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EFFECTS OF CHEMICAL ADDITIVES, STORAGE TIME AND PACKING DENSITY ON THE FERMENTATION QUALITY AND AEROBIC STABILITY OF CORN, SUGARCANE AND GRASS-LEGUME MIXED SILAGES

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Thesis presented to the Universidade Federal Rural da Amazônia as part of the requirements of the Graduate Program on Animal and Health Production in the Amazon, to obtain the title of Doctor Scientiae.
Area of concentration: Animal Production,
Advisor: Prof. Cristian Faturi
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ABSTRACT

Silage is a critical component in the diet of lactating dairy cows, with corn silage being a primary ingredient globally. Despite its favorable characteristics for fermentation, corn silage is prone to aerobic deterioration upon silo opening, unlike sugarcane and grass silages. Similarly, mixed forage silages containing legumes and grasses face challenges due to their higher buffering capacity, which can hinder fermentation and promote the growth of undesirable microorganisms. The use of chemical and microbial additives during the ensiling process has been explored to mitigate these issues, improving fermentation profiles, aerobic stability, and nutrient retention. This study evaluated the effects of Lactobacillus buchneri (4×10^5 cfu.g^{-1}), SAFE (applied at 2, 3, and 4 L.kg^{-1} fresh matter), and sodium-based chemical additives on the fermentative profile, microbiology, aerobic stability, and chemical composition of silages. Additionally, the impact of packing density was assessed in mixed forage silages. Silages were stored for varying durations (3, 10, 21, and 90 days) and analyzed for fermentation parameters, microbial counts, and aerobic stability. SAFE reduced yeast and mold growth and ethanol production during short-term storage, while Buchneri silages exhibited superior yeast inhibition during longer storage periods. In the mixed forage experiment, silages were ensiled with varying packing densities (215, 161, and 108 kg DM/m^3) and treated with a sodium-based additive containing sulfites and fungal amylase. The additive improved fermentation by reducing total acids, lactic acid, acetic acid, and ethanol concentrations compared to untreated silages, though it did not influence yeast or mold counts. Packing density had a significant effect, with poorly compacted silages (108 kg DM/m^3) exhibiting higher pH and microbial counts. These findings highlight the potential of microbial and chemical additives, alongside optimized packing density, to enhance silage quality by modulating fermentation, improving aerobic stability, and minimizing spoilage losses.

KEYWORDS: forage; fermentation; nutritional value

ABBREVIATIONS LIST

ADT: Additive

DML: Dry Matter Losses.

DM: Dry Matter.

ST: Storage time.

WSC: Water soluble carbohydrates.

LAB: Lactic Acid Bacteria.

CP: Crude protein.

AS: Aerobic Stability.

NDF: Neutral detergente fiber.

Summary

1. CONTEXTUALIZAÇÃO

Crops have a certain difference in growth rates, which increase sharply in the rainy season, and decrease considerably during the dry period of the year, making forage production seasonal (Brunetti et al., 2020). Due to this, animal production systems, which are based on the use of pastures, face irregularity in the supply and quality of forage throughout the year.

Thus, the adoption of practices that conserve forage with quality and maintain its nutritional value, such as silage production, is an excellent alternative to provide food throughout the shortage period. Among the favorable ideas for silage production, the following stand out: supply of quality forage for high-producing animals; elimination of daily forage harvesting; crop harvesting done in a uniform manner; and improvement in post-harvest agronomic management.

Several factors can cause losses in ensiling process, including poor compaction of the material, harvesting the forage mass outside the ideal point, as well as inadequate sealing. These factors directly influence fermentation profile and may provide the development of microorganisms and undesirable products. To be efficient and obtain a promising result in silage is necessary to understand the main factors that are involved in fermentation, as well as their influence on the main losses of this process, which directly interfere with the quality of silage (Borreani et al., 2018)

In Brazil, it is not yet possible to estimate the percentage of properties that provide silage to their herds. However, Bernardes and Rêgo (2014) when evaluating dairy farms in Brazil, observed that around 80% of farmers use corn, exclusively or in combination with other crops, for silage production. Next were sorghum, tropical grasses, and sugarcane. Of all the crops used, only 27% of the farmers use additives.

Among the most commonly used silages in Brazil, each forage type exhibits distinct characteristics that directly affect the fermentation pattern of the silage. Additionally, factors inherent to the ensiling process, such as compaction density and storage duration, can significantly impact the effectiveness of additives. Consequently, there has been a growing interest in studying various additives to enhance the fermentative properties of the silage.

Among the various additives tested in silages, many have shown promising results in enhancing fermentative characteristics and improving aerobic stability. However, studies providing consistent evidence of their effectiveness remain limited. Therefore, this study aimed to evaluate the effects of chemical additives, storage duration, and packing density on the fermentation quality and aerobic stability of corn, sugarcane, and grass-legume mixed silages.

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2. CHEMICAL ADDITIVE AND STORAGE TIME CHANGES THE FERMENTATION QUALITY AND AEROBIC STABILITY OF SUGARCANE SILAGES.

ABSTRACT

Sugarcane silages are naturally prone to dry matter (DM) losses due to intense alcoholic fermentation. The use of chemical additives can be efficient in reducing these losses, especially when compared with microbial inoculants that use forage substrates to produce organic acids and then decrease losses. The objective of this study was to determine the effect of microbial and a chemical additive (SAFE; 200 g/kg of sodium benzoate, 100 g/kg of potassium sorbate, and 50 g/kg of sodium nitrite) on the fermentation and aerobic stability of sugarcane silage. A completely randomized block design, in a 6 × 4 factorial arrangement, with five replications was used. The studied factors were additive (ADT) [No additive; Buchneri; CaO; SAFE 2; SAFE 3 and SAFE 4]; and different storage times (ST) [3, 10, 21 and 90 days). The application rates were: *Lactobacillus Buchneri* 4 x 105 cfu.g^{-1}, Calcium oxide (virgin lime) – 10 g.kg^{-1} , SAFE at 2 L.kg^{-1}, 3 L.kg^{-1} and 4 L.kg^{-1} of the fresh matter (FM) of the ensiled mass, and the storage time of silages (3, 10, 21 or 90 days) on the fermentative profile, microbiology, chemical composition and aerobic stability in sugarcane silages. Sugarcane was ensiled in bags for 3 and 10 days of ST and in 20L plastic buckets for 21 and 90 days of ST. The average packing density in the buckets was 550 ± 20 kg /m³ (fresh basis). Fermentative profile, chemical composition and aerobic stability were determined. Storage time and the use of SAFE additive reduced ($P \leq 0{,}05$) LAB counts ($P \leq 0{,}05$) of sugarcane silages. Yeasts' counts decreased with the application of SAFE2, SAFE3, and SAFE4 at 21d of ST, while only SAFE4 was able to decrease yeasts' counts at 90d of ST ($P \leq 0{,}05$). The application of SAFE 4 consistently preserved WSC content, reducing WSC loss regardless of ST ($P \leq 0{,}05$). Silages stored for 21d had lower ($P \leq 0{,}05$) molds count when compared with silages stored for 90d. The use of CaO and SAFE increased aerobic stability. The use of SAFE3 and SAFE4 preserved the WSC even after 7d of aerobic exposure. Additives CaOS AFE at 4 L. Kg^{-1} of fresh matter are effective in inhibiting yeasts during fermentation, reducing DM and WSC losses, and increasing aerobic stability in sugarcane silages.

KEYWORDS: water soluble carbohydrates loss, ethanol, *Lactobacillus Buchneri*, Saccharum officinarum,

1.1.Introduction

Sugarcane (*Saccharum* spp.) in natura is often used as feed for ruminants, especially in periods of shortage of pasture when it is harvested daily and fed to animals (Bernardes et al., 2017). This forage is widely used in animal diets because of its high production of dry matter (25 to 40 t of DM/ha) and high energy concentration, coming from the high concentration of sugars, especially sucrose (250 to 300 g/kg; Avila et al., 2014), and in addition, it has adequate nutritive value at maturity (Daniel et al., 2017). Sugarcane can also be used as silage, and it is a strategy adopted to avoid future lodging, reduce losses by fire and daily harvest (Pedroso et al., 2008). In Brazil, according to Bernardes and Rêgo (2014), in a study evaluating the practice and use of silage in 260 dairy farms, sugarcane was the fourth most cultivated crop for silage. The other most cited species, following the order of use, were corn, sorghum, and tropical grasses. In beef cattle feedlots, sugarcane silage is an effective source of volume because a low inclusion in the diet is sufficient to meet the physically effective fiber requirements (Bernardes et al., 2017).

Although sugarcane silage is widely provided as a feed for ruminants, there is a serious limitation relates the DM losses when ensiling the crop, due to yeast growth that use part of the soluble sugars transforming into high levels of volatile organic compounds, mainly ethanol (Daniel et al., 2013; Pedroso et al., 2005). And consequently, the aerobic stability of sugarcane silages is reduced due to their high concentration of residual soluble sugars and large yeast population.

In this context, the use of additives in sugarcane ensiling is mandatory, but its effects will depend on the additive type. The application of *Lactobacillus buchneri* was able to decrease yeasts population after 30 days of storage, because of the increase of acetic acid concentration, according to Avila et al. (2009). Thus, in shorter storage periods the production of acetic acid by *Lactobacillus buchneri* would be insufficient to improve aerobic stability. For silage supply with shorter fermentation time, the use of chemical additives may be an alternative to improve stability because it is independent of the growth of microorganisms to produce short-chain organic acids that have antifungal characteristics (Kung et al., 2018). However, there is a lack of consistent effects with chemical additives on the fermentation and aerobic stability at the same time of sugarcane silages.

Based on this, we hypothesized that the chemical additive SAFESIL (SAFE) composed of sodium benzoate and potassium sorbate will improve the fermentative characteristics of sugarcane silage by inhibiting alcoholic fermentations, and consequently increase aerobic stability, even at shorter silage storage times. Thus, we aimed to evaluate the effects of the chemical additive Safesil (Safe) on the microbial population, fermentative profile, losses, and chemical composition of corn silage. And compare the effects of adding the SAFE additive with other widely used biological additives on microbial population, fermentative profile, losses, and chemical composition of silages.

1.2.Material e methods

Experimental design and ensiling conditions

A completely randomized block design, in a 6 × 4 factorial arrangement, with five replications was used. The studied factors were additive (ADT) [No additive; Buchneri; CaO; SAFE 2; SAFE 3 and SAFE 4]; and different storage times (ST) [3, 10, 21 and 90 days). Blocks were defined as experimental silos ensiling order. The treatments with chemical and microbiological additives used were defined by:

1- Control (without additive);

2- *Lactobacillus buchneri* (4 x 105 cfu. g^{-1} fresh matter (FM);

3- Calcium oxide (virgin lime) – 10 g.kg $^{-1}$ FM.

4- SAFE 2 – 2 L.ton 1 of FM.

5- SAFE 3 – 3 L.ton 1 of FM.

6- SAFE 4 – 4 L.ton 1 of FM.

The Safesil procuct used was Safesil Ab Hanson & Mohring, Halmstad, Sweden, containing 200 g/kg of sodium benzoate, 100 g/kg of potassium sorbate, and 50 g/kg of sodium nitrite. Sugarcane with approximately 12 months of growth was harvested from field 1 (EXP 1) the Research and Extension Unit in Dairy Cattle of Federal University of Viçosa (UFV). After approximately 12 months of the first harvest (EXP 2), sugarcane was harvested from Department of Animal Science of Federal University of Vicosa (UFV). Year 1 and Year 2 were defined as EXP1 and EXP 2, and all steps were replicated in both experiments. Harvested sugarcane was chopped at a theoretical cut length of 2 cm with a pull type forage harvester.

Ensiling was conducted by repetition and the treatments were applied to the pile of forage made for each experimental unit. *Lactobacillus buchneri* and SAFE treatments were applied to sugarcane at 10 mL/kg of fresh forage, v./w. (according to manufacturer recommendation) and were used variable concentrations according to the treatments. After homogenization, 500g of forage was ensiled in 25.4 cm × 35.56 cm bags (Doug Care Equipment, Springville, CA) for 3 and 10 days of ST or in 20L plastic buckets for 21 and 90 days of ST. The anaerobic condition inside the bag silos was established using a vacuum sealer and buckets were packing to achieve 550 kg/m^3 NM of density. The silos were stored in a covered barn.

The bags were opened at 3 and 10 days of storage, and the buckets were opened at 21 and 90 days of storage. The silage from each silo was mixed and samples were collected before ensiling (Table 1), at silo opening (ST), and after aerobic exposure (7d) for evaluations and laboratory analysis.

Evaluations and analysis

Silage microbiology and fermentation

The numbers of lactic acid bacteria (LAB), yeasts and molds were analyzed in all collected samples. The aqueous extract (1:10) of the sample (25 g) was prepared with the addition of sterile peptone water 0,1% (w./v.), and then homogenized for 3 min in a sterile bag. The microbial counts were determined by pour plating 10-fold serial dilutions (five per sample) of the extract on MRS agar with nystatin (4 ml L 1) to determine LAB and potato dextrose agar (Sigma Aldrich Brasil LTDA), with 10% (v./v.) tartaric acid (15 ml L 1) to determine yeast and molds. After incubation at 35 ºC for 48h (LAB) and at 28 °C for 72h (yeasts) and 120h (molds). The colonies of yeasts and molds were counted for separately, yeasts were distinguished from molds by colony appearance and cell morphology. The pH of each sample was determined using a digital potentiometer (T 1000®, Tekna, Sao Bernardo do Campo, Brazil), in the same water extract. A portion of 2 mL aqueous extract (2 mL) was acidified with 10 μL of 50% (v.v.) H2SO4 and frozen. Aqueous extracts were analyzed to Organic acids (lactic, acetic, propionic, and butyric acids), ethanol, and 1,2 propanediol were analyzed by HPLC (Canale et al., 1984). Water soluble carbohydrates (WSC) were analyzed using the phenol method (Dubois et al., 1956).

Dry matter loss and Aerobic Stability

Silos were weighed prior to storage to determine dry matter loss (DML). The calculation of DML were as the difference between the weight of the material placed in each silo at ensiling and the weight of the silage after the designated length of storage divided by the weight of material at ensiling, corrected for the respective initial or final DM concentration (Jobim et al., 2007). Buckets silos (21 and 90d of ST) (n=60) containing approximately 5kg of remaining silage that had been mixed and loosely replaced in buckets after sample collections were used for determination of aerobic stability.

The buckets were kept in an air-conditioned room at 21 ± 1.6 °C. A data logger was inserted in the geometric center of each forage mass to monitor the temperature every fifteen minutes. After 7 days (168 hours) the data loggers were removed, and the data transferred to a computer to determine the aerobic stability. Aerobic stability was defined as the time required for the silage temperature to exceed 2°C at room temperature (Ranjit and Kung, 2002). After the aerobic stability test, samples were taken for analysis of chemical composition, organic acids, pH and microbiologic population of sugarcane silages after 7 days.

Chemical composition

Fresh and silage samples were dried in a forced air oven set at 55℃ for 72h and grounded to pass through a 1 mm sieve using a Wiley Mill (Thomas Scientific, Swedesboro, NJ), to determine DM (method 950.15, AOAC, 2000 [14]), ash (method 942.05, AOAC, 2000 [14]), crude protein using the Kjedahl method (CP, N × 6.25; method 984.13, AOAC, 2000 [14]). The neutral detergent fiber (NDF) was obtained according to the methodology of Mertens [15] without the use of α amylase and sodium sulfite.

Statistical analysis

The data of fresh sugarcane treatments did not undergo statistical analysis. The microorganism count data were transformed to log 10 to be submitted to statistical analysis. Data were analyzed as a 4 × 6 factorial arrangement with repeated measures over time (3; 10; 21; and 90d of ST) by the SAS® program PROC MIXED procedure observing the effects of additive (ADT), Storage time (ST) and their interaction (ADT × ST). The averages were compared using the Tukey test at 5% probability level and the order of ensiling (block) as a random effect.

1.3. Results

Effects of additive and storage time on the fermentation profile and microbial counts of sugarcane silage are shown in Table 2. Silages In EXP2 treated with ADT buchneri had higher ($P \leq 0.05$) concentrations of lactic acid and ethanol. Also, silages treated with CaO had higher ($P \leq 0.05$) concentrations of lactic acid compared to silages treated with SAFE 4. Silages without additives also had higher ($P \leq 0.05$) concentration of ethanol. Regarding ST, EXP2 sugarcane silages on 3d of ST had lower ($P \leq 0.05$) count of molds and acid lactic concentration. Mold counts had lower counts ($P \leq 0.05$) on 10d as well and raised on 21 and 90d of ST. Ethanol had higher concentration ($P \leq 0.05$) on 10d of ST compared to 21 and 90d.

Effects of additive and storage time on the nutrient loss, aerobic stability and nutrient composition of sugarcane silages are shown in table 3. In EXP1, Sugarcane silages treated with CaO had higher ($P \leq 0.05$) contents of DM and ASH and had the lowest ($P \leq 0.05$) DML on EXP2. In relation to ST, In EXP1 silages stored for 3d had higher ($P \leq 0.05$) DM and lower ($P \leq 0.05$) ash contents compared to 10,21 e 90d of ST silages. In EXP 2 silages of 3 and 10d of ST had lower ($P \leq 0.05$) contents of ash.

Interaction of additive and storage times are shown in figures in sequence. Figures 1 to 5 belongs to experiment 1. Figure 1(a) shows that silages treated with ADT CaO had the highest ($P \leq 0.05$) pH regardless of ST, reaching above 8 on 3d of ST. Silages treated with Buchneri, SAFE 2 and SAFE3 had the lowest ($P \leq 0.05$) pH on 21d of ST. Regardless of ST, all additives, except CaO, showed pH lower than 4. Figure 1 (b) shows that on 3, 10 and 21d of ST, silages treated with CaO had the higher ($P \leq 0.05$) LAB counts, while on 90d of ST the higher ($P \leq 0.05$) count are shown in silages treated with CaO and Buchneri. Silages treated with SAFE 2, SAFE3 and SAFE 4 decreased ($P \leq 0.05$) LAB counts over time on ST.

Silages treated with SAFE 2 and SAFE 3 had the lowest ($P \leq 0.05$) counts of yeasts on 3,10 and 21d of ST (Figure 2 [a]). Silages stored for 3, 10 and 21d had the highest ($P \leq 0.05$) yeast count when treated with Buchneri or without additive. Silages stores for 90d showed the lowest ($P \leq 0.05$) count of yeast in silages control and treated with Buchneri and SAFE 4. Regarding molds counts (Figure 2 [b]) silages stored for 3, 10 and 21d had lower ($P \leq 0.05$) counts of molds unbiased of additive, however Control silages had higher ($P \leq 0.05$) counts compared to other additives on 10d of ST. At 90d of ST, the highest ($P \leq 0.05$) count of molds was in silages treated with CaO and lower ($P \leq 0.05$) in SAFE 2 and SAFE 3 silages.

At 3d of ST the higher ethanol concentration was in SAFE 4 silages (Figure 3 [a]). Silages stored for 10 had higher ($P \leq 0.05$) ethanol concentrations independent of additive. At 21d the lowest ethanol ($P \leq 0.05$) concentration was in silages treated with SAFE 3 and SAFE 4. Highest ($P \leq 0.05$) NDF concentrations was observed in Control and Buchneri silages stored for 3, 10 and 21d. At 90d of ST, silages control, treated with Buchneri and SAFE 2 had the highest ($P \leq 0.05$) NDF content.

At 3 days of storage, Control and Buchneri had the lower ($P \leq 0.05$) WSC content (Figure 4[a]). At 10 and 21d of ST, SAFE 2, SAFE3 and SAFE 4 were effective ($P \leq 0.05$) to preserve WSC content. At 90d of ST this effect was observed for SAFE 4 with the highest ($P \leq 0.05$) WSC content followed by SAFE 3 silages. Silages treated with

CaO had higher ($P \leq 0.05$) WSC content at 3d of storage than decreased ($P \leq 0.05$) the WSC content at 10,21 and 90d of ST. Silages treated with Control, Buchneri and CaO has the highest ($P \leq 0.05$) WSC loss regardless of ST (Figure 4[b]). When stored for 90d, silages treated with SAFE 2 had higher ($P \leq 0.05$) WSC loss than SAFE 3, and SAFE 3 had higher WSC than SAFE 4.

At 21d of storage silages treated with SAFE 3 and SAFE 4 had higher ($P \leq 0.05$) aerobic stability, followed by SAFE 2 (Figure 5[a]). At 90d of ST silages treated with SAFE 2, SAFE 3 and SAFE 4 had higher ($P \leq 0.05$) aerobic stability, followed by CaO silage. Control and Buchneri silages had lowest ($P \leq 0.05$) aerobic stability independent of ST. Control and Buchneri silages has higher DML regardless of ST. At 21d, silages treated with SAFE 3 and SAFE 4 had lower ($P \leq 0.05$) DML (Figure 5[b]), while at 90d the lower ($P \leq 0.05$) DML was observer in SAFE 4 silages.

Interaction of additive and storage times are shown in figures in sequence. Figures 6 to 9 belongs to experiment 2. Silages had pH below 4 regardless of ST (Figure 6 [a]), except for CaO silages that had the highest ($P \leq 0.05$) pH at 3d and decreased ($P \leq 0.05$) over the days of ST. The LAB counts were similar ($P > 0.05$) between additives at 3, 10 and 21d of ST (Figure 6 [b]). At 90d of storage silages treated with Control, Buchneri and CaO had higher ($P > 0.05$) LAB counts compared with SAFE 2 and SAFE 3.

Yeasts counts at 3 and 10d of ST was lower ($P > 0.05$) in silages treated with SAFE 2, SAFE 3 and SAFE 4 (Figure 7 [a]). At 21d the lower ($P > 0.05$) yeasts counts were observed in silages treated with SAFE 3 and SAFE 4. SAFE 4 had the higher ($P > 0.05$) count of yeasts at 90d when compared with other ST. At 3d of ST the DM content was lower ($P > 0.05$) in Control and Buchneri silages (Figure 7 [b]). At 10, 21 and 90d the lower ($P > 0.05$) DM content was in Control, Buchneri and SAFE 2 silages.

CP content has higher ($P > 0.05$) in Control and Buchneri silages at 3d of ST (Figure 7 [a]). At 10 and 21d of ST CP content was similar between additives. At 90d silages treated at SAFE 4 had the lower ($P > 0.05$) CP concentration. NDF content was higher ($P > 0.05$) in Control and Buchneri silages at 3d of ST (Figure 7 [b]). At 10, 21 and 90 the higher ($P > 0.05$) NDF content was in Control, Buchneri and SAFE 2 silages.

Silages treated with SAFE 2, SAFE 3 and SAFE 4 had higher ($P > 0.05$) aerobic stability at 21 d compared to other additives (Figure 8). At 90 the higher ($P > 0.05$) aerobic stability was observed in SAFE 4 silages. SAFE 4 silages reduced ($P > 0.05$) the aerobic stability between ST.

On table 4 are shown the effects of additive and storage time on chemical composition, fermentation profile and microbial counts of sugarcane silage after 7d of aerobic exposure on stability test. Silages treated with CaO had higher ($P \leq 0.05$) counts of molds and ash in EXP1, also had higher ($P \leq 0.05$) content of ash in EXP2. Silages treated with SAFE 4 showed the lowest ($P \leq 0.05$) count of molds and presented highest ($P \leq 0.05$) WSC content in EXP1. Considering ST, silages stored for 21d had higher ($P \leq 0.05$) counts of molds in EXP1 and lower ($P \leq 0.05$) ash content EXP2 compared with 90d. Interaction of additive and storage times after aerobic stability test (7d) are shown in figures in sequence. Figures 9 to 12 belongs to experiment 1 and figures 13 to 15 belongs to experiment 2.

Silages treated with CaO and after AS test had higher ($P \leq 0.05$) pH compared to other additives regardless of ST (Figure 10 [a]). CaO silage reduce ($P \leq 0.05$) pH when stored for 90d when compared to 21d of ST. Yeasts had lower ($P \leq 0.05$) counts when treated with SAFE 4 (Figure 10 [b]). At 90d of ST silages had similar ($P < 0.05$) yeasts counts. CP content was higher ($P < 0.05$) in Control and Buchneri silages at 21d and similar between additives at 90d (Figure 11 [a]). DM content was lower ($P < 0.05$) in

Control, Buchneri and CaO silages regardless of ST (Figure 11 [b]). NDF content was lower ($P < 0.05$) in SAFE 3 and SAFE 4 silages at 21d and lower ($P < 0.05$) in CaO, SAFE 3 and SAFE 4 silages at 90d of ST (Figure 12).

Silages treated with Cao and after AS test had higher ($P \leq 0.05$) pH compared to other additives regardless of ST (Figure 13 [a]). Also, molds had higher ($P \leq 0.05$) counts in CaO silages at 21d of ST. At 90d of ST additives did not differ ($P > 0.05$) (Figure 13 [b]). At 21 d Silages treated with CaO and SAFE 4 had higher ($P \leq 0.05$) DM content, and at 90d CaO had higher ($P \leq 0.05$) DM content (Figure 14[a]). CP content was higher in Buchneri silages at 21d of ST. At 90d silages were similar ($P \leq 0.05$) between additives. NDF was lower ($P \leq 0.05$) in SAFE 4 silages at 21d and lower ($P \leq 0.05$) for CaO silages at 90d of ST.

1.4. Discussion

pH is an important marker of quality of silage fermentation (Kung et al., 2018) In this study, all silages decreased pH below 4, indicating that microorganisms were in favorable environment and substrate enough to decrease pH of silage. In silages treated with CaO the higher pH values are due a function of the alkaline nature of calcium oxide. The raise of pH can reduce yeast and mold population on sugarcane silage, therefore avoiding dry matter loss (Cavali et al., 2010; Jacovaci et al., 2017).

Chemical additive as SAFESIL at high rates can reduces LAB counts, since the use of additives has been reported as inhibitor of LAB growth as well. The use of sodium benzoate, potassium sorbate and sodium nitrite can also influence the reduction of the population of heterofermentative lactic acid bacteria (Benjamin da Silva and Kung, 2022; Muck et al., 2018; Souza et al., 2022; Woolford, 1975). As time of storage increase, LAB are inhibited by concentration of organic acids produced by itself (Pahlow et al., 2003). This also appears to have been the case in the present study.

Silages treated with SAFE at 4L.ton^{-1} showed to be more efficient in inhibit Yeats population. The use of chemical additives based on sodium benzoate, potassium sorbate, and sodium nitrite have already been well established in the industry as inhibitor of undesirable microorganisms. The synergy of these additives has proven to prevent yeast and mold growth (Stanojevic et al., 2009). The fungistatic properties of chemical additives result from their ability to pass across the cell membrane in the undissociated form and release hydrogen in the cell interior. This cause damage to the plasma membrane and the use of ATP to expel H+ result causes the cell to reduce or stop growing (Lambert and Stratford, 1999). However, more resistant microorganisms can recover the damage caused by the additive, regrowth, and cause deterioration of the ensiled mass. (Stanojevic et al., 2009). This may explain why SAFE at 2 or 3 L.ton^{-1} had been less effective when silages were stored for 90d. The dose of 2 or 3 L.ton^{-1} may cause reverse damage and the yeasts grew when recover of the damage.

Also, silages treated with CaO were effective in reducing yeasts growth and decrease DML in sugarcane silages. This effect was widely reported in literature. Silages treated with these products presented reduction of alcoholic fermentation, high concentration of soluble carbohydrates and minor dry matter losses (Balieiro Neto et al., 2009; Cavali et al., 2010)

Fermentation of sugars by yeasts to carbon dioxide and ethanol are primarily responsible for dry matter losses in silage. According to McDonald et al. (1991) yeast metabolic processes lead to approximately 49% dry matter losses. SAFE 4 was effective in reducing DM and WSC losses and had the highest WSC contents despite of ST since it had lower counts of yeasts decreasing ethanol production and losses as well.

The increase in aerobic stability with the use of SAFE at all rates is stated Knicky and Rolf Spörndly et al. 2015, who observed that sodium benzoate, potassium sorbate and sodium nitrite ad additive is effective for increasing aerobic stability and reducing aerobic deterioration in silages. These authors observed that this effect on aerobic stability were most likely a direct result of inhibition of lactate assimilating yeasts even under air-challenged laboratory ensiling conditions. The lowest AS in silages with SAFE was possibly due to the good fermentation and maintenance of silage substrates preserving the nutritional value of the silage.

The difference in the NDF content reflects the overuse of fermentable carbohydrates, silages treated with SAFE at $4L.ton^{-1}$ decreased yeast and LAB populations, avoiding the use of WSC as substrate to production of ethanol and organic acids, respectively. Silages with high NDF, CP and ASH contents reflect the increase of this component proportionally in the dry matter content. Silages that had higher DM loss, reduced DM content and proportionally increased the other components.

The fungistatic effects of silages treated with SAFE at $4L.ton^{-1}$ stored for 21d were also observed when silages were exposed to 7d of aerobic exposure during stability test, showing lower yeast count.

1.5. Conclusion

The use of an additive mixture of sodium benzoate, potassium sorbate, and sodium nitrite reduced changed the fermentation profile and aerobic stability of sugarcane. Silages treated with Safesil in rates of 2 $L.ton^{-1}$ and $3L.ton^{-1}$ or CaO decreased yeast counts, reduced dry matter, WSC losses and improved aerobic stability of sugarcane silage stored for 21d. To improve fermentation profile, prevent yeast grown and raise aerobic stability, the highest dose of Safesil ($4L.ton^{-1}$) is recommended for sugarcane silages.

Lactobacillus Buchneri did not show effect on sugarcane silages stored for 21 or 90d.

Table 1. Characterization of sugarcane treated with additives.

	pH	LAB, log CFU/g	MOLDS, log CFU/g	YEASTS,log CFU/g	DM, %	ASH, % DM	CP, % DM	NDF, % DM	WSC, % DM
EXP 1									
Additive means									
Control	5,84	5,90	4,50	5,37	29,05	3,98	2,60	46,07	31,56
Buchneri	5,98	5,98	4,37	5,59	28,92	3,75	2,98	44,00	32,39
CaO	12,03	4,74	3,83	4,63	31,68	8,11	2,54	40,21	25,73
Safe2	6,07	6,01	4,70	5,71	30,09	3,77	2,90	43,77	27,77
Safe3	5,92	5,92	4,73	5,54	29,79	3,75	2,95	41,81	29,00
Safe4	6,38	5,95	4,63	5,56	29,74	3,75	3,09	41,25	26,63
EXP 2									
Additive means									
Control	5,38	7,00	5,40	6,53	22,07	3,32	4,02	58,35	35,00
Buchneri	5,41	6,86	5,00	6,57	21,35	3,48	2,89	61,43	40,04
CaO	10,81	5,75	5,43	6,59	23,42	6,53	2,89	63,70	32,66
Safe2	5,60	6,98	5,44	6,61	26,61	2,65	2,44	52,81	31,41
Safe3	5,59	7,01	5,15	6,53	27,05	2,97	2,38	51,44	27,86
Safe4	5,68	6,92	5,76	6,59	28,00	2,97	2,38	49,63	32,64

DML, dry matter loss; DM, dry matter; WSC, water soluble carbohydrates; CP, crude protein; NDF, neutral detergent fiber determined. SEM, standard error of the mean. Lowercase letters differ from each other within ADT or ST.

Table 2. Effects of additive and storage time on the fermentation profile and microbial counts of sugarcane silage.

	pH	LAB, log CFU/g	MOLDS, log CFU/g	YEASTS log, CFU/g	Lactic Acid, % DM	Acetic Acid, % DM	Ethanol, % DM	Proprionic Acid, % DM
				EXP 1				
Additive means								
Control	3,50	7,93	2,32	4,96	1,08	3,43	2,66	0,03
Buchneri	3,47	8,31	2,01	5,03	1,41	1,07	2,32	0,08
CaO	5,24	8,72	2,42	4,26	0,75	0,52	1,92	0,27
Safe2	3,42	7,74	2,00	4,28	0,46	0,93	1,94	0,15
Safe3	3,45	7,38	2,10	2,84	0,75	2,95	1,22	0,22
Safe4	3,59	7,01	2,00	2,28	0,65	2,48	2,39	0,22
TA means								
3	4,41	8,98	2,00	4,28	0,31	1,60	1,29	0,02
10	3,65	8,39	2,10	4,16	0,91	2,81	3,22	0,00
21	3,38	7,63	2,00	4,28	1,49	2,60	2,87	0,18
90	3,68	6,39	2,48	3,04	0,69	0,58	0,92	0,46
SEM	0,15	0,19	0,06	0,22	0,11	0,37	0,24	0,04
P-Values								
AD	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	0,40	0,14
TA	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
AD×ST	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	0,14
				EXP 2				
Additive means								
Control	3,50	8,18	2,64	5,77	3,38$_{ab}$	**	13,65$_{a}$	**
Buchneri	3,51	8,19	2,48	5,44	4,66$_{a}$	**	12,78^{a}	**
CaO	4,87	8,53	2,62	5,08	4,32$_{a}$	**	3,95$_{bc}$	**
Safe2	3,52	7,62	2,36	4,59	2,89$_{ab}$	**	5,39$_{b}$	**
Safe3	3,56	7,91	2,14	4,37	3,14$_{ab}$	**	1,66$_{c}$	**
Safe4	3,71	7,66	2,49	3,68	2,09$_{b}$	**	2,85$_{bc}$	**
TA means								
3	4,21	8,61	2,00$_{b}$	4,55	2,39$_{b}$	**	7,08$_{ab}$	**
10	3,62	8,22	2,00$_{b}$	4,56	3,62$_{a}$	**	10,27$_{a}$	**
21	3,62	8,11	3,14$_{a}$	5,13	3,21$_{ab}$	**	6,01$_{b}$	**
90	3,67	7,12	2,69$_{a}$	5,06	4,42$_{a}$	**	3,49$_{b}$	**
SEM	0,06	0,11	0,09	0,10	0,215		0,7874	
P-Values								
AD	<0,01	<0,01	0,28	<0,01	<0,01	**	<0,01	**
TA	<0,01	<0,01	<0,01	<0,01	<0,01	**	0,0137	**
AD×ST	<0,01	<0,01	0,50	<0,01	0,38	**	0,08	**

SEM, standard error of the mean. Lowercase letters differ from each other within ADT or ST.

Table 3. Effects of additive and storage time on the nutrient loss, aerobic stability and nutrient composition of sugarcane silage.

	DML, %DM	WSC Loss, %DM	Aerobic Stability, hours	DM, %	ASH, % DM	CP, % DM	NDF, % DM	WSC, % DM
EXP 1								
Additive means								
Control	28,94	94,72	44,57	22,21$_{c}$	5,49$_{b}$	3,49	64,89	4,76
Buchneri	29,06	94,51	48,55	21,69$_{c}$	5,36$_{b}$	3,41	65,9	5,34
CaO	16,82	94	100,5	26,91$_{a}$	8,89^{a}	2,77	49,67	8,78
Safe2	22,76	54,41	157,71	24,5$_{b}$	4,73$_{c}$	3,07	56,87	24,5
Safe3	15,48	16,68	171,78	25,8$_{ab}$	4,33$_{c}$	2,74	49,7	32
Safe4	11,27	11,05	171,81	26,68$_{a}$	4,74$_{c}$	2,96	49,4	32,63
TA means								
3	**	**	**	26,15$_{a}$	4,94$_{b}$	3,02	52,02	23,72
10	**	**	**	23,86$_{b}$	5,70$_{a}$	3,08	58,62	21,32
21	20,94	53,43	104,29	24,12$_{b}$	5,64$_{a}$	2,89	55,53	17,01
90	20,5	63,36	127,34	24,42$_{b}$	6,08$_{a}$	3,29	58,12	9,97
SEM	1,86	8,83	12,18	0,37	0,24	0,07	18,05	2,32
P-Values								
AD	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
TA	0,6011	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
AD×ST	<0,05	<0,01	<0,01	0,35	0,98	<0,01	<0,01	<0,01
EXP 2								
Additive means								
Control	18,55$_{bc}$	**	49,58	18,82	4,04$_{b}$	3,61	77,07	**
Buchneri	15,31$_{c}$	**	54,86	19,1	3,89$_{b}$	3,57	76,98	**
CaO	1,78$_{d}$	**	49,21	25,25	6,97$_{a}$	2,75	54,16	**
Safe2	27,81^{a}	**	119,87	21,09	4,95$_{ab}$	3,45	63,89	**
Safe3	22,07$_{b}$	**	110,87	23,41	3,33$_{b}$	2,8	51,18	**
Safe4	17,43$_{c}$	**	128,5	24,32	3,28$_{b}$	2,7	55,34	**
TA means								
3	**	**	**	21,85	3,85$_{b}$	2,66	50,9	**
10	**	**	**	23,64	4,3$_{b}$	3,16	65,14	**
21	15,66	**	104,52	21,27	4,36$_{ab}$	3,53	66,97	**
90	18,65	**	66,44	21,23	5,14$_{a}$	3,24	69,42	**
SEM				0,31	0,31	0,08	1,61	
P-Values								
AD	<0,01	**	<0,01	<0,01	<0,01	<0,01	<0,01	**
TA	0,24	**	<0,01	<0,01	<0,01	<0,01	<0,01	**
AD×ST	0,87	**	<0,01	<0,01	0,20	0,04	<0,01	**

DML, dry matter loss; DM, dry matter; WSC, water soluble carbohydrates; CP, crude protein; NDF, neutral detergent fiber determined. SEM, standard error of the mean. Lowercase letters differ from each other within ADT or ST.

Table 4. Effects of additive and storage time on chemical composition, fermentation profile and microbial counts of sugarcane silage after 7d of aerobic exposure on stability test.

	pH	MOLDS, log CFU/g	YEASTS,log CFU/g	DM, %	ASH, % DM	CP, % DM	NDF, % DM	WSC, % DM
EXP 1								
Additive means								
Control	3,63	$5,50_{ab}$	6,84	23,83	$5,28_{bc}$	3,8	66,49	$2,13_{b}$
Buchneri	3,75	$5,76_{ab}$	7,08	23,93	$5,66_{b}$	3,91	64,16	$2,19_{b}$
CaO	5,29	$6,35_{a}$	7,28	29,44	$8,47_{a}$	2,89	48,8	$3,40_{b}$
Safe2	3,44	$4,38_{bc}$	6,36	25,06	$4,89_{bc}$	3,66	61,03	$9,57_{b}$
Safe3	3,36	$4,26_{bc}$	6,52	28	$4,47_{c}$	3,16	45,69	$30,58_{a}$
Safe4	3,54	$3,85_{c}$	4,88	28,94	$4,51_{c}$	2,89	49,05	$31,93_{a}$
TA means								
21	3,9	$5,77_{a}$	6,86	26,19	5,48	3,4	57,02	10,58
90	3,69	$4,24_{b}$	6,18	26,77	5,6	3,39	54,86	13,64
SEM	0,10	0,23	0,21	0,37	0,19	0,07	1,54	2,32
P-Values								
AD	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
TA	<0,01	<0,01	0,034	0,12	0,4	0,93	0,33	0,88
AD×ST	<0,01	0,54	<0,01	0,02	0,54	<0,01	0,02	0,77
EXP 2								
Additive means								
Control	3,64	2,44	7,35	20,38	$3,90_{b}$	3,27	81,67	**
Buchneri	3,67	2,91	6,41	19,72	$4,35_{b}$	3,73	77,5	**
CaO	7,67	4,63	8,21	25,13	$6,77_{a}$	2,91	61,48	**
Safe2	3,76	2,4	7,46	20,19	$3,91_{b}$	3,6	77,76	**
Safe3	3,91	2,9	6,59	21,6	$3,96_{b}$	3,61	73,42	**
Safe4	3,7	2,5	5,72	22,91	$3,76_{b}$	3,36	58,58	**
TA means								
21	4,18	2,57	6,17	21,94	$4,21_{b}$	3,07	68,13	**
90	4,60	3,1	7,78	21,37	$4,67_{a}$	3,75	75,01	**
SEM	0,2	0,22	0,21	0,35	0,16	0,09	2,33	**
P-Values								
AD	<0,01	0,04	0,21	<0,01	<0,01	<0,01	<0,01	**
TA	<0,01	0,53	0,03	0,29	<0,01	<0,01	<0,01	**
AD×ST	<0,01	<0,01	0,32	0,01	0,12	0,02	0,02	**

DM, dry matter; WSC, water soluble carbohydrates; CP, crude protein; NDF, neutral detergent fiber determined. SEM, standard error of the mean. Lowercase letters differ from each other within ADT or ST.

Figure 1: Effect of ADT×ST on (a) pH and (b) LAB of sugarcane silages EXP1.

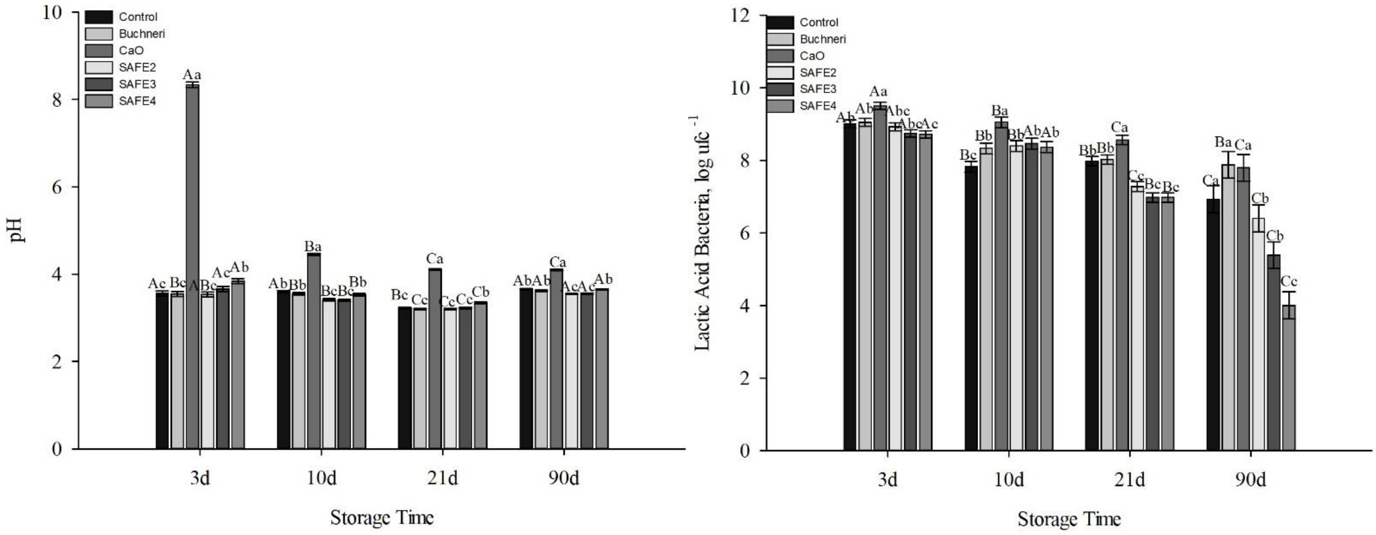


Figure 2: Effect of ADT×ST on (a) yeasts and (b) molds counts of sugarcane silages EXP1.

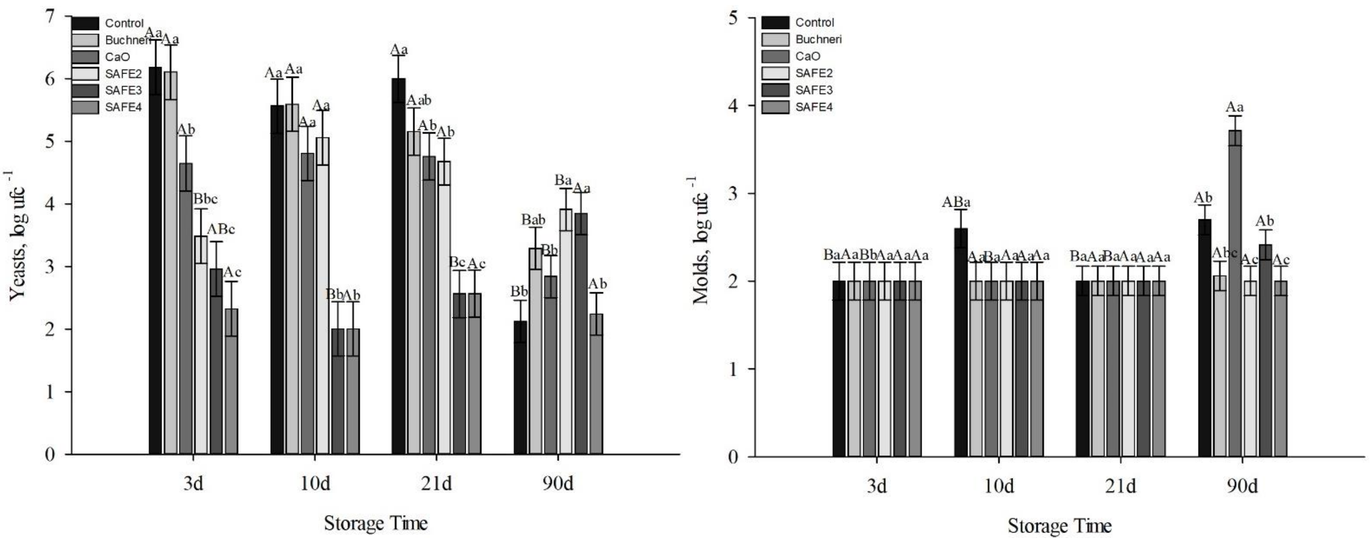


Figure 3: Effect of ADT×ST on (a)ethanol and (b) NDF concentration of sugarcane silages EXP1.

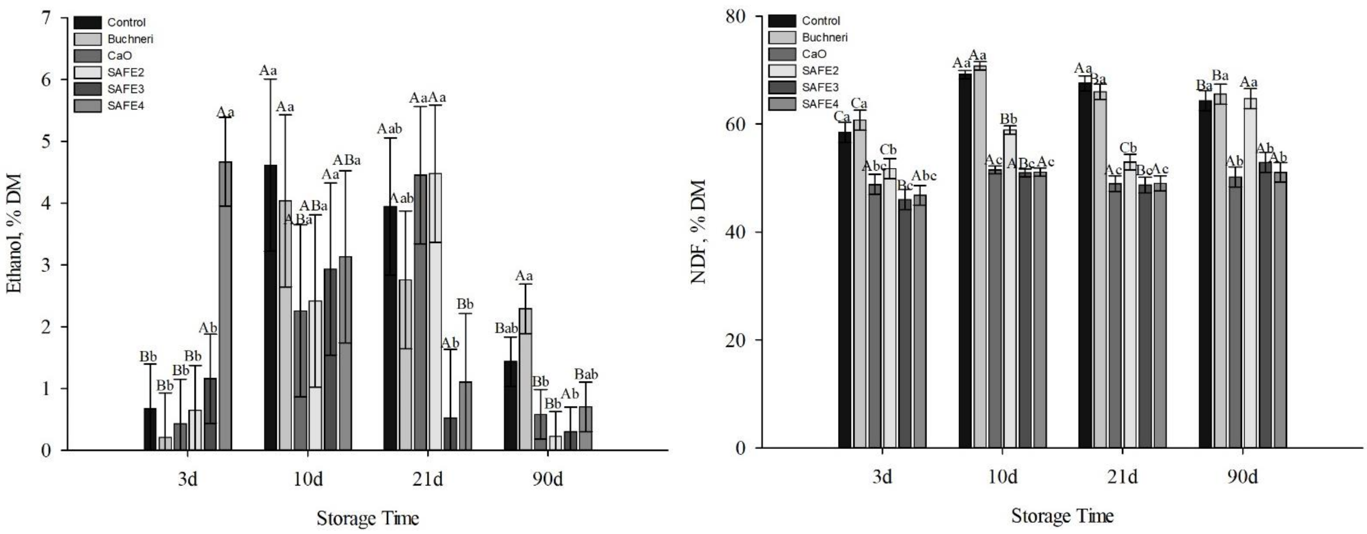


Figure 4: Effect of ADT×ST on (a) WSC content and (b) WSCLoss of sugarcane silages EXP1.

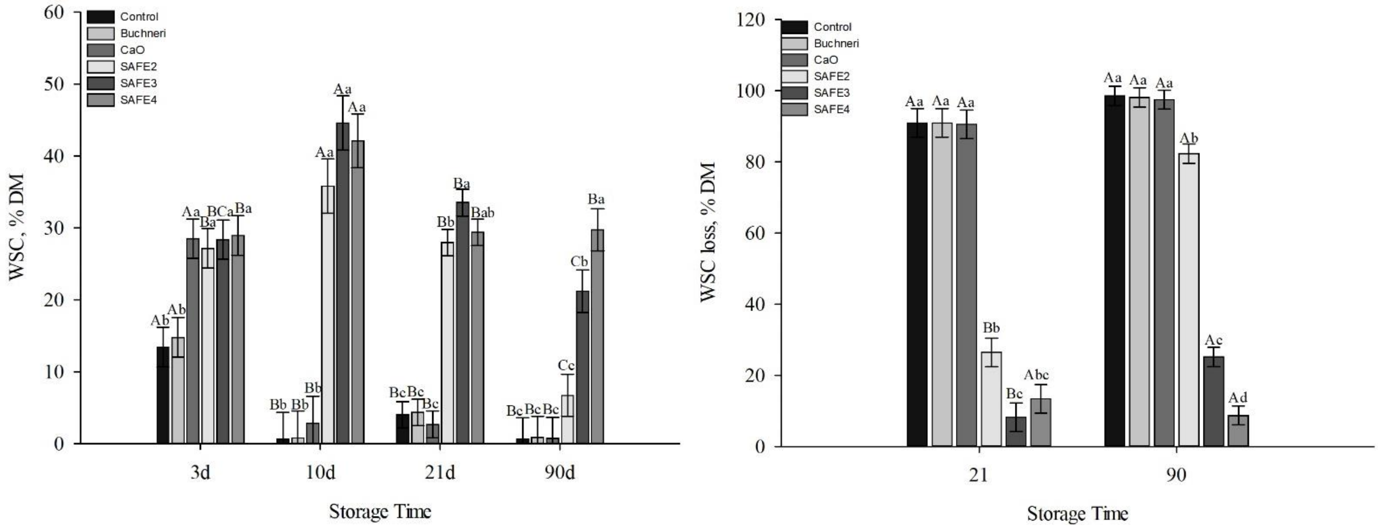


Figure 5: Effect of ADT×ST on (a) aerobic stability and (b) dry matter loss of sugarcane silages EXP1.

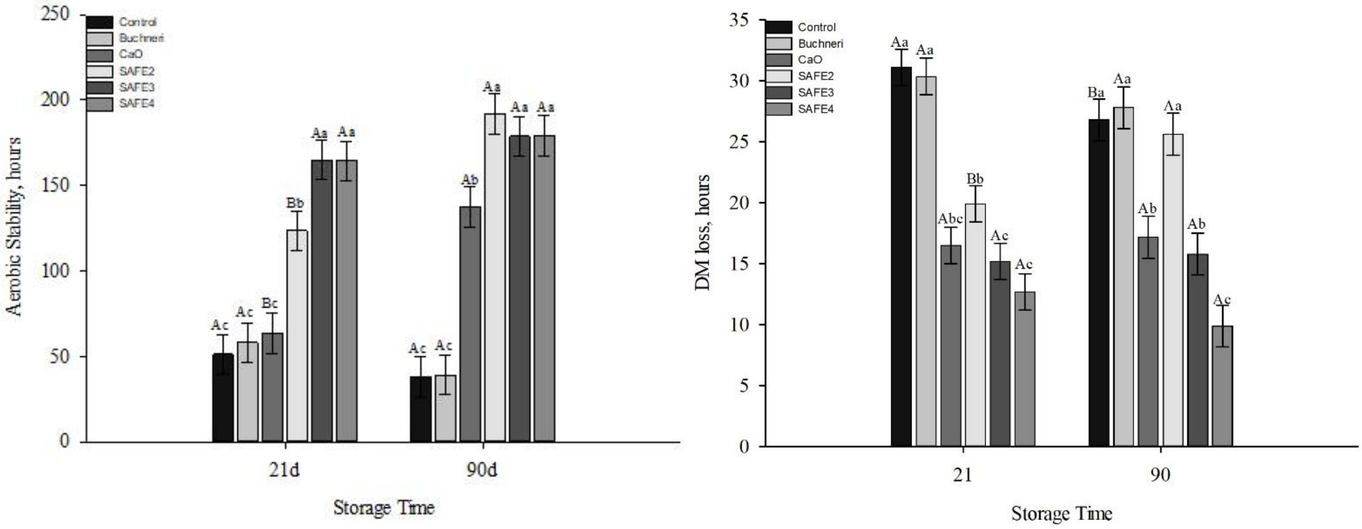


Figure 6: Effect of ADT×ST on (a) pH and (b) LAB count of sugarcane silages EXP2.

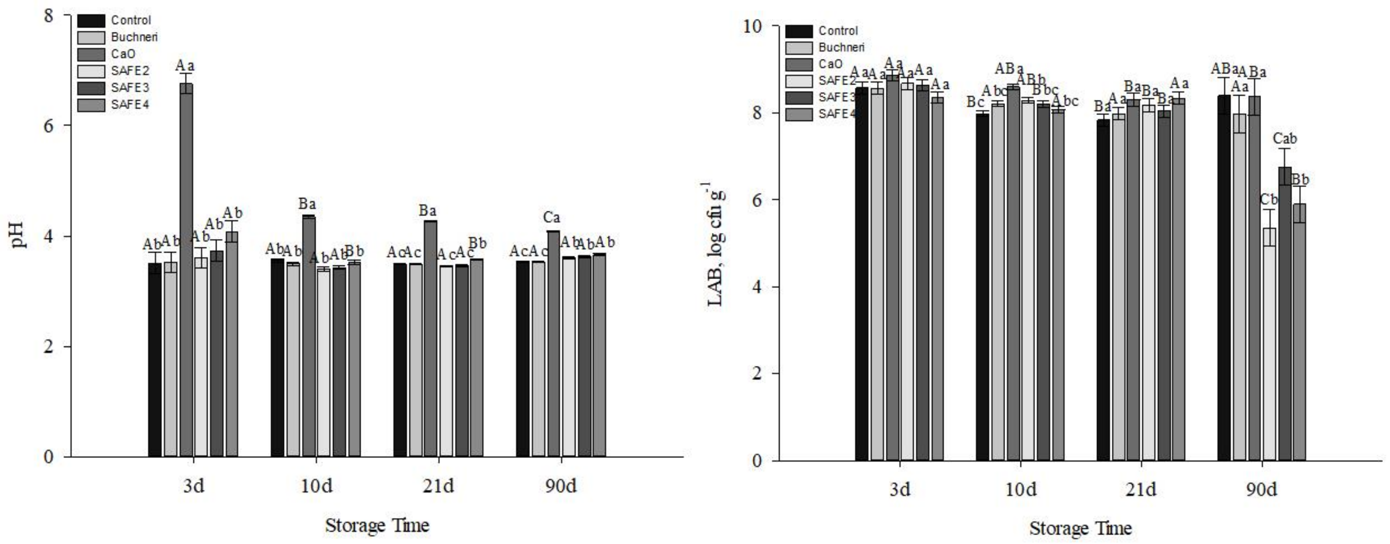


Figure 7: Effect of ADT×ST on (a) Yeasts count and (b) DM content of sugarcane silages EXP2.

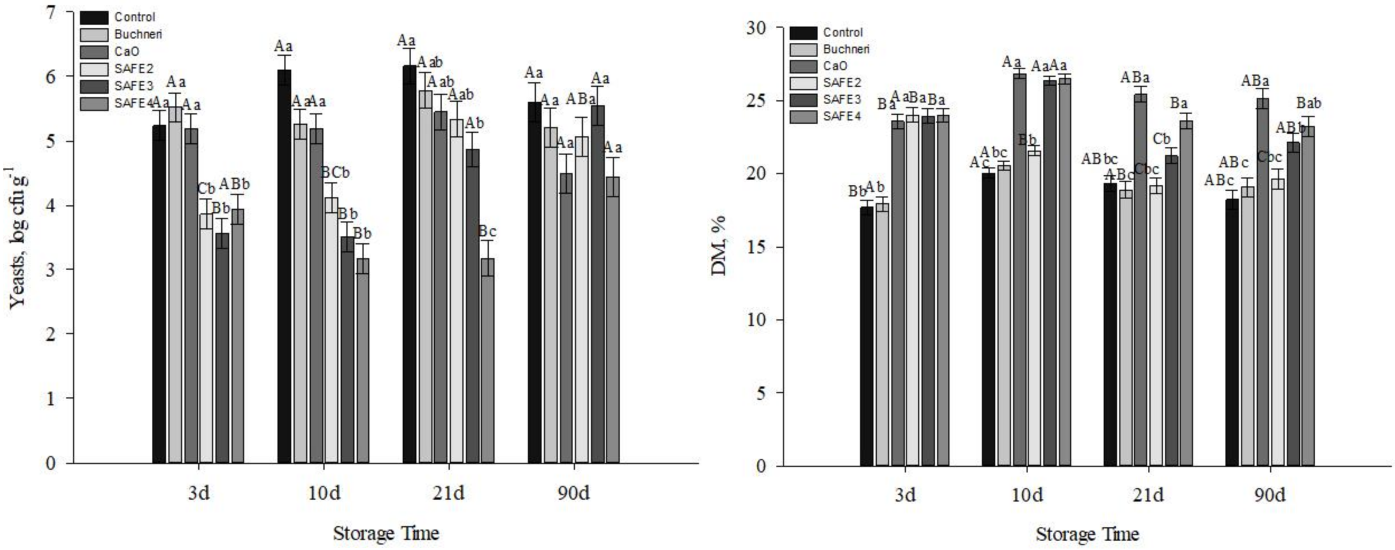


Figure 8: Effect of ADT×ST on (a) CP and (b) NDF contents of sugarcane silages EXP2.

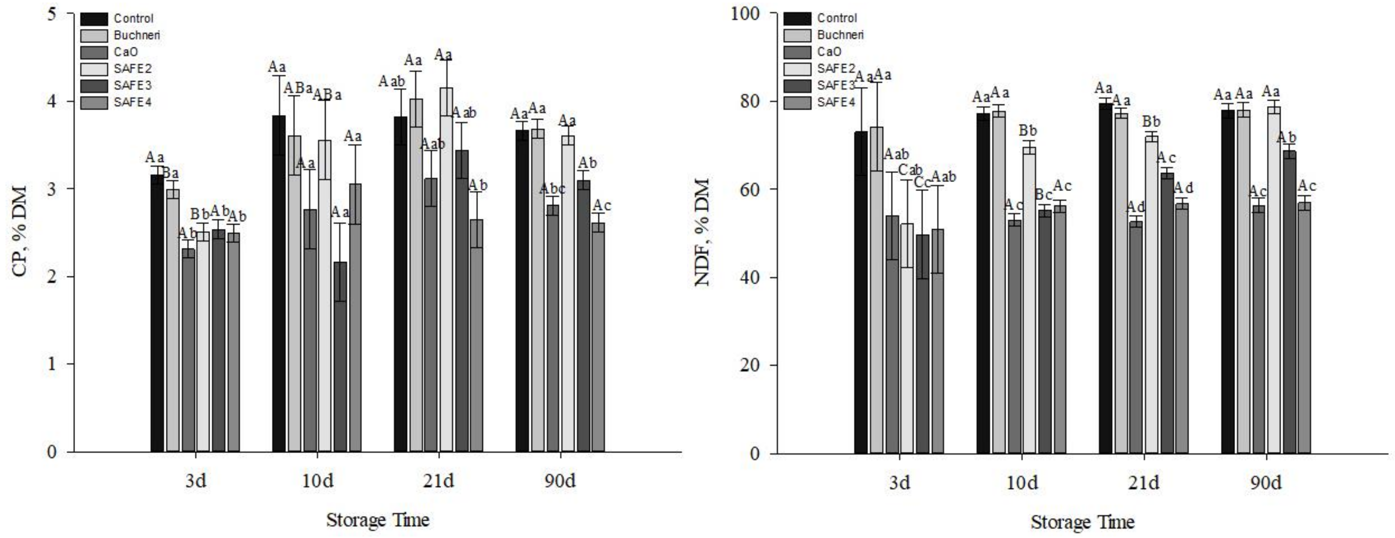


Figure 9: Effect of ADT×ST on aerobic stability of sugarcane silages EXP2.

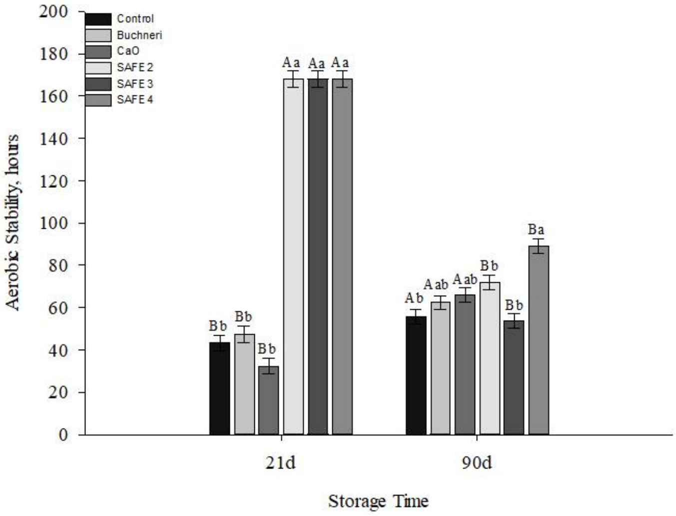


Figure 10: Effect of ADT×ST on (a) pH and (b) yeasts counts of sugarcane silages after 7d of AS EXP1.

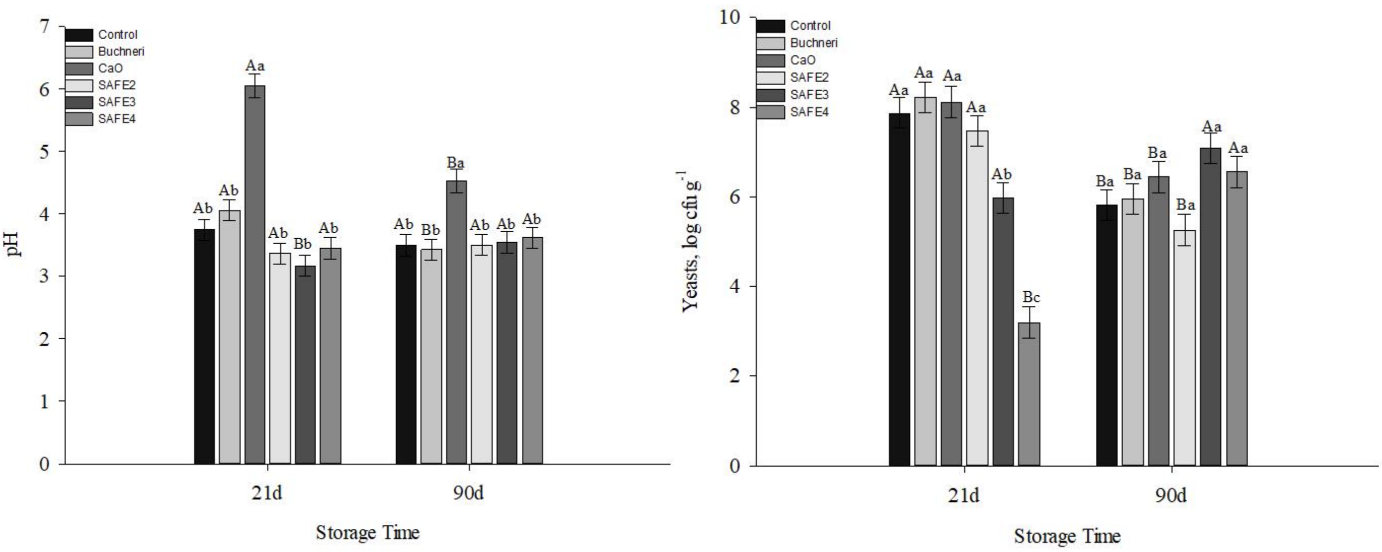


Figure 11: Effect of ADT×ST on (a) CP and (b) DM content of sugarcane silages after 7d of AS EXP1.

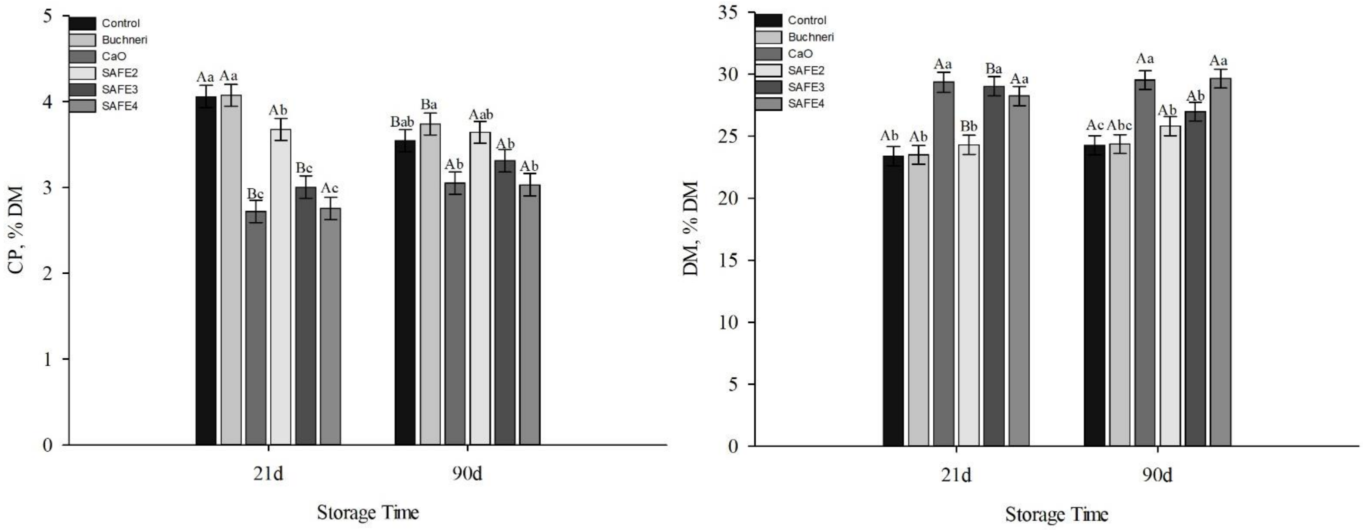


Figure 12: Effect of ADT×ST on NDF content of sugarcane silages after 7d of AS EXP1.

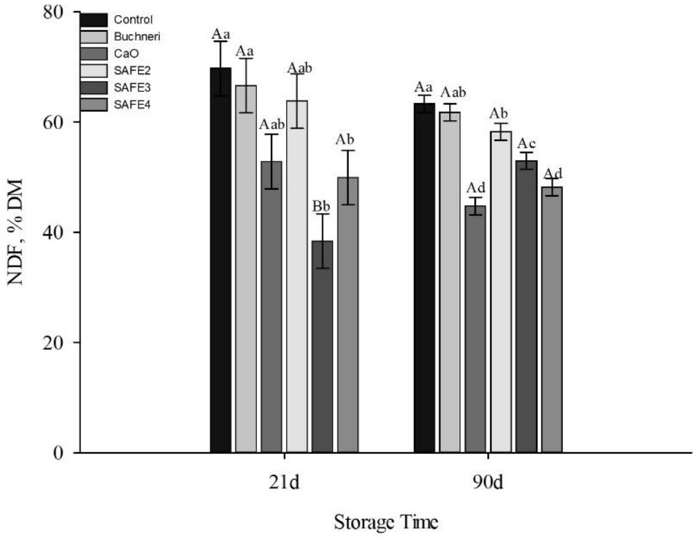


Figure 13: Effect of ADT×ST on (a) pH and (b) molds counts of sugarcane silages after 7d of AS EXP2.

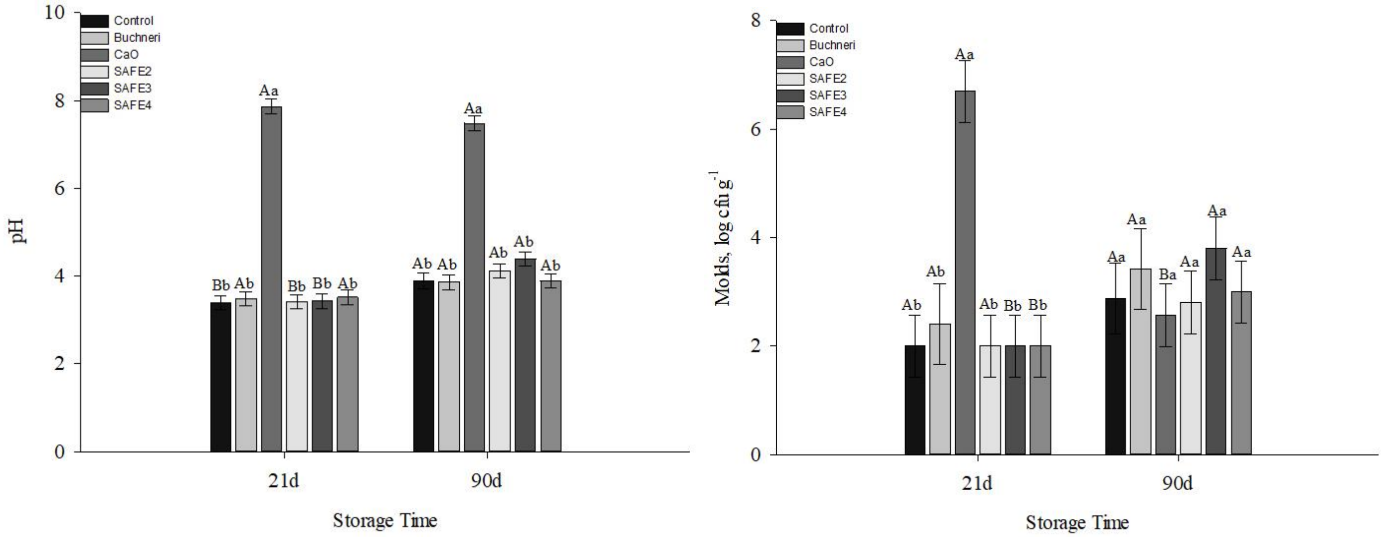


Figure 14: Effect of ADT×ST on (a) DM and (b) CP content of sugarcane silages after 7d of AS EXP2.

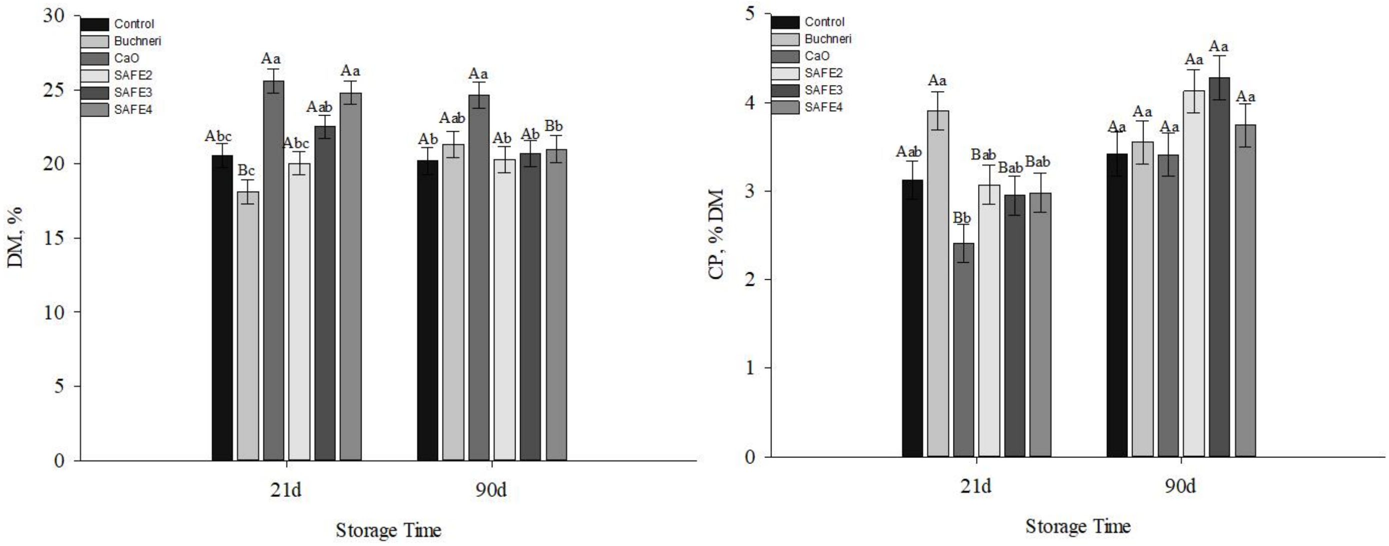
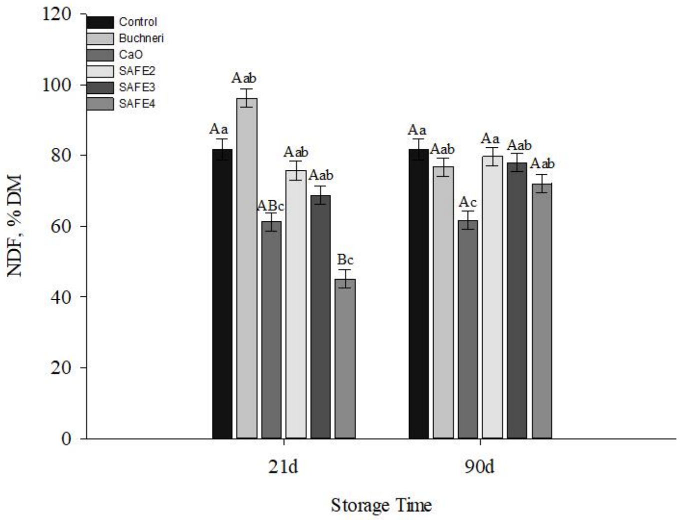


Figure 15: Effect of ADT×ST on NDF concentration of sugarcane silages after 7d of AS EXP2



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2. CHEMICAL ADDITIVE AND STORAGE TIME CHANGES THE FERMENTATION QUALITY AND AEROBIC STABILITY OF CORN SILAGES

ABSTRACT

Corn silage is the main ingredient in the diet of lactating cows worldwide; Although corn has favorable characteristics to silage production, promoting a good fermentative profile, when opening the silo corn silage is more favorable to aerobic deterioration when compared to sugar cane and grass silage. Therefore, the use of additives during the ensiling process has been widely used to increase aerobic stability and reduce losses. The objective of this study was to determine the effect of additive application *Lactobacillus Buchneri* at 4 x 105 cfu.g^{-1}, SAFE at 2 L.kg^{-1}, 3 L.kg^{-1} and 4 L.kg^{-1} of the fresh matter (NM) of the ensiled mass, and the storage time of silages (3, 10, 21 or 90 days) on the fermentative profile, microbiology, chemical composition and aerobic stability of corn silages. Chopped corn from two fields (1 and 2) was ensiled in for 3 and 10 days of ST or in 20L plastic buckets for 21 and 90 days of ST. Densities of 550 ± 20 kg of MN/m^3 were used. Fermentative profile, chemical composition and aerobic stability were determined. LAB count decreased when storage time was longer. However, Buchneri silages had higher ($P \leq 0,05$) LAB counts despite of ST. SAFE 0.5, SAFE1.5 E SAFE 2.5 decreased ($P \leq 0,05$) yeast growth until 10d of ST. After this period, SAFE 2.5 was effect in reducing ($P \leq 0,05$) yeast count on 21d of ST, and at 90 d of ST only Buchneri was effective in reducing ($P \leq 0,05$) yeast growth. Molds were inhibited and had minimal counts on 21 and 90d of storage time. When ST for 3 or 10d SAFE 0.5, SAFE1.5 E SAFE 2.5 reduces ($P \leq 0,05$) mold population. SAFE 2.5 was effective in reducing ($P \leq 0,05$) ethanol production at 10,21 and 90d of Storage Time.

2.1. Introduction

Corn is one of the most widely used silage for animal feeding (Ferrarettto 2018; Daniel et al., 2019; Gamble et al. 2022). Associated with this gradual increase in the use and production of corn silage, there is an exponential need to improve the quality, productivity and utilization of this silage, which due to intrinsic factors, the product and the ensiling process, suffers from considerable losses caused among other reasons, by the proliferation of fungi and yeast, depreciative factors that directly affect the nutritional value of silage (Arriola et al., 2020; Kung et al. 2018).

The use of additives, microbiological or chemical, allow the modulation of fermentation, increase stability, and change nutritional characteristics of silage (Kung et al. 2018). There is a range of additives amenable to use, being distributed into 4 categories, defined from the proposed effects, where act on the fermentative parameters of the ensiled material, stimulating or inhibiting; act inhibiting aerobic deterioration or act on the availability of nutrients (Muck et al., 2018). However, it is important to evaluate not only the efficiency in improving the ensiled material, but also the economic return (Kung et al., 2018).

Among the most widespread microbiological additives, the bacterial inoculant of the species *Lactobacillus buchneri* is one of the most used, acting in the multiplication of beneficial microorganisms which promotes an improvement in aerobic stability due to the profile of acids produced, besides acting in the inhibition of yeast development reducing the degradation of the ensiled material. However, for the process to be successful, L. buchneri needs a period longer than 40 days. (Kung et al., 2018; Muck et al., 2018). In order to obtain more readily available results, the use of chemical additives to improve the aerobic stability of silage, has been confirmed, by numerous studies, in reducing undesirable microorganisms improving the stability of the ensiled material overall (Knicky and Sporndly, 2011; Knicky and Sporndly, 2015; Kung et al. 2018; Muck et al., 2018; Daniel et al., 2022).

Based on this, we hypothesized that the chemical additive SAFESIL (SAFE) will improve the fermentative characteristics of corn silages by inhibiting yeast and mold growth, and consequently increase aerobic stability, even at shorter silage storage times. Thus, we aimed to evaluate the effects of the chemical additive Safesil (Safe) on the microbial population, fermentative profile, losses, and chemical composition of corn silage. And compare the effects of adding the SAFE additive with other widely used biological additives on microbial population, fermentative profile, losses, and chemical composition of silages.

2.2. Material and methods

Experimental design and ensiling conditions

A completely randomized block design, in a 5 × 4 factorial arrangement, with five replications was used. The studied factors were additive (ADT) [No additive; Buchneri; SAFE 0.5; SAFE 1.5 and SAFE 2.5]; and different storage times (ST) [3, 10, 21 and 90 days). Blocks were defined as experimental silos ensiling order. SAFE product used in this study was Safesil Ab Hanson & Mohring, Halmstad, Sweden; contained 200 g/kg of sodium benzoate, 100 g/kg of potassium sorbate, and 50 g/kg of sodium nitrite. The treatments with chemical and microbiological additives used were defined by:

1- Control (without additive);
2- *Lactobacillus buchneri* (4 x 105 cfu.g^{1} fresh matter (FM);
3- SAFE 0.5 – 0.5 L.ton^{1} of FM;
4- SAFE 1.5 – 1.5 L.ton^{1} of FM; It is
5- SAFE 2.0 – 2.0 L.ton^{1} of FM;

Corn with approximately 32% DM was harvested from two farms (Field 1 and Field 2) in the municipality of Coimbra, MG. Harvested corn were transported to Department of Animal Science of UFV where it was chopped at a theoretical cut length of 20mm with a pull type forage harvester. All steps were replicated in both experiments (Field 1 and Field 2).

Ensiling was conducted by repetition and the treatments were applied to the pile of forage made for each experimental unit. *L. buchneri* and SAFE treatments were applied to corn at 10 mL/kg of fresh forage, v./w. (according to manufacturer recommendation) and were used variable concentrations according to the treatments. After homogenization, 500g of forage was ensiled in 25.4 cm × 35.56 cm bags (Doug Care Equipment, Springville, CA) for 3 and 10 days of ST or in 20L plastic buckets for 21 and 90 days of ST. The anaerobic condition inside the bag silos was established using a vacuum sealer and buckets were packing to achieve 550 kg/m^3 of density. The silos were stored in a covered barn.

Silos bags were opened at 3 and 10 days of storage, and buckets silos were opened at 21 and 90 days of storage. The silage from each silo was mixed and samples were collected before ensiling (Table 1), at silo opening (ST), and after aerobic exposure (7d) for evaluations and laboratory analysis.

Evaluations and analysis

Silage microbiology and fermentation

The numbers of lactic acid bacteria (LAB), yeasts and molds were analyzed in all collected samples. The aqueous extract (1:10) of the sample (25 g) was prepared with the addition of sterile peptone water 0,1% (w./v.), and then homogenized for 3 min in a sterile bag. The microbial counts were determined by pour plating 10-fold serial dilutions (five per sample) of the extract on MRS agar with nystatin (4 ml L 1) to determine LAB and potato dextrose agar (Sigma Aldrich Brasil LTDA), with 10% (v./v.) tartaric acid (15 ml L 1) to determine yeast and molds. After incubation at 35 ºC for 48h (LAB) and at 28 °C for 72h (yeasts) and 120h (molds). The colonies of yeasts and molds were counted for separately, yeasts were distinguished from molds by colony appearance and cell morphology. The pH of each sample was determined using a digital potentiometer (T 1000®, Tekna, Sao Bernardo do Campo, Brazil), in the same water extract. A portion of 2 mL aqueous extract (2 ml) was acidified with 10 μL of 50% (v.v.) H2SO4 and frozen. Aqueous extracts were analyzed to Organic acids (lactic, acetic, propionic, and butyric acids), ethanol, and 1,2 propanediol were analyzed by HPLC (Canale et al., 1984). Water soluble carbohydrates (WSC) were analyzed using the phenol method (Dubois et al., 1956).

Dry matter loss and Aerobic Stability

Silos were weighed prior to storage to determine dry matter loss. DML were calculated as the difference between the weight of the material placed in each silo at ensiling and the weight of the silage after the designated length of storage divided by the weight of material at ensiling, corrected for the respective initial or final DM concentration (Jobim et al., 2007). Buckets silos (21 and 90d of ST) (n=60) containing approximately 5kg of remaining silage that had been mixed and loosely replaced in buckets after sample collections were used for determination of aerobic stability.

The buckets were kept in air-conditioned room at 21 ± 1.6 °C. A data logger was inserted in the geometric center of each forage mass to monitor the temperature every fifteen minutes. After 7 days (168 hours) the data loggers were removed, and the data transferred to a computer to determine the aerobic stability. Aerobic stability was defined as the time required for the silage temperature to exceed 2°C at room temperature (Ranjit and Kung, 2002).

After the aerobic stability test, samples were taken for analysis of chemical composition, organic acids, pH and microbiologic population of corn silages after 7 days.

Chemical composition

Fresh and silage samples were dried in a forced air oven set at 55℃ for 72h and grounded to pass through a 1 mm sieve using a Wiley Mill (Thomas Scientific, Swedesboro, NJ), to determine DM (method 950.15, AOAC, 2000 [14]), ash (method 942.05, AOAC, 2000 [14]), crude protein using the Kjedahl method (CP, N × 6.25; method 984.13, AOAC, 2000 [14]). The neutral detergent fiber (NDF) was obtained according to the methodology of Mertens [15] without the use of α amylase and sodium sulfite.

Statistical analysis

The data of fresh corn treatments did not undergo statistical analysis. The microorganism count data were transformed to log 10 to be submitted to statistical analysis. Data were analyzed as a 4 × 5 factorial arrangement with repeated measures over time (3; 10; 21; and 90d of ST) by the SAS® program PROC MIXED procedure observing the effects of additive (ADT), Storage time (ST) and their interaction (ADT × ST). The averages were compared using the Tukey test at 5% probability level and the experimental error as a random effect.

2.3.Results

Effects of additive and storage time on the fermentation profile and microbial counts of corn silage are shown in table 2. Sugarcane ensiled for 3 days had lower ($P \leq$ 0.05) concentrations of lactic acid (Field 1 and 2), acetic acid and propionic acid (Field

2) compared to others ST. In the field 1 silages 10d of ST had the highest ($P \leq 0.05$) concentrations of lactic acid and acetic acid, however in the field 2 silage had the highest ($P \leq 0.05$) concentrations of lactic acid and propionic acid on 90d of ST.

Effects of additive and storage time on the nutrient loss, aerobic stability and nutrient composition of corn silages are shown in table 3. Silages treated with ADT Buchneri had elevated ($P \leq 0.05$) DML compared with SAFE 1.5 and SAFE 2.5 (Field 1). Silages treated with SAFE 2.5 had lower ($P \leq 0.05$) DML (Field 1) and the highest ($P \leq 0.05$) WSC concentration (Field 2). Regarding to ST, Silages stored for 3d had the higher ($P \leq 0.05$) CP (Field 1), WSC and ASH concentration (Field 2). Silages stored for 10d had the higher ($P \leq 0.05$) DM content (Field 2) and silages stored for 90d had higher ($P \leq 0.05$) DML than silages stored for 21d (Field 1).

Interaction of additive and storage times are shown in figures in sequence. Figures 1 to 4 belongs to field 1 and figures 5 to 7 belongs to field 2. pH had lower ($P \leq 0.05$) values for silages treated with SAFE 1.5 and SAFE 2.5 (Figure 1 [a]). At 10, 21 and 90d of ST silages did not differ ($P < 0.05$) from each other. Silages treated with buchneri had the highest ($P < 0.05$) counts o LAB for all storage times (Figure 1 [b]). Silages stored for 3d has the higher ($P < 0.05$) LAB counts compared with silages stored for 10, 21 and 90d.

At 3 and 10d of ST, silages treated with Control and Buchneri had the highest ($P < 0.05$) counts of yeast (Figure 2 [a]). At 21d, Control, Buchneri and SAFE 2 had higher ($P < 0.05$) yeasts count. At 90d the use of Buchneri in corn silage reduced ($P < 0.05$) the yeast counts. At 3d of ST the use of SAFE 0.5, SAFE 1.5 and SAFE 2.5 controlled the mold counts and presented the lowest ($P < 0.05$) values (Figure 2 [b]). At 10 d of ST the only SAFE 1.5 and SAFE 2.5 reduced ($P < 0.05$) the mold counts. At 21 and 90d the molds count reduced ($P < 0.05$) regardless of additives.

Silages treated with SAFE 2.5 (Figure 3 [a]) had lower (P < 0.05) ethanol concentration at 10, 21 and 90d of ST and had the higher (P < 0.05) aerobic stability at 21 d compared with other additives (Figure 3 [b]). SAFE 2.5 reduced (P < 0.05) aerobic stability when stored for 90d if compared with 21d. At 90d of ST Buchneri and SAFE 2.5 had the higher (P < 0.05) aerobic stability. NDF content was higher (P < 0.05) in SAFE 2.5 silages than in SAFE 1.5 Silages at 3d of storage (Figure 4 [a]). At 10, 21 and 90d silages had similar NDF content. WSC content was higher (P < 0.05) at 3d of ST independent of additive use (Figure 4 [b]). At 3d and 90d of ST, the higher (P < 0.05) WSC was in SAFE 2.5 silages, while at 10 and 21d SAFE 1.5 and SAFE 2.5 had higher (P < 0.05) WSC contents.

Silages stored for 3d has low (P < 0.05) pH values (Figure 5 [a]) and high (P < 0.05) LAB counts (Figure 5 [b]) when compared to the other times of storage. Lab counts reduced (P < 0.05) in Control, SAFE 1.5 and SAFE 2.5 silages when stored for 10 and 21d. The lowest (P < 0.05) LAB count was observed for SAFE 2.5 silages at 90d of ST. Yeasts counts was higher (P < 0.05) at 21d of ST for all additives (Figure 6 [a]). At 10d the lowest (P < 0.05) yeast count was observed in silages treated with SAFE 2.5. At 90d the lowest (P < 0.05) yeast count was in silages treated with Buchneri. Ethanol was higher (P < 0.05) at 90d of storage, regardless the additive (Figure 6 [b]). Control and SAFE 2.5 had low (P < 0.05) ethanol concentration at 90d when compared to other additives. CP content was lower (P < 0.05) in SAFE 2.5 silages stored for 90d and NDF content was higher (P < 0.05) in SAFE 0.5 silages when 10d ST (Figure 7 [a] and [b], respectively).

On table 4 are shown the effects of additive and storage time on chemical composition, fermentation profile and microbial counts of corn silage after 7d of aerobic exposure on stability test. Silages treated (Field 1) with ADT Buchneri and SAFE 2.5 had the lowest ($P \leq 0.05$) pH, 4,9 and 4,4, respectively. Silages treated with Buchneri and

SAFE 0.5 had the higher ($P \leq 0.05$) NDF content. After 7d of aerobic stability test, silages treated with SAFE 2.5 had the highest ($P \leq 0.05$) WSC content compared with other additives. Concerning ST silages stored for 90d had lower pH (Field 1) and highest CP (Field 1 and 2). Interaction of additive and storage times are shown in figures in sequence. Figure 8 belongs to field 1 and figures 9 to 11 belongs to field 2.

Yeasts counts was reduced ($P \leq 0.05$) when corn silage was treated with SAFE 2.5 and stored for 21d (Figure 8 [a]). However, when stored for 90d Buchneri reduced ($P \leq 0.05$) yeasts counts on corn silage (Figure 8 [b]). pH was lower ($P \leq 0.05$) in silages treated with SAFE 2.5 at 21d of ST, but in 90d of ST, the silages treated with Buchneri SAwas observed in SAFE 2.5 silages (Figure 9 [b]). Still at 90d of ST the lowest ($P \leq 0.05$) count was found in silages treated with Buchneri, followed for silages treated with SAFE 2.5. Control, SAFE 0.5 and SAFE 1.5 had the highest ($P \leq 0.05$) counts. WSC content was higher ($P \leq 0.05$) in silages treated with Control, SAFE 0.5 and SAFE 2.5 at 21d of ST (Figure 10). At 90d of ST, the higher ($P \leq 0.05$) WSC content was in silages treated with Buchneri.

2.4. Discussion

In the ensiling process, the population of LAB increase when anaerobiosis is established. Silages LAB populations in control treatments are arising from the epiphytic microflora of corn. Silages treated with *Lactobacillus Buchneri* showed higher counts of LAB. When microbial inoculant containing populations of heterofermentative Acid Lactic bacteria is used, the population of LAB in the ensiled forage increases, which explains why treatment with L. buchneri showed a higher population of these microorganisms at longer storage times. On the other hand, silages treated with SAFE had the lowest counts at 21 and 90 days, because additives can cause a decrease in the population of LAB (Benjamin da Silva and Kung, 2022; Muck et al., 2018).

The reduction of LAB in corn silages may turn favorable the conditions for yeast proliferation. Due to the fungistatic effect, lower rates of SAFE on corn silages can reduce the effectiveness of the additive as storage time increase. SAFE at higher rates was efficient in reduce proliferation of undesirable microorganism (Knicky and Spörndly, 2015). This may explain the lower yeast counts only in silages treated with SAFE 2.5 at 21 days of storage.

As widely discussed in literature, L. Buchneri showed effectiveness after 90d of storage time. The lower yeast counts in silages for 90 days occurred due to the heterofermentative nature of L. buchneri and the slow conversion of lactate to acetate that has fungicidal action (Muck, 2010; Muck et al., 2018). Yeasts are responsible for converting sugars into ethanol, and since the SAFE 2.5 treated silages were the most effective in controlling yeast, consequently, was observed less ethanol production.

The control on yeast population in silages positively affected aerobic stability. At 90d of ST, silages treated with SAFE 2.5 and L. buchneri remained longer stable compared to the other treatments. This is because yeasts are the first microorganisms to initiate the spoilage process in silage during the period when the silage is unloaded, since they are able to develop in wide pH ranges and use lactic acid as substrate, being responsible for initiating the spoilage process (Pallow et al., 2003. Therefore, the lower the yeast count, the longer the time in stability.

The reduction in the LAB population and inhibition of the yeast population, resulted in lower consumption of fermentable substrate, which explains the higher concentration of WSC in silages treated with SAFE 2.5.

The difference in the NDF content reflects the overuse of fermentable carbohydrates, silages treated with SAFE at 2.5L.ton^{-1} decreased yeast and LAB populations, preventing the use of WSC as substrate to production of ethanol and organic acids, respectively. Silages with high NDF, CP and ASH contents reflect the increase of this component proportionally in the dry matter content. Silages that had higher DM loss, reduced DM content, and proportionally increased the other components.

Even after 7d of aerobic exposure on stability test, silages treated with SAFE 2.5 and stored for 21d had lower yeast counts and presented the WSC content, proving the high effect of this dose in preserve nutritional content of sugarcane silages. On the other hand, silages treated with L. Buchneri had lower counts of yeast at 90d but had lower WSC content, because the use of this substrate to growth and organic acid production.

2.5.Conclusion

In conclusion, application of 2.5 $L.ton^{-1}$ of an additive mixture of sodium benzoate, potassium sorbate, and sodium nitrite reduced undesirable microorganisms in corn silages stored for 21d provided decrease in undesirable microorganisms and prolonged aerobic stability and prevent WSC loss during fermentation, even after 168h of aerobic exposure. Lactobacillus Buchneri showed been effective in reduce yeast counts and improve aerobic stability after 90d of storage, therefore reduced WSC content of silages.

Table 1. Characterization of fresh corn treated with additives.

	pH	LAB, log CFU/g	MOLDS, log CFU/g	YEASTS,log CFU/g	DM, %	ASH, % DM	CP, % DM	NDF, % DM	WSC, % DM
Field 1									
Additive means									
Controle	5,18	7,46	6,47	7,00	27,32	4,39	7,37	56,72	16,54
Buchneri	5,14	7,54	6,25	6,96	28,47	4,37	7,23	56,55	12,28
Safe0.5	5,19	7,49	6,44	6,94	27,15	4,45	7,44	56,70	13,40
Safe1.5	5,19	7,72	6,26	6,79	26,83	4,79	7,34	55,70	14,45
Safe2.5	5,16	7,56	6,47	6,96	27,49	4,38	7,26	56,64	14,58
Field 2									
Additive means									
Controle	5,39	7,13	5,40	6,18	34,08	4,12	6,90	53,95	10,00
Buchneri	5,50	7,24	5,88	6,20	34,32	4,17	7,37	57,62	10,51
Safe0.5	5,49	7,48	5,50	6,22	35,02	4,19	7,48	54,52	9,23
Safe1.5	5,61	7,15	6,04	6,37	35,09	4,35	7,39	54,55	10,12
Safe2.5	5,59	7,09	6,03	6,32	35,89	4,10	7,37	44,50	9,65

LAB, Lactic Acid Bacteria; DML, dry matter loss; DM, dry matter; WSC, water soluble carbohydrates; CP, crude protein; NDF, neutral detergent fiber determined. SEM, standard error of the mean. Lowercase letters differ from each other within ADT or ST.

Table 2. Effects of additive and storage time on the fermentation profile and microbial counts of corn silage.

	pH	LAB, log CFU/g	MOLDS, log CFU/g	YEASTS log, CFU/g	Lactic Acid, % DM	Acetic Acid, % DM	Ethanol, % DM	Proprionic Acid, % DM
				Field 1				
Additive means								
Controle	3,73	7,86	2,88	5,30	2,72	1,10	2,58	**
Buchneri	3,71	8,69	2,92	4,83	2,93	1,17	2,48	**
Safe0.5	3,72	8,02	2,61	4,86	2,65	1,16	1,93	**
Safe1.5	3,70	7,96	2,40	4,17	2,68	1,23	1,34	**
Safe2.5	3,68	7,88	2,39	3,51	2,28	1,02	0,88	**
TA means								
3	3,72	9,18	3,57	4,16	$2,28_{b}$	$0,92_{b}$	0,97	**
10	3,74	8,25	3,00	4,22	$3,02_{a}$	$1,14_{a}$	1,16	**
21	3,70	7,77	2,00	5,24	$2,73_{ab}$	$1,2_{a}$	2,11	**
90	3,69	7,13	2,00	4,51	$2,6_{ab}$	$1,28_{a}$	3,13	**
SEM	0,01	0,11	0,08	0,13	0,07	0,05	0,16	
P-Values								
AD	<0,01	<0,01	<0,01	<0,01	0,06	0,37	<0,01	**
TA	<0,01	<0,01	<0,01	<0,01	0,01	0,02	<0,01	**
AD×ST	<0,01	<0,01	<0,01	<0,01	0,40	0,32	<0,01	**
				Field 2				
Additive means								
Controle	3,81	8,10	2,23	4,88	1,21	0,75	0,63	0,29
Buchneri	3,82	8,74	2,18	4,60	1,34	1,01	3,08	0,36
Safe0.5	3,82	8,43	2,23	4,60	1,19	0,92	2,80	0,44
Safe1.5	3,83	8,14	2,21	3,97	1,29	0,81	2,03	0,49
Safe2.5	3,84	7,66	2,22	3,76	1,36	0,52	0,34	0,39
TA means								
3	3,92	9,15	$2,67_{a}$	3,68	$1,34_{b}$	0,68	0,30	$0,09_{b}$
10	3,80	8,52	$2,00_{bc}$	3,73	$0,91_{b}$	0,45	0,21	$0,18_{b}$
21	3,77	8,00	$2,00_{c}$	6,31	$1,08_{b}$	0,64	0,98	$0,18_{b}$
90	3,81	7,18	$2,18_{b}$	3,72	$1,79_{a}$	1,44	5,63	$1,12_{a}$
SEM	0,01	0,13	0,04	0,18	0,07	0,08	0,51	0,06
P-Values								
AD	0,09	<0,01	0,97	<0,01	0,89	0,22	0,18	0,15
TA	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
AD×ST	<0,01	<0,01	0,48	<0,01	0,31	0,03	0,02	0,29

LAB, Lactic Acid Bacteria; SEM, standard error of the mean. Lowercase letters differ from each other within ADT or ST.

Table 3. Effects of additive and storage time on the nutrient composition, dry matter loss and aerobic stability of corn silage.

	DML, %DM	Aerobic Stability, hours	DM, %	ASH, % DM	CP, % DM	NDF, % DM	WSC, % DM
			Field 1				
Additive means							
Controle	$6{,}92_{ab}$	37,48	26,18	4,37	6,73	58,8	3,69
Buchneri	$13{,}43_{a}$	81,50	25,79	4,56	6,9	58,79	3,79
Safe0.5	$7{,}74_{ab}$	38,46	26,43	4,43	6,91	58,36	4,56
Safe1.5	$4{,}58_{b}$	58,83	26,54	4,43	6,78	58,87	4,71
Safe2.5	$5{,}86_{b}$	158,84	26,56	4,19	6,55	58,15	6,27
TA means							
3	**	**	27,01	4,33	$7{,}38_{a}$	56,44	9,16
10	**	**	27,01	4,27	$6{,}28_{c}$	57,89	3,57
21	$5{,}29_{b}$	61,66	26,15	4,25	$6{,}6_{c}$	58,08	2,88
90	$10{,}12_{a}$	88,31	25,03	4,74	$6{,}84_{b}$	59,58	2,8
SEM	0,74	7,9	0,11	0,07	0,05	0,3	0,4
P-Values							
AD	0,01	<0,01	<0,01	0,33	0,08	<0,01	0,22
TA	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
AD×ST	0,37	<0,01	0,05	0,80	0,08	<0,01	<0,01
			Field 2				
Additive means							
Controle	3,4	40	34,38	4,22	7,68	49,65	$1{,}55_{bc}$
Buchneri	3,59	106,8	34,26	4,32	7,69	49,06	$1{,}82_{bc}$
Safe0.5	3,98	53,8	344,67	4,26	7,46	49,95	$1{,}93_{b}$
Safe1.5	2,43	75,4	35,21	4,08	7,32	46,3	$1{,}52_{c}$
Safe2.5	4,9	171,9	34,89	4,2	7,36	46,53	$2{,}47_{a}$
TA means							
3	**	**	$34{,}78_{ab}$	$4{,}36_{a}$	7,33	51,82	$2{,}93_{a}$
10	**	**	$35{,}69_{a}$	$4{,}35_{a}$	7,3	51,74	$1{,}31_{c}$
21	1,76	61	$34{,}49_{b}$	$4{,}03_{b}$	7,45	44,89	$1{,}13_{c}$
90	5,46	118,5	$33{,}72_{b}$	$4{,}13_{ab}$	7,94	44,74	$2{,}06_{b}$
SEM	0,7	7,91	0,18	0,04	0,05	0,62	0,09
P-Values							
AD	0,75	<0,01	0,36	0,11	<0,01	<0,01	<0,01
TA	<0,01	<0,01	<0,01	0,01	<0,01	<0,01	<0,01
AD×ST	0,03	<0,01	0,12	0,24	<0,01	<0,01	0,23

DML, dry matter loss; DM, dry matter; WSC, water soluble carbohydrates; CP, crude protein; NDF, neutral detergent fiber determined. SEM, standard error of the mean. Lowercase letters differ from each other within ADT or ST.

Table 4. Effects of additive and storage time on the fermentation profile and microbial counts of corn silage after 7d of aerobic exposure on stability test.

	pH	MOLDS, log CFU/g	YEASTS log, CFU/g	DM, %	ASH, % DM	CP, % DM	NDF, % DM	WSC, % DM
Field 1								
Additive means								
Controle	$5{,}60_a$	<2,00	9,04	26,74	4,85	7,54	$59{,}94_{ab}$	$0{,}78_b$
Buchneri	$4{,}92_b$	<2,00	8,13	26,21	4,94	7,35	$61{,}93_a$	$0{,}85_b$
Safe0.5	$5{,}81_a$	<2,00	8,92	26,27	4,96	7,41	$62{,}85_a$	$0{,}68_b$
Safe1.5	$5{,}54_a$	<2,00	8,80	26,31	4,99	7,43	$59{,}78_{ab}$	$0{,}83_b$
Safe2.5	$4{,}40_b$	<2,00	7,30	27,05	4,84	7,16	$58{,}19_b$	$2{,}32_a$
TA means								
21	$5{,}56_a$	<2,00	8,57	26,78	$5{,}09_a$	$7{,}24_b$	60,68	0,93
90	$4{,}95_b$	<2,00	8,37	26,25	$4{,}74_b$	$7{,}51_a$	60,40	1,21
SEM	0,12	0,00	0,13	0,17	0,05	0,06	0,49	0,14
P-Values								
AD	<0,01	**	<0,01	0,38	0,81	0,38	<0,01	<0,01
TA	<0,01	**	0,48	0,10	<0,01	0,03	0,73	0,32
AD×ST	0,06	**	<0,01	0,42	0,39	0,44	0,10	0,92
Field 2								
Additive means								
Controle	6,26	<2,00	8,84	33,54	4,61	8,23	52,41	1,52
Buchneri	5,11	<2,00	7,13	34,07	4,39	7,72	54,01	1,52
Safe0.5	6,26	<2,00	8,65	31,48	4,36	7,54	49,97	1,06
Safe1.5	5,85	<2,00	8,85	34,31	4,52	7,76	53,51	1,07
Safe2.5	4,52	<2,00	7,09	35,05	4,36	7,98	49,11	1,40
TA means								
21	5,89	<2,00	8,53	34,77	4,45	7,59b	50,37	1,40
90	5,26	<2,00	7,70	32,58	4,43	8,09a	52,96	1,20
SEM	0,17	0,00	0,21	0,71	0,06	0,08	1,29	0,07
P-Values								
AD	<0,01	**	<0,01	0,54	0,68	0,06	0,74	<0,01
TA	<0,01	**	<0,01	0,16	0,95	<0,01	0,27	0,36
AD×ST	<0,01	**	<0,01	0,64	0,49	0,05	0,03	<0,01

Figure 1: Effect of ADT×ST on (a) pH and (b) LAB of corn silages EXP1.

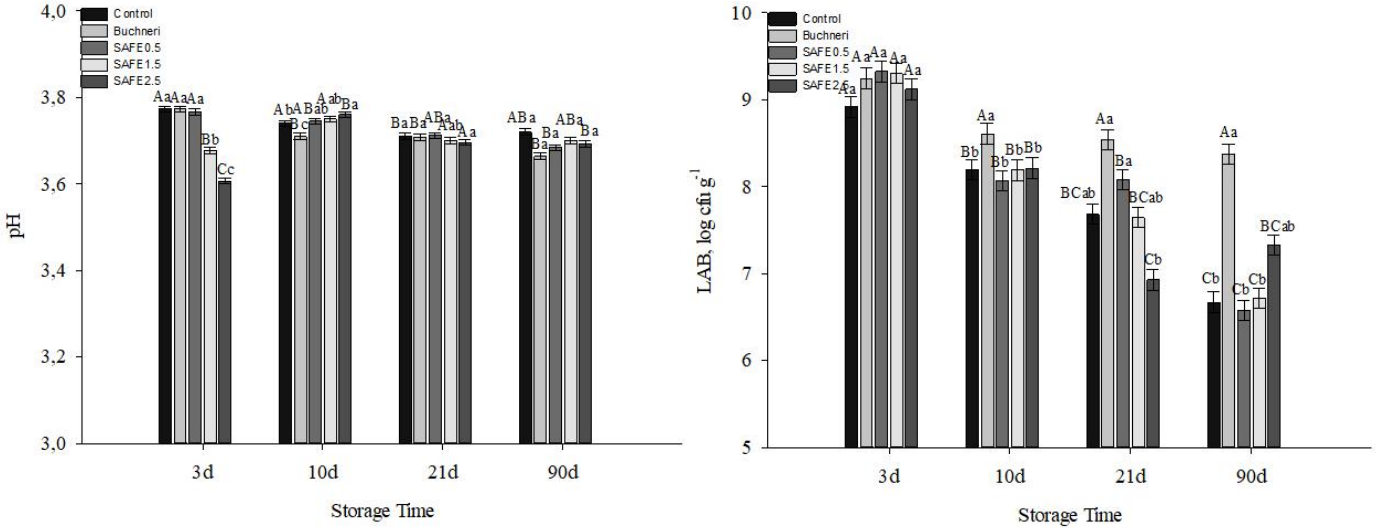


Figure 2: Effect of ADT×ST on (a) Yeasts and (b) Molds counts of corn silages EXP1.

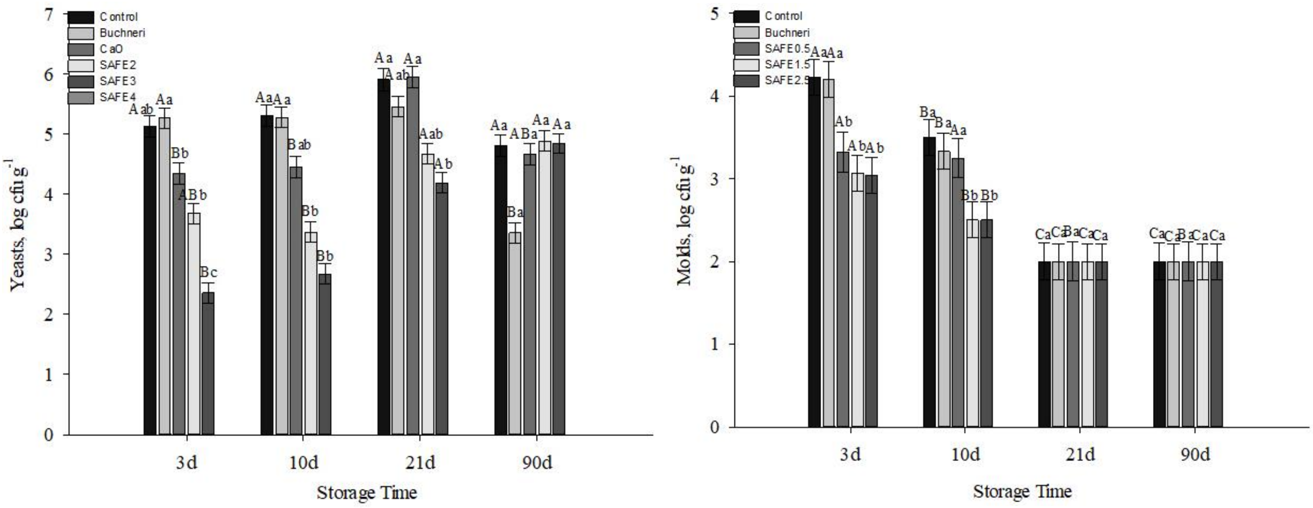


Figure 3: Effect of ADT×ST on (a)ethanol and (b) Aerobic stability of corn silages EXP1.

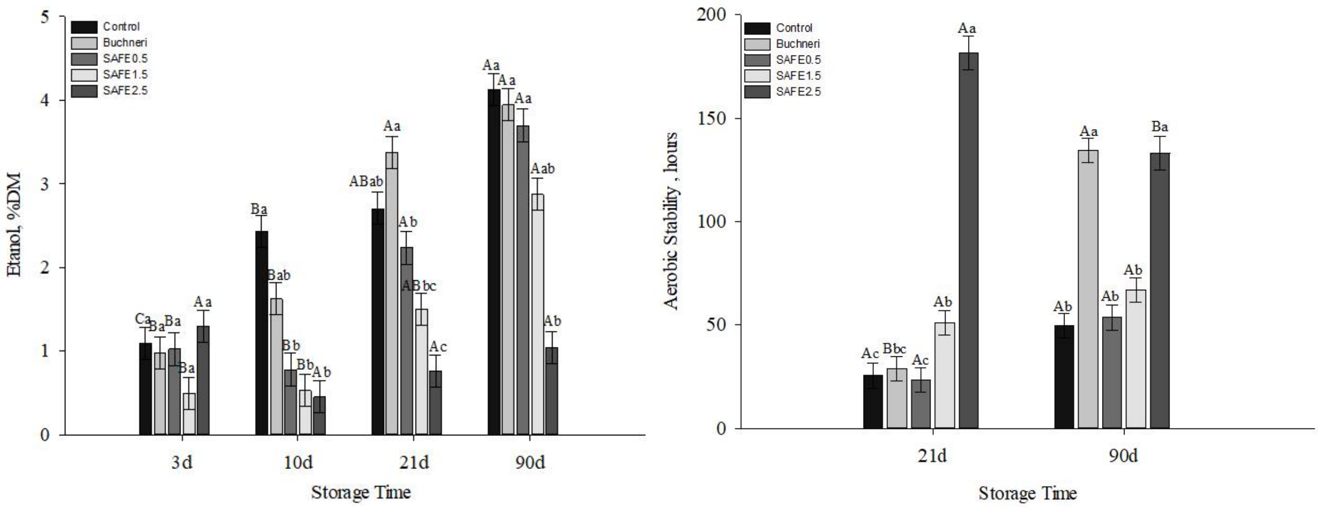


Figure 4: Effect of ADT×ST on (a) NDF and (b) WSC content of corn silages EXP1.

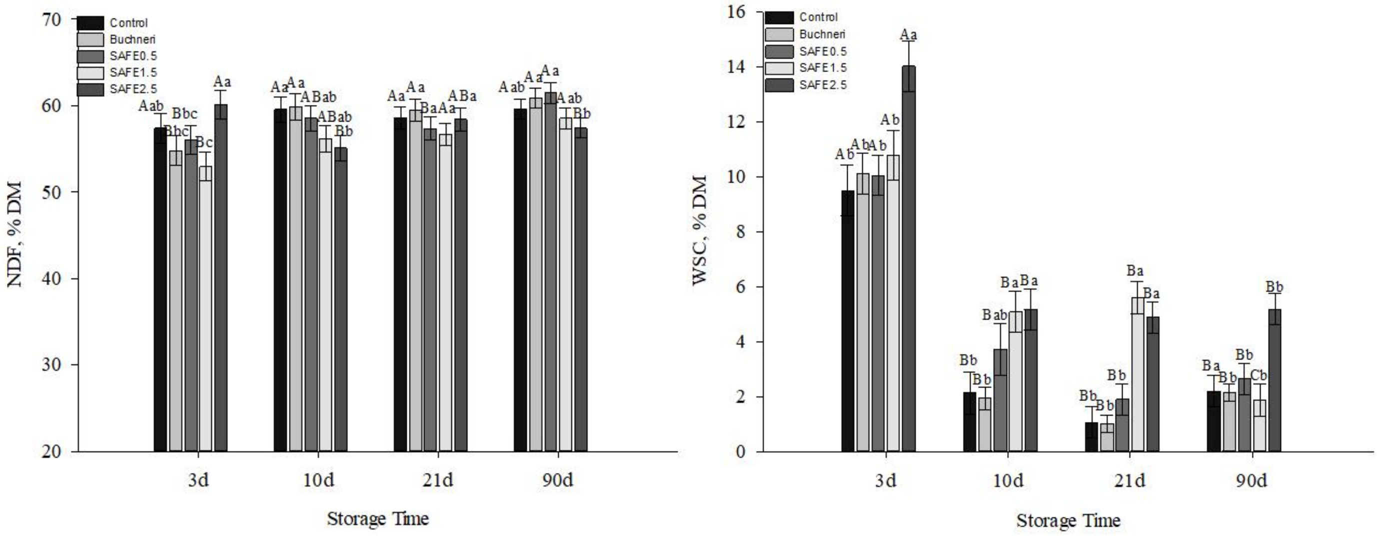


Figure 5: Effect of ADT×ST on (a) aerobic stability and (b) dry matter loss of corn silages EXP2.

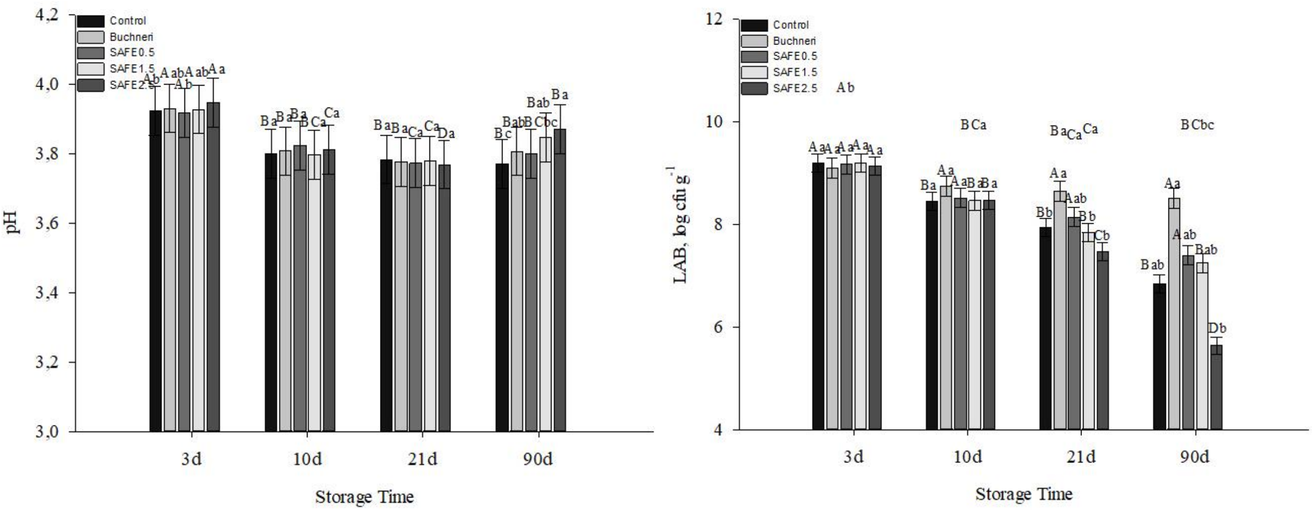


Figure 6: Effect of ADT×ST on (a) yeasts count and (b)ethanol concentration of corn silages EXP2.

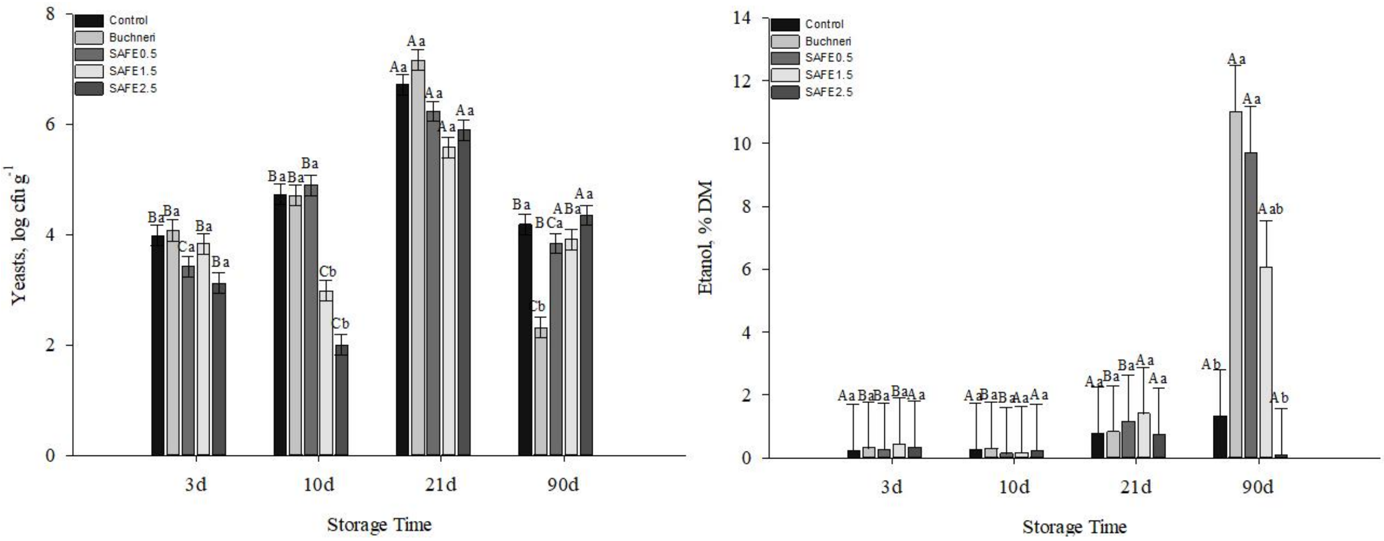


Figure 7: Effect of ADT×ST on (a) CP and (b) NDF content of corn silages EXP2.

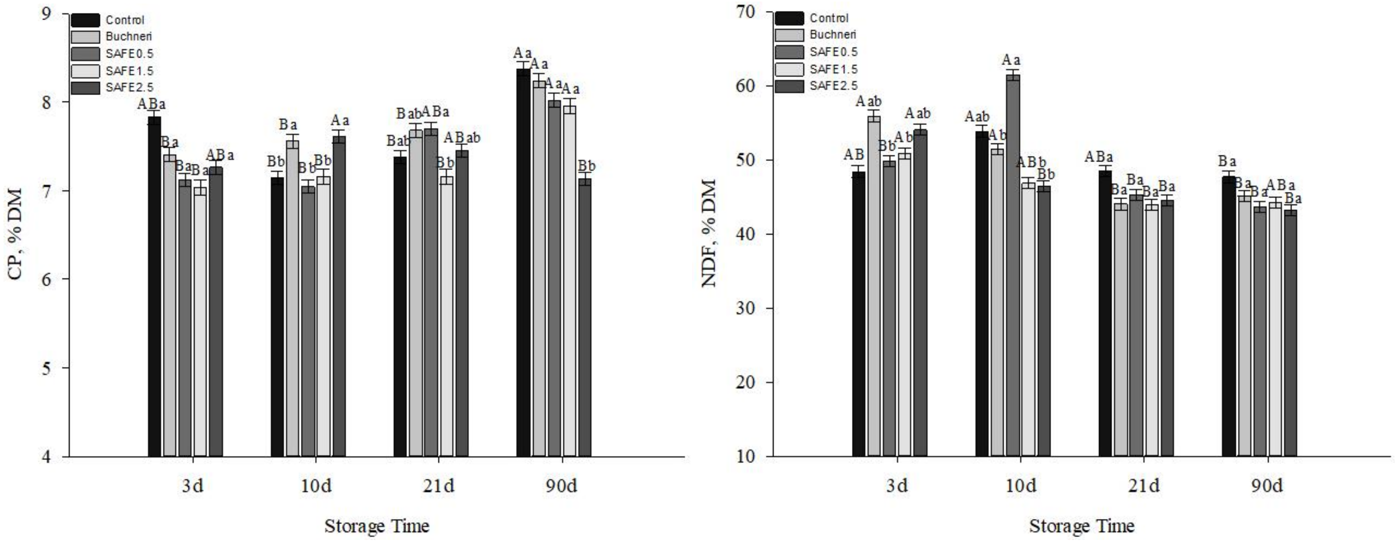


Figure 8: Effect of ADT×ST on yeasts counts of corn silages after 7d of AS EXP1.

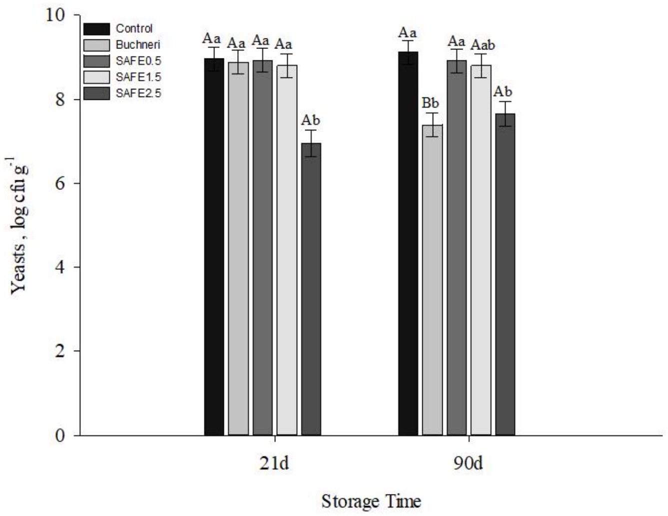
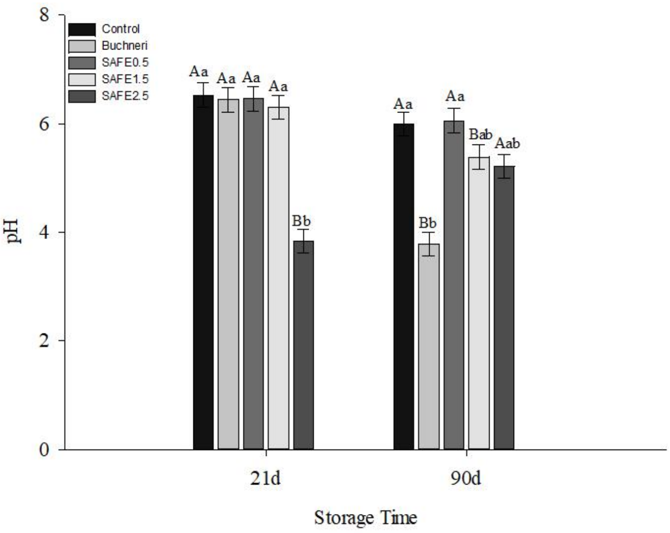


Figure 9: Effect of ADT×ST on (a) pH and (b) yeasts count of corn silages after 7d of AS EXP2.



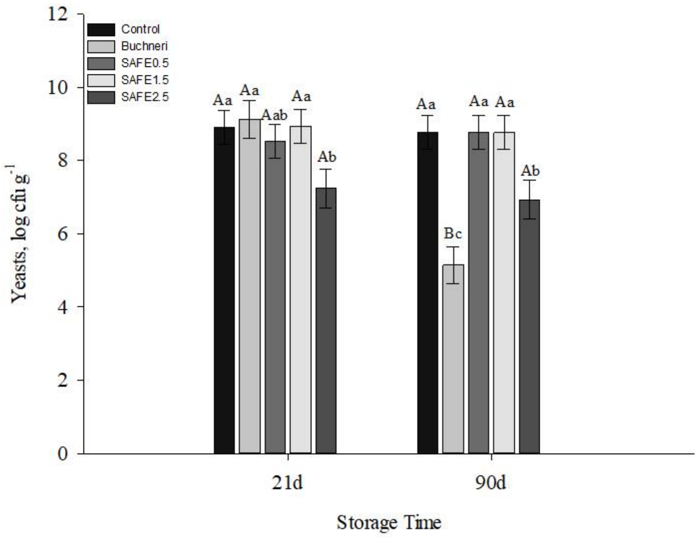
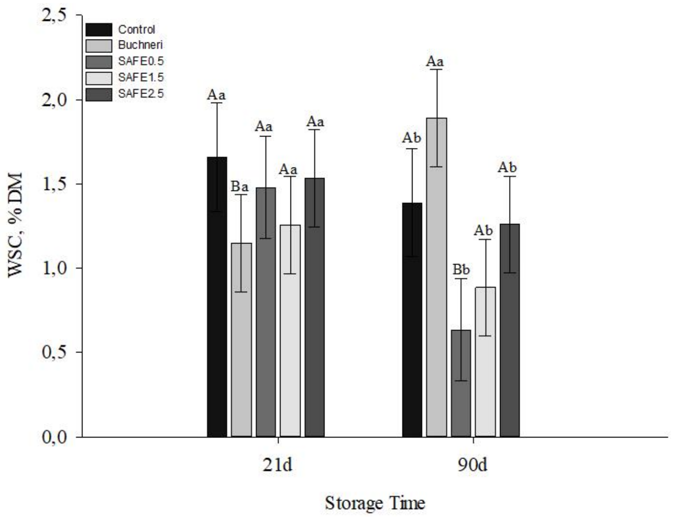


Figure 10: Effect of ADT×ST on WSC content of corn silages after 7d of AS EXP2.



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3. EFFECTS OF CHEMICAL ADDITIVE AND PACKING DENSITY ON THE FERMENTATION PROFILE AND NUTRIENT COMPOSITION OF ENSILED COCKTAIL FORAGE MIXABSTRACT

ABSTRACT

Effect of chemical additives and packing density on mixed forage silage quality. Successful silage production can be challenging for dairy farmers because some legumes and grasses included in forage mixes have a higher buffering capacity compared to other crops commonly grown for dairy cattle. The use of additives in the mixed forage ensiling process can modulate fermentation, decreasing production of total acids in the silage and the growth of undesirable microorganisms, such as yeasts and molds. Thus, the objective of this experiment was to evaluate the effects of a chemical additive and packing density on the fermentation profile and nutrient composition of mixed forage silage. Our hypothesis is that the chemical additive would improve the silage fermentation profile, but this response would be greater when the silage was poorly compacted. The experiment was conducted in a randomized block design with a 2 × 3 factorial arrangement with 6 treatments (two chemical additives × three packing densities) and four replications. Each silo was treated individually without (Control [CON]; 30 ml of distilled water) or with an additive solution of sodium sulfite, sodium metabisulphite and fungal amylase (Additive [ADD]; containing a minimum of 8% sulfur and aiming 0.11% of fresh forage. The forage was ensiled in silos with maximum packing density (D100, 215 kg of DM/m3), 75% of the maximum density (D75, 161 kg of DM/m3) or 50% of the density maximum (D50, 108 kg DM/m3). The silos were opened after 30 days of storage. pH, microbial counts, fermentation profile and nutrient characterization were evaluated. The silage treated with additive showed lower ($P \leq 0.01$) concentrations of total acids, lactic acid, acetic acid and ethanol compared to control. No chemical additive effect was observed on yeast and mold counts. In the experiment, D50 treatments had higher ($P \leq 0.01$) pH and counts of yeast and molds compared to D100 silage. Although there was a higher contagion in yeast and mold on D50 silage, there were minimal changes in nutrient composition, regardless of treatment.

KEYWORDS: buffering capacity, aerobic stability, grasses, yeasts

3.1. Introduction

Cocktail forage mixes are often used as cover crops to provide physical, chemical, and biological benefits to the soil (Fageria et al., 2005). However, selecting species within a mixture for forage quality could offer additional benefits by supporting the requirements of dairy cattle through a lowcost forage source. Although forage mixes containing cool season crops seeded later in the growing season may yield forage with high nutritive value (Neville et al., 2008), successful production of silage can be challenging for dairy producers because some legumes and grasses included in these cocktail forage mixes (i.e. ryegrass or clover) have a greater buffering capacity compared to other crops commonly grown for dairy cattle (Harrison et al., 2003). High buffering capacity (i.e. high ammonia and/or cation mineral [Ca, K, Mg] concentrations) can make silage more susceptible to DM and nutrient losses, undesirable fermentation (particularly clostridial or butyric acid based fermentation), and promotes aerobic spoilage in the ensiled material (Wilkinson and Davies, 2013; Kung et al., 2018).

Packing density is important to maintain silage nutritive value by avoiding air exposure, which increases aerobic respiration and heat production (Muck et al., 2004). Greater porosity delays anaerobic fermentation, allowing the growth of aerobic microorganisms that consume readily available carbohydrates and organic acids (Pitt and Muck, 1993). Ruppel et al. (1995) found that the packing density was negatively correlated to storage losses, which increased up to 20 percentage units when packing density decreased from 320 to 160 kg DM/m^3. Poorly packed silage may benefit from the use of chemical additives, reducing nutrient losses from the silo and improving silage palatability. Sodium metabisulfite has strong reducing capacity, consuming large amounts of O_2, and stimulating rapid anaerobic conditions in the silos (Kung et al., 2003). Besides, sulfur dioxide, a product of the reaction between sulfur metabisulfite and moisture or acids, is a powerful antimicrobial agent (Kung et al., 2003). McDonald et al. (1991) reported that silages treated with sodium metabisulfite had greater pH, lower concentration of fermentation acids, reduced proteolysis and deamination, and decreased DM and nutrient losses compared with the corresponding untreated silages. Likewise, Ahmadi et al. (2021) observed that sodium metabisulfite can reduce fermentation, decreasing total acid production in silage, and the growth of undesirable microorganisms, such as yeast and molds. To our knowledge, studies evaluating the effects of packing density and sulfur-based additives in cocktail mixed forages are lacking in the literature.

Thus, the objective of this experiment was to evaluate the effects of a chemical additive and packing density on the fermentation profile and nutrient composition of cocktail mix silage. We hypothesized that the chemical additive would improve fermentation profile of cocktail mix silage, but this response would be greater when silage was poorly packed.

3.2. Material e Methods

This experiment was carried out in a completely randomized block design with a 2 × 3 factorial arrangement of treatments and consisted of 6 treatments (two chemical additives × three packing densities) with four replications (order of forage load emptying considered a block) for a total of 24 laboratory silos.

Cocktail mix forage was seeded in a field of the University of Wisconsin Marshfield Agricultural Research Station (Marshfield, WI) with 22, 10, 5, 5, and 5 kg/ha of brown midrib (BMR) sorghum sudangrass (Nutri King BMR 6, Gayland Ward Seed, Hereford, TX), Italian ryegrass (Kingfisher Allegro mix, Byron Seeds, Rockville, IN), red clover (Marathon, Welter Seed and Honey Co., Onslow, IA), berseem clover (Frosty, GO Seed, Salem, OR), and hairy vetch (AU Merit, Smith Seed Services, Halsey, OR), respectively. Second cut cocktail forage mix was mowed on September 15, 2021, using a, MacDon Model R113 pull type mower/conditioner (MacDon Industries Ltd.), to a 10 cm stubble height. This mower conditioner is configured with a maximum 4.0 m cutting width, and forage is subsequently conditioned with 3.3 m wide steel intermeshing conditioning rollers. Forage was allowed to wilt (18 h) to approximately 39% DM, and 2 windrows merged immediately before harvest on September 16th, 2021, at 1000 h using a pull type forage harvester (model F 41, Dion AG, Boisbrian, QC, Canada) set for a theoretical length of cut of 1.27 cm. Forage was chopped directly into a forage wagon and immediately transported to the lab. No inoculant or other additives were added to the forage during harvest. Forage subsamples were collected during unloading of forage from the forage wagon (1 sample/block) and analyzed for pH, microbial counts, and nutrient characterization of fresh, unfermented material.

Chopped forage was unloaded from the forage wagon in amounts sufficient for each block of silos to be made at a time to minimize drying effects on the forage. Forage subsamples (2.04, 1.59, and 1.14 kg for maximum density, 75% of maximum density, and 50% of the maximum density, on average, respectively) from each unloading time were collected and each laboratory silo was individually treated without (CON; 30 ml distilled water) or with a sodium sulfite, sodium metabisulfite, and fungal amylase additive solution (ADD; containing a minimum of 8% Sulfur and targeting for 0.11% of fresh forage according to manufacturer recommendation), and hand mixed with harvested forage immediately before ensiling. The amount of ADD was adjusted depending on the forage amount used in each packing density treatment. The chemical additive contained a combination of sodium sulfite, sodium metabisulfite, and fungal amylase.

Forage was ensiled in laboratory silos (3.79 L plastic buckets) with maximum packing density (D100, 215 kg of DM/m^3), 75% of the maximum density (D75, 161 kg of DM/m^3) or 50% of the maximum density (D50, 108 kg of DM/m^3). All silos were sealed with a lid and tape, weighed, and then stored at room temperature (22.3 ± 0.3 ˚C) until reaching 30 d of storage length. After reaching 30 d of fermentation, laboratory silos were weighed, opened, and the top 4 cm of material was discarded. Subsamples were collected for pH, microbial counts, fermentation profile and nutrient characterization. Dry matter losses were calculated as the difference between the weight of the material placed in each laboratory silo at ensiling and the weight of the silage after the designated length of storage divided by the weight of material at ensiling, corrected for the respective initial or final DM concentration (Jobim et al., 2007). Laboratory silos containing approximately 350 g of remaining silage that had been mixed and loosely replaced after sample collections were used for determination of aerobic stability. Wireless temperature sensors (HOBO temperature data logger 64 k; Onset Computer Corporation, Cape Cod, MA, USA) set to record temperatures every 30 min for 240 h were placed in the geometric center of laboratory silos. Three additional sensors were also placed to record ambient temperature, which averaged 22.3 ± 0.3 ˚C for the duration of the aerobic stability assay. Aerobic stability was defined as the number of hours before silage increased 2˚C above the baseline of room temperature.

Extracts of fresh and silage samples were obtained from 20 g of an undried, unground sample diluted 10-fold (mass basis) in 0.1% peptone water (Oxoid CM0090), blended for 60 s in a high-speed blender, and filtered through two layers of cheese cloth. The extract was divided into two subsamples. The first subsample was used to measure pH in duplicate using a pH electrode (Accumet AP85 Portable Waterproof; Thermo Fisher Scientific Inc.). The second subsample was evaluated for yeast and mold enumeration via a pour plating method in a 10-fold serial dilution on malt extract agar (Difco 211220) acidified with 85% lactic acid. Agar plates were incubated at 32°C for 48 h for yeast, and an additional 72 h for mold counts. Yeast and mold counts were evaluated on the same plates and separated by visually examining the growth of colonies.

Silage samples were submitted to a commercial laboratory (Dairyland Laboratories Inc., Arcadia, WI) for fermentation profile analysis. Organic acids (lactic, acetic, propionic, and butyric acids), ethanol, and 1,2 propanediol were analyzed by HPLC (Canale et al., 1984). Ammonia nitrogen (ammonia N) was determined by colorimetry using a Technicon Automatic Analyzer (RFA 300, Alpkem Corporation, Clackamas, OR) with a method adapted from Noel and Hambleton (1976).

Fresh and silage samples were dried in a forced air oven set at 60℃ for 48 h to determine DM concentration, ground to pass through a 1 mm sieve using a Wiley Mill (Thomas Scientific, Swedesboro, NJ), and sent to a commercial laboratory (Dairyland Laboratories Inc., Arcadia, WI) for nutrient characterization. Dried, ground samples were analyzed by near infrared spectroscopy (Foss model 5000; Foss North America Inc., Eden Prairie, MN) with calibration equations for ash, water soluble carbohydrates (WSC), ether extract, crude protein (CP), neutral detergent fiber treated with alpha amylase and sodium sulfite (aNDF), neutral detergent fiber treated with alpha amylase and sodium sulfite, corrected for residual ash (aNDFom), and starch based on wet chemistry procedures described in AOAC method 942.05 (AOAC, 2005), Derias (1961), AOAC method 920.39 (AOAC, 2005), AOAC method 990.03 (AOAC, 2005), AOAC method 2002.04 (AOAC, 2005), AOAC method 2002.04 (AOAC, 2005), and Vidal et al. (2009), respectively. The digestibility of NDF after 30 h ($NDFD_{30h}$) was determined using a method adapted from Goering and Van Soest (1970) and residue was analyzed for aNDFom concentration by near infrared spectroscopy as described before.

The data of fresh corn treatments did not undergo statistical analysis. The microorganism count data were transformed to log 10 to be submitted to statistical analysis. Data were analyzed as a 4 × 5 factorial arrangement with repeated measures over time (3; 10; 21; and 90d of ST) by the SAS® program PROC MIXED procedure observing the effects of additive (ADT), Storage time (ST) and their interaction (ADT × ST). The averages were compared using the Tukey test at 5% probability level and the experimental error as a random effect.

3.3. Results

Effects of chemical additive and packing density on fermentation profile and microbial counts are shown in Table 2. Silage treated with ADD had lower ($P \leq 0.01$) concentrations of total acids, lactic acid, acetic acid, and ethanol compared with CON. However, the pH of ADD silage was greater ($P = 0.001$) than CON silage. Cocktail mix silage ensiled at D50 had lower ($P \leq 0.03$) concentrations of total acids, lactic, and acetic acids compared with D100. Conversely, no effects of chemical additive on yeast and mold counts were observed in this experiment. In the current experiment, D50 treatments had greater pH, and yeast and mold counts compared with D100 silage. The lower production of total acids may have limited pH decline in D50 silages. In general, D100 and D75 had a more extensive fermentation than D50, evidenced by lower pH and greater production of organic acids.

Effects of chemical additive and packing density on the nutrient composition and dry matter recovery of ensiled cocktail forage mix are shown in Table 3. Although there were greater yeast and mold counts in D50 (Table 2), silage had minimal changes in nutrient composition regardless of treatment. Silage treated with ADD had greater ($P = 0.01$) DM concentrations compared with CON, whereas DM recovery was lower ($P = 0.04$) for ADD than CON. Despite the effects of DM recovery for ADD treated silages, all cocktail mix silages treatments had DM recovery values above 99% DM and this effect is unlikely to be biologically meaningful.

3.4. Discussion

Chemical additives containing sodium sulfite and sodium metabisulfite are readily available and effective preservatives with known bactericidal properties. Similar products are widely used in the food industry for preservation by suppressing microbial growth (Natskoulis et al., 2018). These additives are also used to influence fermentation in silage to restrict cellular respiration in the ensiled material. Cellular respiration is restricted by the liberation of sulfur dioxide (SO_2), which contributes to the formation of an anaerobic environment (Knodt, 1950). Previous studies reported sodium sulfite and sodium metabisulfite can suppress microorganisms involved in protein degradation and ammonia formation (Gould and Russel, 2003; Ahmadi et al., 2018). Also, literature evaluating sodium metabisulfite as a preservative for legume grass silage observed a reduction in organic acids and ammonia, increasing silage pH (Mathison et al., 1979). Similarly, Romero Gil et al. (2016) found that sodium metabisulfite was an efficient additive to reduce the growth of lactic acid bacteria (LAB) and yeast. Conversely, no effects of chemical additive on yeast and mold counts were observed in this experiment.

. Zhang and Yu (2015) evaluated the effects of two different levels of packing density (500 vs. 600 kg as fed/m^3) on the fermentation profile of grass silage and observed a greater lactic acid concentration, coupled with a lower pH, butyric acid concentration, and ammonia N concentration in silage with the greater density. Likewise, Savage et al. (2015) observed greater lactic acid concentration in corn silage ensiled at 240 kg of DM/m^3 compared with 170 kg of DM/m^3. Additionally, the lower lactic acid concentration in poorly packed silage observed in the current study was likely related to delayed growth of lactic acid producing bacteria due to greater oxygen entrapped in the silage mass (Pahlow et al., 2003), allowing for greater yeast and mold proliferation in comparison to D100. Greater packing in silage removes oxygen and impairs air penetration in the forage mass (Borreani et al., 2018), encouraging a desirable fermentation, promoting LAB growth, increasing organic acid production, and promoting a rapid decrease in pH, which can delay yeast and mold growth (Pahlow et al., 2003).

3.5.Conclusion

Overall, this study highlights the importance of well packed silage. Even though fermentation was adequate for all silage samples, poorly packed cocktail forage mix silages had greater yeast and molds counts and a less pronounced fermentation. Contrary to our hypothesis, the use of a chemical additive restricted bacterial fermentation and did not improve aerobic stability. Future studies must evaluate the efficacy of other chemical and bacterial additives as fermentation modulators in cocktail mix silage.

Table 1. Nutrient composition, pH, and yeast and mold counts of harvested, fresh cocktail forage mix (average ± SD).

Item[1]	Forage cocktail mix
pH	6.1 ± 0.04
DM, % as fed	39.1 ± 3.47
CP, % of DM	11.6 ± 0.25
EE, % of DM	3.6 ± 0.02
NDF, % of DM	53.1 ± 0.52
Starch, % of DM	3.2 ± 0.31
Ash, % of DM	8.1 ± 0.21
WSC, % of DM	10.7 ± 0.14
Yeast counts, log CFU/g	3.9 ± 0.25
Mold counts, log CFU/g	3.8 ± 0.10

[1]DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; WSC, water-soluble carbohydrates, CFU: colony-forming unit.

Table 2. Effects of chemical additive and packing density on the fermentation profile and microbial counts of ensiled cocktail forage mix[1].

Item[2]	CON			OS			SEM[3]	*P*-values		
	Full	75%	50%	Full	75%	50%		AD	DEN	AD × DEN
pH	4.26	4.28	4.32	4.31	4.34	4.40	0.02	0.001	0.001	0.64
Total acids, % DM	8.2	8.2	6.8	6.3	5.0	4.3	0.50	0.001	0.001	0.17
Lactic Acid, % DM	6.6	6.7	5.5	5.2	4.1	3.3	0.42	0.001	0.001	0.16
Acetic acid, % DM	1.5	1.5	1.4	1.1	0.9	0.9	0.09	0.001	0.03	0.25
Ethanol, % DM	0.5	0.6	0.6	0.5	0.3	0.5	0.08	0.01	0.31	0.08
1,2 Propanediol, % DM	0.06	0.09	0.12	0.02	0.01	0.07	0.02	0.001	0.01	0.54
Ammonia-N, % CP	3.7	3.8	3.7	3.7	3.7	3.6	0.22	0.62	0.60	0.73
Yeast, log CFU/g	3.55	4.60	5.36	4.25	4.74	6.10	0.71	0.21	0.01	0.80
Mold, log CFU/g	3.88	3.39	4.57	3.61	3.52	5.28	0.71	0.64	0.02	0.62

[1]Treatments consist of AD: effect of additive (CON: 30 ml distilled water; ADD: sodium sulfite, sodium metabisulfite, and fungal amylase; 0.11% of fresh forage]); DEN: effect of packing density ([D100 (maximum amount of material in laboratory silo), D75 (75% of full density, as fed basis) and D50 (50% of full density, as fed basis)]).[2]DM, dry matter; Ammonia-N, ammonia nitrogen; CP, crude protein; CFU, colony-forming unit.
[3]Greatest standard error of the mean.

Table 3. Effects of chemical additive and packing density on the nutrient composition and dry matter recovery of ensiled cocktail forage mix[1].

Item[2]	CON			OS			SEM[3]	*P*-values		
	Full	75%	50%	Full	75%	50%		AD	DEN	AD × DEN
DM, % as fed	39.9	40.0	39.2	41.7	42.0	41.3	0.92	0.002	0.49	0.99
DM Recovery, % DM	99.2	99.3	99.3	98.8	99.2	99.2	0.19	0.04	0.13	0.43
Ash, % DM	9.7	9.4	9.5	9.5	9.5	9.9	0.19	0.29	0.18	0.16
WSC, % DM	5.0	5.0	4.8	4.8	5.2	5.3	0.20	0.30	0.38	0.06
EE, % DM	4.4	4.2	4.2	4.3	4.2	4.2	0.09	0.33	0.31	0.66
CP, % DM	12.7	12.6	12.7	12.5	12.6	12.5	0.24	0.52	0.97	0.82
aNDFom, % DM	49.8	50.6	50.6	50.8	50.5	50.2	0.76	0.70	0.90	0.51
Starch, % DM	2.8	2.7	3.3	2.8	2.5	3.0	0.27	0.31	0.06	0.82
NDF_{30h}, % aNDFom	69.4	69.6	68.5	67.9	68.7	68.3	0.70	0.06	0.31	0.49

[1]Treatments consist of AD: effect of additive (CON: 30 ml distilled water; ADD: sodium sulfite, sodium metabisulfite, and fungal amylase; 0.11% of fresh forage); DEN: effect of packing density ([D100 (maximum amount of material in laboratory silo), D75 (75% of full density, as fed basis) and D50 (50% of full density, as fed basis)]).

[2]DM, dry matter; WSC, water soluble carbohydrates; EE, ether extract; CP, crude protein; aNDF, neutral detergent fiber determined using α-amylase and sodium sulfite; $NDFD_{30h}$, neutral detergent fiber digestibility after 30 h of incubation in rumen fluid.[3]Greatest standard error of the mean

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FINAL CONCLUSION

The results of both experiments demonstrate the significant impact of additives, storage time, and packing density on the fermentation quality and aerobic stability of silages. In the corn silage experiment, the microbial additive Lactobacillus buchneri effectively reduced yeast growth and improved aerobic stability during extended storage, while SAFE additives showed greater efficacy in suppressing yeast, mold growth, and ethanol production during shorter storage periods. This highlights the importance of additive application to optimize silage preservation.

In the mixed forage silage experiment, the chemical additive containing sodium sulfite, sodium metabisulphite, and fungal amylase improved fermentation by reducing the production of acids, ethanol, and undesirable microbial growth, particularly when applied to silages with lower packing densities. However, silages with higher packing densities demonstrated superior overall fermentation quality and lower yeast and mold counts, emphasizing the critical role of proper compaction in silage preservation.

These findings underscore the combined influence of additives, storage management, and compaction on silage quality. They provide valuable insights for optimizing ensiling practices across different forage types to improve nutrient retention, reduce spoilage losses, and enhance aerobic stability.