB showed a significant development due to the abundant source of soluble carbohydrates in

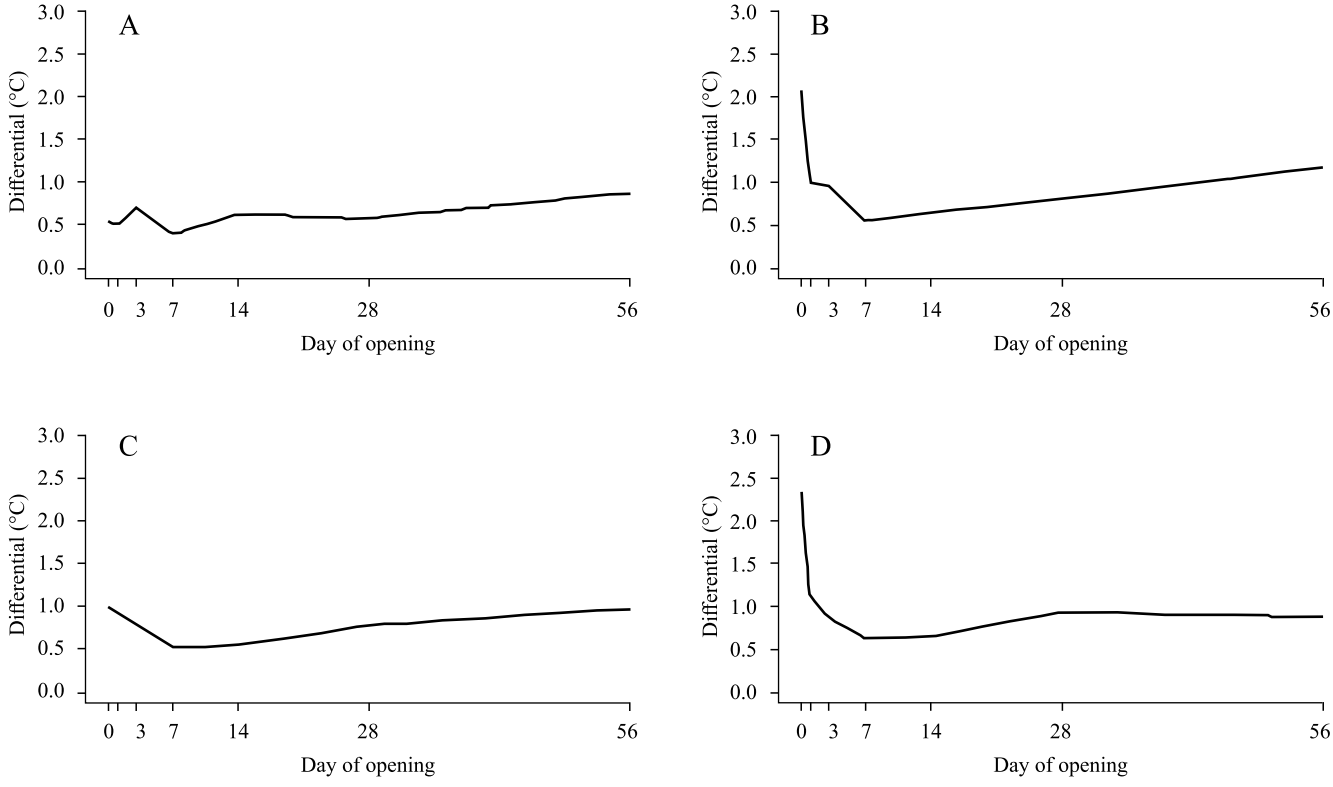

Figure 1. Temperature differentials between sorghum (*Sorghum bicolor*) silage and the ambient, on each day of opening, for the following treatments: A, sorghum silage without additives (SS); B, sorghum silage enriched with the inoculant (SSI); C, sorghum silage enriched with urea (SSU); and D, sorghum silage enriched with urea and inoculant (SSUI).

the ensiled material, as observed from the original material (4.82 log CFU g^{-1}) up to the day 1 of opening (7.29 log CFU g^{-1}), exhibiting a quadratic effect ($p \leq 0.05$) (Table 3). LAB population decreased on day 3, in comparison with day 1, remaining constant until day 28, with a reduction only observed at day 56. These values indicate that the favorable conditions for the development of these bacteria (pH, SC, DM) were appropriate.

The LAB population growth and activity are intense, when ideal conditions are present, such as temperature, substrate, anaerobiosis, and moisture. With all these characteristics, as observed in the present study, most of the fermentation processes should be completed within a period from 7 to 10 days after ensiling (Feng & Wang, 2020).

In the present study, the additives showed no effect on the LAB population, due to the ideal conditions for growth and action on the ensiled mass. The lack of effect can be attributed to the high content of soluble carbohydrates present in the original material (116.13 g kg^{-1}), which contributed to a high LAB development, with the population remaining high (5.95 log CFU g^{-1}) until day 28.

The use of homofermentative or facultative LAB inoculation in sorghum silages, especially forage and sweet sorghum, aims to increase the lactic acid fermentation (Arriola et al., 2021), to inhibit undesirable microorganisms, to lower pH, and to preserve the nutritional value of the ensiled material (Ogunade et al., 2016).

However, in the literature, results regarding the action of inoculants vary. A positive effect of inoculation was observed on the reduction of pH and lactic acid production in treated silages, in comparison with untreated ones (Filya et al., 2000). Nevertheless, Ogunade et al. (2016) reported that no effect of inoculation was verified in sweet sorghum silages, which was justified by the fact that sorghum has a high concentration of soluble carbohydrates, which theoretically contributed to a rapid reduction of pH in silage treatments with or without the use of additives.

There was also a negative effect of bacterial inoculation on the quality of forage sorghum. Kung Junior et al. (2018) observed some aerobic deterioration in silage, due to the inoculation with LAB, which was attributed to the inhibition of acetic acid bacteria responsible for acetate production that has antifungal properties, and a high concentration of lactate, which serves as a substrate for yeast development.

In the case of enterobacteria, higher counts were also observed in the original material ($p \leq 0.05$). The population declined from the day of opening, showing some fluctuations as the fermentation profile continued, but with values lower than those of the original material. The yeast population in the original material (3.18 log CFU g^{-1}) was higher than that of other opening days of the forage sorghum silage, with or without additives ($p \leq 0.05$), which exhibited a reduction, in comparison with the original material.

Enterobacteria are responsible for aerobic deterioration of silage because they compete with LAB for available substrates (sugars), producing acetic and succinic acids. They can also degrade proteins, reducing the nutritional value and palatability of the silage (Weiss et al., 2016).

The treatments with additives did not differ from the control material for population of enterobacteria. The reduction of their population as the days of opening progressed is a result of the sharp pH drop of the ensiled material, as their development was inhibited under conditions of pH lower than 4.5 (Xu et al., 2017).

Table 3. Means and results of Tukey's test of means[(1)] of cell counting (log CFU g^{-1}) of lactic acid bacteria (LAB), enterobacteria, and yeasts in PDA medium spread with diluted extracts of sorghum (*Sorghum bicolor*) silage of samples collected at each day of opening.

Variables	Day of opening						
	0	1	3	7	14	28	56
LAB[(2)]	4.82c	7.29a	6.56b	6.48b	6.21b	5.95b	3.35d
Enterobacteria	5.68a	4.36b	0.26e	1.35d	2.26c	2.30c	0.66de
Yeasts	3.18a	0.54b	0.83b	0.89b	0.93b	0.70b	0.76b

[(1)]Means followed by different letters in the same row differ statistically ($p \leq 0.05$). [(2)]Regression equation of LAB = $3.5314 + 1.961x - 0.2783x^2$, where x is the day of opening.

Similarly, Gonçalves et al. (2014) pointed out that this reduction of pH also influences the activity of other harmful microorganisms, including clostridia and fungi.

Gandra et al. (2017) reported that the addition of urea restricts the growth of this group of microorganisms, stimulating the production of other acids (acetic and propionic) produced by heterofermentative LAB.

Yeast is an undesirable microorganism responsible for initial aerobic deterioration in silage. Yeasts consume soluble carbohydrates and organic acids, converting them into ethanol and CO_2 that volatilize, resulting in DM losses (Driehuis et al., 2001). This process raises the temperature and pH, creating favorable conditions for the development of fungi, clostridia, and enterobacteria.

In a data compilation on additives in sorghum silage, Oliveira et al. (2017) found that inoculated silages had a higher yeast population than untreated silages, a result that was not observed in the present study. Santos et al. (2018) evaluated the addition of urea in sorghum silages and observed a reduction of the yeast population, in comparison with the control treatment. This reduction can be attributed to the toxic effect of ammonia released during the hydrolysis of urea on yeasts and fungi (Neumann et al., 2010). In the present study, there was no influence of additives on the yeast population. However, the rapid growth and action of LAB converting sugars into lactate, consequently reducing pH, inhibited the development and activity of yeasts, indicating a proper fermentation process.

Regarding the population of clostridia, differences were observed between treatments and opening days, with an interaction between treatment and day ($p \leq 0.05$). Clostridia populations were not influenced by additives within the first seven days after ensiling (Table 4). However, on day 14, treatments with urea inclusion reduced the clostridia population ($p \leq 0.05$) and, after day 28, the silages treated with additives reduced the population, in comparison with silage without additives.

Concerning the opening period, the development of the clostridia population after ensiling, in the treatment without additives, remained elevated up to day 14, decreasing on day 28, and then increasing again on day 56. For the silage treated with bacterial inoculant (SSI), the highest clostridia population counts were observed on day 1, day 7, and day 14, with a reduction on day 28, and an increase again on day 56. As for the silages treated with urea (SSU) and urea combined with the inoculant (SSUI), the highest development occurred on day 1, subsequently decreasing with the progression of days and fermentation.

The presence of clostridia on all days of opening, throughout the fermentation process, indicates the occurrence of undesirable fermentations, possibly due to the low initial DM content, which is one of the ideal parameters for the development of these microorganisms (McDonald et al., 1991), even under low pH conditions as observed in the present study.

Clostridia are Gram-positive bacteria that primarily develop in environments modified by the presence of fungi and yeasts, leading to a reduction of lactic acid, amino acids, and an increase of pH. Forage has low concentrations of clostridia, but their high presence in the ensiled material is mainly due to the high population in the soil (Auerbach & Nadeau, 2020).

Additives can have different effects on the ensiled material. As observed in the present study, the population of clostridia in silages with the addition of

Table 4. Mean and results of Tukey's test of means[(1)] of colony counting of *Clostridium* (log CFU g^{-1}) in samples of sorghum (*Sorghum bicolor*) silage collected at each day of opening for each treatment.

Treatment[(2)]	Days of opening						
	0	1	3	7	14	28	56
SS	5.93c	6.81ab	6.64ab	6.65ab	7.07Aa	6.38Abc	6.68Aab
SSI	5.82c	7.38a	6.67b	6.94ab	7.15Aab	5.49Bc	5.74Bc
SSU	5.89cd	7.41a	6.52b	6.39b	6.26Bbc	5.68Bd	5.74Bd
SSUI	5.79cd	7.38a	6.52b	6.44b	6.38Bbc	5.37Bd	5.87Bcd

[(1)]Means followed by same letters do not differ statistically ($p>0.05$) from each other, upper case in the column and lowercase in the row. [(2)]SS, sorghum silage without additives; SSI, sorghum silage enriched with the inoculant; SSU, sorghum silage enriched with urea; and SSUI, sorghum silage enriched with urea and inoculant.

urea and bacterial inoculant decreased, in comparison with the control treatment on day 14 and day 28, respectively. The decrease of the clostridial population contributes to the reduction of butyric, ethyl, and acetic fermentations caused by these microorganisms, along with fungi and yeasts (Rezende et al., 2011).

Seven genera of fungi (*Trichoderma, Fusarium*, *Cladosporium*, *Penicillium*, *Aspergillus*, *Phoma*, and *Pithomyces*) were identified in the forage sorghum silage treated with additives throughout the fermentation profile (Table 5). In the original material, all treatments exhibited a high population of the mentioned fungi, except for treatments SSI

Table 5. Counting of seven species of fungal colonies (log CFU g^{-1}), and the sum of all species counted in samples of sorghum (*Sorghum bicolor*) silage collected on each day of opening, for each treatment.

Treatment[1]	Species of fungi[2]							Sum
	Trich	Fusar	Clado	Penic	Asper	Phoma	Pitho	
				Day 0				
SS	2.75	2.08	2.84	1.16	1.10	1.46	2.93	14.32
SSI	2.37	2.31	2.57	1.55	1.21	0.00	1.93	11.94
SSU	1.51	2.86	2.64	1.58	1.78	0.90	2.25	13.52
SSUI	2.06	2.81	2.77	0.87	2.29	0.00	0.91	11.71
				Day 1				
SS	0.44	2.57	1.45	0.25	1.85	0.50	0.00	7.06
SSI	0.00	1.41	0.90	0.96	2.62	0.00	0.25	6.14
SSU	0.50	1.13	0.75	0.95	1.60	0.00	0.50	5.43
SSUI	0.00	0.50	0.75	0.62	1.75	0.00	1.00	4.62
				Day 3				
SS	0.00	1.50	1.60	0.63	2.27	0.00	0.75	6.75
SSI	0.25	2.50	0.75	0.65	1.97	0.00	0.00	6.12
SSU	1.19	2.36	1.13	0.81	2.71	0.75	0.50	9.45
SSUI	0.33	2.58	2.00	1.31	2.38	0.00	0.00	8.60
				Day 7				
SS	0.00	0.00	0.00	0.46	0.85	0.00	0.00	1.31
SSI	1.35	1.70	2.04	1.00	1.92	0.00	0.00	8.01
SSU	0.00	1.70	1.95	1.49	1.46	1.50	1.33	9.43
SSUI	0.00	2.00	0.25	0.00	1.27	0.00	0.00	3.52
				Day 14				
SS	0.00	0.97	0.00	0.50	1.25	0.00	0.50	3.22
SSI	0.00	0.50	1.25	1.12	1.37	0.00	1.25	5.49
SSU	0.00	1.33	1.62	0.00	2.37	0.00	1.75	7.07
SSUI	0.00	1.12	0.75	0.63	1.08	0.00	1.58	5.16
				Day 28				
SS	0.00	0.32	0.65	0.75	1.33	0.00	1.58	4.63
SSI	0.75	1.25	0.25	0.00	0.00	0.00	0.50	2.75
SSU	0.00	2.07	0.62	1.58	1.69	0.00	1.60	7.56
SSUI	0.00	0.50	0.25	0.00	1.38	0.00	1.58	3.71
				Day 56				
SS	1.82	2.46	0.75	0.00	1.67	0.00	2.07	8.77
SSI	0.25	0.54	0.00	0.00	0.87	0.00	1.74	3.40
SSU	0.50	0.83	0.50	1.28	1.91	0.00	0.25	5.27
SSUI	0.00	1.25	0.00	0.00	0.33	0.00	0.75	2.33

[1]Treatment: SS, sorghum silage without additives; SSI, sorghum silage enriched with inoculant; SSU, sorghum silage enriched with urea; and SSUI, sorghum silage enriched with urea and inoculant. [2]Tricho, *Trichoderma* spp.; Fusar, *Fusarium* spp.; Clado, *Cladosporium* spp.; Penic, *Penicillium* spp.; Asper, *Aspergillus* spp.; Phoma, *Phoma* spp.; Pitho, *Pithomyces* spp.

and SSUI that included bacterial inoculant, where the presence of the *Phoma* genus was not determined. This absence of the *Phoma* genus continued until the end of the opening days in the silages treated with the inoculant.

From the day 1 opening, a reduction of the population of fungi, especially those of the *Trichoderma* and *Phoma* genera, was observed in the silage, with *Phoma* still present up to day 14. Fungi frequently detected throughout all opening days were mainly *Fusarium*, *Cladosporium*, *Aspergillus*, and *Pithomyces*. Over the fermentation profile, the addition of additives to the silage led to the disappearance of certain fungal populations. This fact was observed on day 7, day 14, and day 28, when silages with urea inhibited the presence of *Trichoderma*, while on day 28, the bacterial inoculant inhibited the *Penicillium* genus, and on day 56, both *Penicillium* and *Cladosporium* genera were inhibited.

Fungi are also involved in undesirable reactions in silage, as they consume sugars, like lactic acid production, and they cause changes in the fiber composition of the forage. According to Schocken-Iturrino et al. (2005), the genera *Aspergillus*, *Fusarium*, and *Penicillium* are the most common genera in forage silages, as observed in the present study, with a higher population of these genera, along with *Phitomyces* and *Cladosporium* which appeared more frequently.

Araki et al. (2017) evaluated the addition of bacterial inoculant and chemical additives (CaO, NaCl, and urea), either individually or in combination, in forage sorghum silage, and found that the additives, whether alone or in combination, reduced the population of fungi, in comparison with the control treatment. They justified the results by the antifungal compounds produced by some strains of heterofermentative LAB and the action of chemical additives on the silage, creating an anaerobic environment suitable for conservation and unsuitable for the development of such microorganisms (Gandra et al., 2017).

Conclusions

1. The use of additives is unnecessary, considering the original characteristics of the forage of sorghum (*Sorghum bicolor*).

2. The silage at day 28 is suitable for feeding animals because of the stabilization of the fermentation process.

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References

ARAKI, H.M.C.; OLIVEIRA, E.R. de; GANDRA, J.R.; GOES, R.H.T.B. de; TAKIYA, C.S.; JACAÚNA, A.G.; OLIVEIRA, K.M.P. de; VASQUES, D.N.; CÔNSOLO, N.R.B.; DEL VALLE, T.A.; ORBACH, N.D. Association of biological and chemical additives on nutrient composition, total losses, microbiological and fermentative profile of sugarcane silage. **Iranian Journal of Applied Animal Science**, v.7, p.577-584, 2017.

ARAÚJO, M.L.G.M.L.; SANTOS, E.M.; CARVALHO, G.G.P. de; PINA, D. dos S.; OLIVEIRA, J.S. de; TOSTO, M.S.L.; SILVA, D.S.; PERAZZO, A.F.; PEREIRA, D.M.; NASCIMENTO, T.V.C.; FERREIRA, D. de J.; CAVALCANTI, H.S.; SANTOS, F.N. de S.; RIBEIRO, M.D.; ZANINE, A. de M. Ensiling sorghum with urea, aerobic exposure and effects on intake, digestibility, ingestive behaviour and blood parameters of feedlot lambs. **Animals**, v.13, art.2005, 2023. DOI: https://doi.org/ 10.3390/ani13122005.

ARRIOLA, K.G.; OLIVEIRA, A.S.; JIANG, Y.; KIM, D.; SILVA, H.M.; KIM, S.C.; AMARO, F.X.; OGUNADE, I.M.; SULTANA, H.; PECH CERVANTES, A.A.; FERRARETTO, L.F.; VYAS, D.; ADESOGAN, A.T. Meta-analysis of effects of inoculation with *Lactobacillus buchneri*, with or without other bacteria, on silage fermentation, aerobic stability, and performance of dairy cows. **Journal of Dairy Science**, v.104, p.7653-7670, 2021. DOI: https://doi.org/10.3168/jds.2020-19647.

AUERBACH, H.; NADEAU, E. Effects of additive type on fermentation and aerobic stability and its interaction with air exposure on silage nutritive value. **Agronomy**, v.10, art.1229, 2020. DOI: https://doi.org/10.3390/agronomy10091229.

BALIEIRO NETO, G.; SIQUEIRA, G.R.; REIS, R.A.; NOGUEIRA, J.R.; ROTH, M. de T.P.; ROTH, A.P. de T.P. Óxido de cálcio como aditivo na ensilagem de cana-de-açúcar. **Revista Brasileira de Zootecnia**, v.36, p.1231-1239, 2007. DOI: https://doi.org/10.1590/S1516-35982007000600003.

BORBA, L.F.P.; FERREIRA, M. de A.; GUIM, A.; TABOSA, J.N.; GOMES, L.H. dos S.; SANTOS, V.L.F. dos. Nutritive value of different silage sorghum (*Sorghum bicolor* L. Moench) cultivars. **Acta Scientiarum: Animal Science**, v.34, p.123-129, 2012.

BORREANI, G.; TABACCO, E.; SCHMIDT, R.J.; HOLMES, J.B.; MUCK, R.E. Silage review: factors affecting dry matter and quality losses in silages. **Journal of Dairy Science**, v.101, p.3952-3979, 2018. DOI: https://doi.org/10.3168/jds.2017-13837.

CATTANI, M.; GUZZO, N.; MANTOVANI, R.; BAILONI, L. Effects of total replacement of corn silage with sorghum silage on milk yield, composition, and quality. **Journal of Animal Science and Biotechnology**, v.8, art.15, 2017. DOI: https://doi.org/10.1186/s40104-017-0146-8.

DRIEHUIS, F.; OUDE ELFERINK, S.J.W.H.; VAN WIKSELAAR, P.G. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. **Grass and Forage Science**, v.56, p.330-343, 2001. DOI: https://doi.org/10.1046/j.1365-2494.2001.00282.x.

FAZAELI, H.; GOLMOHHAMMADI, H.A.; AL-MODDARES, A.; MOSHARRAF, S.; SHOAEI, A.A. Comparing the performance of sorghum silage with maize silage in feedlot calves. **Pakistan Journal of Biological Sciences**, v.9, p.2450-2455, 2008. DOI: https://doi.org/10.3923/pjbs.2006.2450.2455.

FENG, T.; WANG, J. Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. **Gut Microbes**, v.12, e1801944, 2020. DOI: https://doi.org/10.1080/19490976.2020.1801944.

FILYA, I.; ASHBELL, G.; HEN, Y.; WEINBERG, Z.G. The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. **Animal Feed Science and Technology**, v.88, p.39-46, 2000. DOI: https://doi.org/10.1016/S0377-8401(00)00214-5.

FRANÇA, A.F. de S.; OLIVEIRA, R. de P.; MIYAGI, E.S.; SILVA, A.G. da; PERÓN, H.J.M.C.; BASTO, D. de C. Características fermentativas da silagem de híbridos de sorgo sob doses de nitrogênio. **Ciência Animal Brasileira**, v.12, p.383-391, 2011. DOI: https://doi.org/10.5216/cab.v12i3.540.

GANDRA, J.R.; MIRANDA, G.A.; GOES, R.H.T.B.; TAKIYA, C.S.; DEL VALLE, T.A.; OLIVEIRA, E.R.; FREITAS JUNIOR, J.E.; GANDRA, E.R.S.; ARAKI, H.M.C.; SANTOS, A.L.A.V. Fibrolytic enzyme supplementation through ruminal bolus on eating behavior, nutrient digestibility and ruminal fermentation in Jersey heifers fed either corn silage- or sugarcane silage-based diets. **Animal Feed Science and Technology**, v.231, p.29-37, 2017. DOI: https://doi.org/10.1016/j.anifeedsci.2017.06.009.

GONÇALVES, J.A.G.; ZAMBOM, M.A.; FERNANDES, T.; MESQUITA, E.E.; SCHIMIDT, E.; JAVORSKI, C.R.; CASTAGNARA, D.D. Composição químico-bromatológica e perfil de fermentação da silagem de resíduo úmido de fécula de mandioca. **Bioscience Journal**, v.30, p.502-511, 2014.

JOBIM, C.C.; NUSSIO, L.G.; REIS, R.A.; SCHMIDT, P. Avanços metodológicos na avaliação da qualidade de forragem conservada. **Revista Brasileira de Zootecnia**, v.36, p.101-119, 2007. Suplemento especial. DOI: https://doi.org/10.1590/S1516-35982007001000013.

KUNG JUNIOR, L.; SHAVER, R.D.; GRANT, R.J.; SCHMIDT, R.J. Silage review: interpretation of chemical, microbial, and organoleptic components of silages. **Journal of Dairy Science**, v.101, p.4020-4033, 2018. DOI: https://doi.org/10.3168/jds.2017-13909.

MCDONALD, P.; HENDERSON, A.; HERON, S.J.E. **The biochemistry of the silage**. 2nd ed. Marlow: Chalcombe Publications, 1991. 340p.

MOHD-SETAPAR, S.H.; MOHAMAD-AZIZ, S.N.; HARUN, N.H.; MOHD-AZIZI, C.Y. Review on the extraction of biomolecules by biosurfactant reverse micelles. **APCBEE Procedia**, v.3, p.78-83, 2012. DOI: https://doi.org/10.1016/j.apcbee.2012.06.050.

MUCK, R.E.; NADEAU, E.M.G.; MCALLISTER, T.A.; CONTRERAS-GOVEA, F.E.; SANTOS, M.C.; KUNG JR, L. Silage review: recent advances and future uses of silage additives. **Journal of Dairy Science**, v.101, p.3980-4000, 2018. DOI: https://doi.org/10.3168/jds.2017-13839.

NEUMANN, M.; OLIBONI, R.; OLIVEIRA, M.R.; FARIA, M.V.; UENO, R.K.; REINERH, L.L.; DURMAN, T. Aditivos químicos utilizados em silagens. **Pesquisa Aplicada & Agrotecnologia**, v.3, p.187-195, 2010.

NUSSIO, L.G.; CAMPOS, F.P. de; LIMA, M.L.M. de. Metabolismo de carboidratos estruturais. In: BERCHIELLI, T.T.; PIRES, A.V.; OLIVEIRA, S.G. de. (Ed.). **Nutrição de ruminantes**. Jaboticabal: Funep, 2011. p.193-234.

OGUNADE, I.M.; KIM, D.H.; JIANG, Y.; WEINBERG, Z.G.; JEONG, K.C.; ADESOGAN, A.T. Control of *Escherichia coli* O157:H7 in contaminated alfalfa silage: effects of silage additives. **Journal of Dairy Science**, v.99, p.4427-4436, 2016. DOI: https://doi.org/10.3168/jds.2015-10766.

OLIVEIRA, A.S.; WEINBERG, Z.G.; OGUNADE, I.M.; CERVANTES, A.A.P.; ARRIOLA, K.G.; JIANG, Y.; KIM, D.; LI, X.; GONÇALVES, M.C.M.; VYAS, D.; ADESOGAN, A.T. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. **Journal of Dairy Science**, v.100, p.4587-4603, 2017. DOI: https://doi.org/10.3168/jds.2016-11815.

PERAZZO, A.F.; CARVALHO, G.G.P.; SANTOS, E.M.; BEZERRA, H.F.C.; SILVA, T.C.; PEREIRA, G.A.; RAMOS, R.C.S.; RODRIGUES, J.A.S. Agronomic evaluation of sorghum hybrids for silage production cultivated in semiarid conditions. **Frontiers in Plant Science**, v.8, art.1088, 2017. DOI: https://doi.org/10.3389/fpls.2017.01088.

PUPO, N.I.H. **Manual de pastagens e forrageiras**: formação, conservação, utilização. Campinas: Instituto Campineiro de Ensino Agrícola, 2002. p.274-303.

REZENDE, A.V. de; RABELO, C.H.S.; RABELO, F.H.S.; NOGUEIRA, D.A.; FARIA JUNIOR, D.C.N.A. de; BARBOSA, L. de A. Perdas fermentativas e estabilidade aeróbia de silagens de cana-de-açúcar tratadas com cal virgem e cloreto de sódio. **Revista Brasileira de Zootecnia**, v.40, p.739-746, 2011. DOI: https://doi.org/10.1590/S1516-35982011000400006.

RIBEIRO, C.G.M.; GONÇALVES, L.C.; RODRIGUES, J.A.S.; RODRIGUEZ, N.M.; BORGES, I.; BORGES, A.L.C.C.; SALIBA, E.O.S.; CASTRO, G.H.F.; RIBEIRO JUNIOR, G.O. Fermentation pattern of silages from five sorghum genotypes. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.59, p.1531-1537, 2007. DOI: https://doi.org/10.1590/S0102-09352007000600028.

SANTOS, A.P.M. dos; SANTOS, E.M.; OLIVEIRA, J.S. de; RIBEIRO, O.L.; PERAZZO, A.F.; PINHO, R.M.A.; MACÊDO, A.J. da S.; PEREIRA, G.A. Effects of urea addition on the fermentation of sorghum (*Sorghum bicolor*) silage. **African**

Journal of Range & Forage Science, v.35, p.55-62, 2018. DOI: https://doi.org/10.2989/10220119.2018.1458751.

SCHMIDT, P.; MARI, L.J.; NUSSIO, L.G.; PEDROSO, A. de F.; PAZIANI, S. de F.; WECHSLER, F.S. Aditivos químicos e biológicos na ensilagem de cana-de-açúcar. 1. Composição química das silagens, ingestão, digestibilidade e comportamento ingestivo. **Revista Brasileira de Zootecnia**, v.36, p.1666-1675, 2007. Supl. DOI: https://doi.org/10.1590/S1516-35982007000700027.

SCHOCKEN-ITURRINO, R.P.; REIS, R.A.; COAN, R.M.; BERNARDES, T.F.; PANIZZI, R. de C.; POIATTI, M.L.; PEDREIRA, M. dos S. Alterações químicas e microbiológicas nas silagens de Capim-Tifton 85 após a abertura dos silos. **Revista Brasileira de Zootecnia**, v.34, p.464-471, 2005. DOI: https://doi.org/10.1590/S1516-35982005000200013.

SIQUEIRA, G.R.; REIS, R.A.; SCHOCKEN-ITURRINO, R.P.; BERNARDES, T.F.; PIRES, A.J.V.; ROTH, M. de T.P.; ROTH, A.P. de T.P. Associação entre aditivos químicos e bacterianos na ensilagem de cana-de-açúcar. **Revista Brasileira de Zootecnia**, v.36, p.789-798, 2007. DOI: https://doi.org/10.1590/S1516-35982007000400006.

WEISS, K.; KROSCHEWSKI, B.; AUERBACH, H. Effect of air exposure, temperature and additives on fermentation characteristics, yeasts count, aerobic stability and volatile organic compounds in corn silage. **Journal of Dairy Science**, v.99, p.8053-8069, 2016. DOI: https://doi.org/10.3168/jds.2015-10323.

XU, Z.; HE, H.; ZHANG, S.; KONG, J. Effects of inoculants Lactobacillus brevis and *Lactobacillus parafarraginis* on the fermentation characteristics and microbial communities of corn stover silage. **Scientific Reports**, v.7, art.13614, 2017. DOI: https://doi.org/10.1038/s41598-017-14052-1.

ZHOU, Y.; DROUIN, P.; LAFRENIÈRE, C. Effect of temperature (5-25°C) on epiphytic lactic acid bacteria populations and fermentation of whole-plant corn silage. **Journal of Applied Microbiology**, v.121, p.657-671, 2016. DOI: https://doi.org/10.1111/jam.13198.