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# Effect of cactus pear mucilage as a moistening additive for corn grain silage

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**Abstract.** The purpose of this study was to evaluate the effects of different proportions of cactus pear mucilage as a moistening additive on chemical composition, ammonia nitrogen, volatile fatty acids, microbial dynamics, fermentation losses, pH, and aerobic stability of corn grains in the form of silage throughout storage periods. The study was carried out in a completely randomized design with a factorial arrangement (5×2), with four replications. The factors corresponded to five forms of moistening for corn grain silage, 0, 5, 10, 20, and 40% of cactus pear mucilage (MA) associated or not with urea (U) in the level of 1.5% of DM. The lowest DM content was found in the silage moistened with 40% of cactus pear mucilage, and the greatest CP content was found in the same treatment but when urea was added. Lower contents of propionic and butyric acids were observed in the silages moistened with 5 and 10% of cactus pear without and with the addition of urea. Dry matter recovery was higher in the treatments with water and 10% of cactus pear. The use of urea affected the LAB population only at 15 d of silo opening, providing a bigger population (6.58 logs CFU $g^{-1}$) when compared to silages without urea (6.27 logs CFU $g^{-1}$). The lower concentrations of propionic and butyric acids observed in the silage moistened with 5 and 10% of cactus pear mucilage, regardless of the addition of urea. However, it is recommended to use 10% of cactus pear mucilage without the addition of urea, as it provides better indicators of good-quality silage.

***Keywords:*** *anaerobiosis, Cactaceae, energy, fermentation, forage.*

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## Introduction

Corn grain (*Zea mays* L.) is the main feed in animal production. Its use is almost indispensable in diets supplied to ruminants, as it the main energy source responsible for maintaining adequate production rates. However, this ingredient presents digestion difficulties due to the starch composition and its physical relationship with the protein matrix (Ngonyamo-Majee *et al.,* 2008). Therefore, depending on the texture, the grains can influence the performance of the animals and in the quality of the ensiled ingredient, compromising the productive performance of the animals and the profit from the activity.

One of the options to improve the nutritional quality of corn grain silage, is the moistening process, which consists of moistening the grain, aiming to reach the desired moisture content for the fermentation process in the silo, enabling adequate storage conditions. This is an effective procedure, as in addition to improving digestibility, it conserves corn by reducing material losses and deterioration as a result of pest action, temperature, and humidity changes, reduces transportation costs and presents minimal effects of market price fluctuations (Arcari *et al.,* 2016). Therefore, the moist corn silage technique is an alternative to maximize its use by the animals (Junges *et al.,* 2017). Thus, to improve economic and storage conditions, the farmers can choose to buy corn grains at times when the price is affordable, then adopt the ensiling process with grain moistening and use this silage strategically in periods of forage scarcity and elevated prices, working as a maneuver to avoid excessive expenses. Another option has been proposed in the use of a wide variety of additives aiming to improve the fermentation process and aerobic stability, through the inhibition of undesirable microorganisms after opening the silo (Morais *et al.,* 2017). The moistening additive is used in grains to improve digestibility (Kung Junior *et al.*, 2014) and silage quality (Saylor *et al.,* 2020). However, for silages rich in energy such as cereal grains, the control over the population of unwanted microorganisms is also important to ensure feed safety (Sadhasivam *et al.,* 2017).

Cactus pear-based silages are already used in ruminant feeding (Pereira *et al.,* 2021), therefore it can be an option in the moistening process of corn grain silage. The mucilage of cactus pear consists of glycoproteins, organic acids, sugars, and other carbohydrates, that enable the capacity to retain water and implies in the low resistance of the cactus to pH drop, inhibiting the development of yeasts (Carvalho *et al.,* 2020), as well as the proliferation of clostridium and enterobacteria (Toit *et al.,* 2018). Cactus pear-based silages maintain the fermentation pattern and can, therefore, adequately preserve the ensiled material (Macêdo *et al.,* 2018) in addition to being an alternative to feed scarcity in years of drought.

Another additive often associated with low-protein silages, such as the corn grain silage, is urea, because it improves the protein value of the feed, besides acting effectively in moist silages ensuring the supply of substrate for the microorganisms throughout the storage process, minimizing fermentative losses, and increasing the aerobic stability (Zambom *et al.,* 2014; Mombach *et al.,* 2018). Thus, facing the need to produce better quality feed and reduce losses, it was hypothesized that corn grain silage moistening with cactus pear mucilage, combined with commercial urea, may be a viable strategy, as they are low-cost additives, easy to access and handle, and can conserve the material and improve quality by reducing losses and increasing the protein content and energy constituents of the silage. The purpose of this study was to evaluate the effects of different proportions of cactus pear mucilage as a moistening additive on chemical composition, ammonia nitrogen, volatile fatty acids, microbial dynamics, fermentation losses, pH, and aerobic stability of corn grains in the form of silage throughout storage periods.

## Material and Methods

### *Design and treatments*

The study was carried out in a completely randomized design with factorial arrangement (5 × 2), with four replications. The first factor corresponded to five (5) moistening additives, which were 0, 5, 10, 20 and 40% of cactus pear mucilage on as fed basis. The treatment of 0% corresponded to the non-inclusion of cactus pear mucilage, in which the ground corn grain silage was moistened with 250 mL

$kg^{-1}$ of water. The second factor was the inclusion 0 or 1.5% of urea in the corn grain silage, on as fed basis based on the recommendations by Vilela *et al.* (2014).

***Ingredients and ensiling process***

The cactus pear used was *Opuntia stricta* variety *"Orelha de Elefante Mexicana"*. The corn grains (*Zea mays* hybrid BRS 3046) were ground in a forage chopper machine using a 5.0 mm sieve before ensiling and had their chemical composition analyzed (Table 1). After grinding the material, the corn was rehydrated with water or cactus pear mucilage and the urea was added or not according to the proposed treatments. The material was constantly mixed during rehydration to ensure homogeneity and the final resulting mass was ensiled in experimental silos of PVC tubes of 0.10 m of diameter and 0.50 m of length. The silages were stored with an average density of 1.244 kg $m^{-3}$.

**Table 1.** Chemical composition of corn grain and cactus pear mucilage.

| Chemical composition (g $kg^{-1}$ DM) | Ingredients | |
|---|---|---|
| | Corn grain | Cactus pear mucilage |
| Dry matter (as fed) | 834 | 894 |
| Crude protein | 105 | 77.8 |
| Neutral detergent fiber | 150 | 311 |
| Acid detergent fiber | 45.3 | 221 |
| Mineral matter | 2.68 | 21.7 |
| Ether extract | 46.9 | 57.9 |
| Non-fiber carbohydrates | 695 | 400 |

*DM= Dry-matter.

***Chemical composition determination***

The samples of ground corn grains and *in natura* cactus pear mucilage were collected before ensiling, and silage samples were collected 90 d after the silo closure. The samples were placed in aluminum foil trays, identified, weighed, and subjected to pre-drying in a forced air circulation oven at 55 ºC for 72 h. Then they were ground in a Willey knife mill with sieve of 1.0 mm and analyzed for dry matter (DM, method 967.03), crude ash (method 942.05), crude protein (CP, method 981.10) and ether extract (EE, method 920.29), according to Association of Official Analytical Chemistry (AOAC, 2012).

The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined through the methods of Van Soest *et al.* (1991) with the modification proposed by Senger *et al.* (2008). The autoclave had the temperature adjusted to 110 °C for 1 h. The NDF content was corrected for ashes and protein ($NDF_{ap2}$) and, for this purpose, the residue from boiling in neutral detergent was incinerated in a muffle at 600 °C for 4 h, and the correction for protein was carried out by deducting the content of neutral detergent insoluble protein (NDIP). The Non-fibrous carbohydrates (NFC) were calculated according to Weiss (1999) as, NFC (%) = 100 - (%$NDF_{ap}$ + %CP + %EE + % crude ash). The NFC of the silages with urea was calculated as proposed by Hall (2000): NFC = 100 - [(%CP - %CP from urea + %urea) + %$NDF_{ap}$ + %EE + % crude ash].

***Dry matter loss and recovery determination***

To obtain the values of losses through gases and effluents, a Bunsen valve was adapted to the lid of the experimental silos, to allow the escape of gases from fermentation. The sand was deposited at the bottom of each silo, separated from the grains by a layer of TNT fabric, so that the total weight, together with the lid, reached 1.0 kg, making it possible to measure the amount of effluent retained.

The silos were opened after a period of 90 d from the closure. The silage dry matter losses through effluents and dry matter recovery were calculated according to the equations described by Jobim *et al.* (2007): EL = (Wo - We) ÷ (GMef) × 1000, EL = Effluent loss (kg $t^{-1}$ of green mass); Wo = Weight of the set (silo + sand + fabric + lid) at opening (kg); We = Weight of the set (silo + sand + fabric + lid) at ensiling (kg); GMeF = Green mass of ensiled forage (kg) and DMR = (FMo × DMo) ÷ (FMc × DMc) × 100, DMR = dry matter recovery index; FMo= forage mass at opening; DMo = DM content at opening; FMc = forage mass at closure; DMc = DM content of forage at closure. For gas losses, it was used the equation described by Zanine *et al.* (2010): GL = (FWc - FWo) ÷ (FMc × DMc) × 10000, GL = gas loss (%DM); FWc = weight of full silo at closure (kg); FWo = weight of full silo opening (kg); FMc = forage mass at closure (kg); DMc = forage dry matter concentration at closure (%).

***Ammonia nitrogen ($N\text{-}NH_3$) and pH determination***

The pH was determined in the silo opening periods of 0, 15, 45 and 90 d. The pH was analyzed in an aqueous extract prepared with 25 g of a sample of the ensiled material from each treatment and 100 mL of distilled water. The pH was measured in a pHmeter (DM-22; Digimed®, São Paulo, SP, Brazil). For the determination of $N\text{-}NH_3$, 30 g of fresh silage were collected 90 d after ensiling, to which 270 mL of distilled water were added and homogenized for 4 min in a digital LED hotplat magnetic stirrer (MS-H280-Pro®). After this process, the extract was filtered and went through distillation with magnesium oxide and calcium chloride using the Kjeldahl method according to AOAC (2012).

***Volatile fatty acids determination***

In order to quantify the content of volatile fatty acids (VFA), a portion of each collected sample was used, and the lactic, acetic, propionic, and butyric acids contents were determined in the silages after 90 d of storage, through the method mentioned by Kung Jr. *et al.* (2018), where juice was extracted using a manual press. The samples were centrifuged and later, the VFA were analyzed by high-resolution liquid chromatography in a high-performance liquid chromatograph (HPLC), model SPD-10ª VP detector coupled to an ultraviolet (UV) detector, using a wavelength of 210 *n*m. Decimal alcohol degree of boiling was determined using an ebulliometer as recommended by Maia and Campelo (2006).

***Aerobic stability***

After a period of 90 d, the silos were opened and 10 cm of the silage surface layer was discarded, then the material from each silo was homogenized and exposed to air in an acclimatized room at 25 ºC. The surface temperature of the ensiled mass was measured by a digital infrared thermometer, and the internal temperature (10 cm) by a digital skewer thermometer at times 0 h and every 4 h up to 120 h. The data from 0, 8, 16, 24, 32, 40, 80 and 120 h after opening the silo were used. The stability was calculated as the time observed for the silage, after exposure to air, to increase 2 °C in comparison to the room temperature (Taylor and Kung Jr., 2002).

***Microbial population evaluation***

The counting of microorganisms in the silages was carried out during the opening periods of the silos at 0, 15, 45 and 90 d. The samples were obtained to specify the microbial groups, from the homogenization of all replications of each treatment, adding 90 mL of distilled water and homogenizing in an industrial blender for 1 min, to obtain a $10^{-1}$ dilution. Then, successive dilutions were performed, aiming to obtain dilutions ranging from $10^{-1}$ to $10^{-9}$, with plaques that presented values between 30 and

300 colony forming units (CFU) being considered susceptible to counting. The plating was performed in duplicate in sterile petri dishes.

The microbial populations were quantified using selective culture media for each microbial group listed as follows: agar rugose (Difco) for lactic acid bacteria (LAB) after incubation for 48 h in a B.O.D. oven at 37 °C; Brilliant Green Agar (Difco), for enterobacteria (ENT) after incubation for 24 h in a B.O.D. oven at 35 °C; and Potato Dextrose Agar, adding 1 $kg^{-1}$ of 1% tartaric acid after sterilization to count molds and yeasts (M and Y) after incubation for between 3 and 7 d at room temperature. The plates considered susceptible to counting were those in which there were values between 30 and 300 CFU in the petri dish, and the means of the plates of the selected dilution were considered. The yeasts differentiation was given by the physical structure of the colonies (González and Rodríguez, 2003).

***Statistical analysis***

The data were subjected to analysis of variance and subsequent Tukey's test, where the means were compared at a level of significance of 5%. The means of moistening additives (MA) and use of urea (U) were analyzed through the Tukey's test and compared with a significance level of $P$ <0.05. The version 5.0 of the SISVAR software developed by the Federal University of Lavras (Ferreira, 2011) was used.

The statistical model adopted was: $Yijk = \mu + \tau i + \gamma j + (\tau\gamma)\, ij + \varepsilon ijk$, where: Yijk = record referring to different proportions of moistening additive for corn grain silage *i* and additive use of urea *j*; μ = general constant; τi = effect of different proportions of moistening additive *i*; *i* = 1, 2, 3, 4 and 5 (0%; 5%; 10%; 20%; 40%)); γj = effect of the use of urea 1 and 2 (0%, 1.5% ); (τγ)ij = effect of the interaction different proportions of moistening additive for corn grain silage *i* additive use urea *j*; εijk = random error associated to each different proportions of moistening additive for corn grain silage and additive use urea.

## Results and Discussion

***Chemical composition***

Significant effect of interaction was found ($P$ <0.01) between the different proportions of moistening additives and the use of urea in the corn grain silage (Table 2) on the contents of dry matter (DM), crude ash and crude protein (CP). The DM content was lower in the silage moistened with 40% of cactus pear mucilage, regardless of the use of urea. As for crude ash, a higher concentration was observed in the silages moistened with 5, 10 and 40% of cactus pear mucilage without urea (0%), while with 1.5% of urea the silages containing cactus pear showed higher values. The greatest CP content in the corn grain silage was found with the addition of 1.5% urea and 40% of cactus pear mucilage, which was 159 g $kg^{-1}$.

**Table 2**. Chemical composition of corn grain silages with different moistening additives and urea, at 90 d of ensiling.

| Urea (U) | Moistening additive (MA) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Water | 5% cactus | 10% cactus | 20% cactus | 40% cactus | | |
| | Dry matter (DM, g kg$^{-1}$) | | | | | Mean | SEM[1] |
| Without (0%) | 676bB | 819aA | 796aA | 657bB | 554cA | 713 | 5.9 |
| With (1.5%) | 704cA | 825aA | 790bA | 702cA | 544dA | 702 | |
| Mean | 690 | 827 | 793 | 680 | 549 | | |
| | Mineral matter (g kg$^{-1}$ DM) | | | | | | |
| Without (0%) | 18.2bA | 22.1aA | 23.9aA | 18.7bB | 26.1aA | 21.8 | 0.8 |
| With (1.5%) | 19.4bA | 23.2aA | 22.4aA | 26.0aA | 27.1aA | 23.6 | |
| Mean | 18.8 | 22.6 | 23.1 | 22.3 | 26.6 | | |
| | Crude protein (g kg$^{-1}$ DM) | | | | | | |
| Without (0%) | 103aB | 95aB | 102aB | 99aB | 100aB | 100 | 2.8 |
| With (1.5%) | 137cA | 151bA | 135cA | 147bA | 159aA | 146 | |
| Mean | 119 | 129 | 118 | 123 | 129 | | |
| | Ether extract (g kg$^{-1}$ DM) | | | | | | |
| Without (0%) | 84.9 | 38.6 | 44.8 | 39.0 | 58.4 | 53.1 | 7.8 |
| With (1.5%) | 50.9 | 60.0 | 59.0 | 56.1 | 36.5 | 52.4 | |
| Mean | 67.9 | 49.3 | 51.9 | 47.5 | 47.3 | | |
| | Neutral detergent fiber (g kg$^{-1}$ DM) | | | | | | |
| Without (0%) | 104 | 114 | 104 | 100 | 98 | 104B | 6.8 |
| With (1.5%) | 143 | 138 | 137 | 138 | 138 | 138A | |
| Mean | 123 | 126 | 121 | 119 | 118 | | |
| | Acid detergent fiber (g kg$^{-1}$ DM) | | | | | | |
| Without (0%) | 23.1 | 23.4 | 43.4 | 50.9 | 50.5 | 38.3B | 19.0 |
| With (1.5%) | 34.4 | 24.9 | 50.4 | 58.0 | 59.9 | 45.4A | |
| Mean | 28.7c | 24.1c | 46.7b | 54.4ab | 55.2a | | |
| | Non-fiber carbohydrates (g kg$^{-1}$ DM) | | | | | | |
| Without (0%) | 702 | 745 | 735 | 750 | 725 | 731A | 6.3 |
| With (1.5%) | 661 | 683 | 696 | 689 | 699 | 686B | |
| Mean | 682b | 712a | 716a | 719a | 712a | | |
| | $N-NH_3$ (%) | | | | | | |
| Without (0%) | 1.33aB | 1.03aB | 0.96aB | 1.41aB | 1.76aB | 1.30 | 0.24 |
| With (1.5%) | 12.7bA | 3.50dA | 8.24cA | 16.73aA | 11.87bA | 10.61 | |
| Mean | 7.03 | 2.26 | 4.60 | 9.07 | 6.81 | | |
| | P-value | | | | | | |
| | | | MA | U | MA × U | | |
| Dry matter | | | <0.01 | 0.05 | <0.01 | | |
| Mineral matter | | | <0.01 | 0.02 | 0.01 | | |
| Crude protein | | | 0.07 | <0.01 | <0.01 | | |
| Ether extract | | | 0.37 | 0.92 | 0.10 | | |
| NDF | | | 0.91 | <0.01 | 0.93 | | |
| ADF | | | <0.01 | <0.01 | 0.44 | | |
| NF-Carbohydrates | | | <0.01 | <0.01 | 0.22 | | |
| $N-NH_3$ (%) | | | NS[2] | <0.01 | <0.01 | | |

[1]SEM: standard error of the mean. Different lowercase letters in the row represent significance of $P < 0.05$ by the Tukey's test for the type of moistening of the corn grain silage. Different uppercase letters in the column represent significance of $P < 0.05$ by the Tukey's test for the use of urea in the corn grain silage.

The lowest dry matter content was found in the corn grain silage moistened by 40% of cactus pear mucilage, as the cactus pear has a high moisture content (Table 1). The elevated water composition associated with the hydrocolloid content (gummy exudates) with hydrophilic function of the cactus pear mucilage, involved the mass of the corn grain silage, moistening the material and blocking the water leaching, thus reducing the DM content in the corn grain silage (Gusha *et al.,* 2014). The silages hydrated with water and 20% of cactus pear associated with urea had greater dry matter content, and this result may be related to its hygroscopicity and the high effectiveness of this additive in reducing losses during the fermentation period, as observed by Dias *et al.* (2014) and Gois *et al.* (2019).

The different types of moistening additives used in the corn grain silages affected ($P$ <0.01) the contents of acid detergent fiber (ADF) and non-fiber carbohydrates (NFC) but did not change (P>0.05) the composition of ether extract (EE) and neutral detergent fiber (NDF). The silage moistened with 40% of cactus pear had greater content of ADF, and the silage with 1.5% of urea had greater ADF value than the silage without urea. The corn grain silage moistened with cactus pear mucilage had greater NFC contents than the silage moistened with water, ranging from 712 g $kg^{-1}$ to 719 g $kg^{-1}$, and the silage without urea presented the greatest NFC value, which was 731 g $kg^{-1}$.

The increase in mineral matter concentration observed in corn grain silages moistened by cactus pear mucilage with urea may have occurred due to the formation of organic salts during ensiling, most of the buffering power of plants can be attributed to anions such as salts of organic acids, orthophosphates, sulfates, nitrates (Catchpool and Henzell, 1971), due to the high mineral content in the cactus pear (Table 1) and urea. Ryley (1969) referred to the partial binding of ammonia produced from the degradation of urea with organic acids to form organic salts during the anaerobic fermentation of the ensiled material. This increase in mineral content was also observed by Macêdo *et al.* (2008). Cordova-Torresa *et al.* (2015) stated that the cactus pear has a high concentration of mineral macro-elements, such as Ca (52.6 ± 70.2 g $kg^{-1}$), K (4.4 ± 19.0 g $kg^{-1}$) and P (1.8 ± 2.1 g $kg^{-1}$ DM). Carvalho *et al.* (2020) and Edvan *et al.* (2020) also observed a high concentration of minerals in different species of cactus pear.

The increase in the crude protein content of the silages that had urea is linked to the retention of nitrogen in the ensiled mass, from the ureolytic action responsible for transforming urea into ammonia, corroborating with Silva *et al.* (2014) who stated that urea can enhance the nutritional value of the ensiled material, by increasing the CP content. This can be beneficial to the growth of rumen microorganisms by promoting greater protein synthesis, improving fiber use, and increasing forage digestibility. In any case, the protein composition of corn grain silage found with the addition of 1.5% of urea can be considered satisfactory. The silage moistened with 40% of cactus pear associated with urea obtained the highest amount of crude protein, and this occurs due to chemical reactions in the silage with cactus pear mucilage when associated with urea as observed by Nogueira *et al.* (2019). In the production of ammonia ($NH_3$) the presence of urease, which is the enzyme responsible for catalyzing the hydrolysis of urea into carbon dioxide and ammonia, due to the partial transformation of urea into ammonia during silage fermentation, increasing the crude protein (Lopes *et al.,* 2007).

In general, the addition of cactus pear increased the acid detergent fiber content of the silages, which was because the cactus pear itself has high levels of this fiber in its chemical composition (Table 1) as observed by Monteiro *et al.* (2018) who also reported similar values of acid detergent fiber in cactus pear. The silages moistened by cactus pear also showed higher amount of non-fiber carbohydrates, and that probably occurred due to the preservation of these carbohydrates in the fermentation process of these silages. According to Silva and Sampaio (2015) both cactus pear and corn grain have a high concentration of non-fiber carbohydrates, characterizing them as energy feeds. The addition of urea and the respective increase in pH caused an increase in the acid detergent fiber content in the silage, which may be related to the reduction of acid hydrolysis. This reduction in acid hydrolysis is directly related to elevated pH levels, as reported by Cheeke (1994).

The interaction effect was found ($P$ <0.01) between the different proportions of moistening additives and the use of urea on the concentration of ammoniacal nitrogen ($N\text{-}NH_3$). The silages with urea had higher concentration of $N\text{-}NH_3$ (Table 2), and the silage moistened with 20% of cactus pear obtained the greatest amount. No influence was found ($P$ >0.05) on the $N\text{-}NH_3$ content in silages without urea, regardless of the type of moisturizer used, noting that in these silages the values were lower than 1.76%.

The addition of urea in the corn grain silage resulted in higher concentration of $N\text{-}NH_3$ in the silage, as urea, in addition to being a source of non-protein nitrogen, acts in the fermentation dynamics, changing the pH of the ensiled mass. Costa *et al.* (2020) related this increase to the transformation of urea into ammonia, which occurs from the action of urease on urea, noting that initially this reaction seems undesirable. However, the presence of ammonia can be seen as a positive aspect in the control of undesirable microorganisms, which can be reflected in minimal losses. In contrast, Calixto-Junior *et al.* (2017) observed lower values than the present study in high moisture corn grain silage adding the same additive. $N\text{-}NH_3$ values below 10% are indicative of adequate fermentation, according to Gois *et al.* (2019). The silages moistened with water, 20 and 40% of cactus pear mucilage with urea presented values above 10% of $N\text{-}NH_3$, while the other silages showed $N\text{-}NH_3$ values below 10%.

A significant effect of interaction ($P$ <0.05) was found between the different proportions of moistening additives and the use of urea on the pH values of the corn grain silages according to the silo opening periods (0, 15, 45 and 90 d) (Figures 1). The silages with 20 and 40% of cactus pear mucilage without urea had lower pH values at 0, 15, 45 and 90 d of silo opening, presenting pH values between 4.6 and 3.1, whereas with urea had pH values between 5.06 and 7.89.

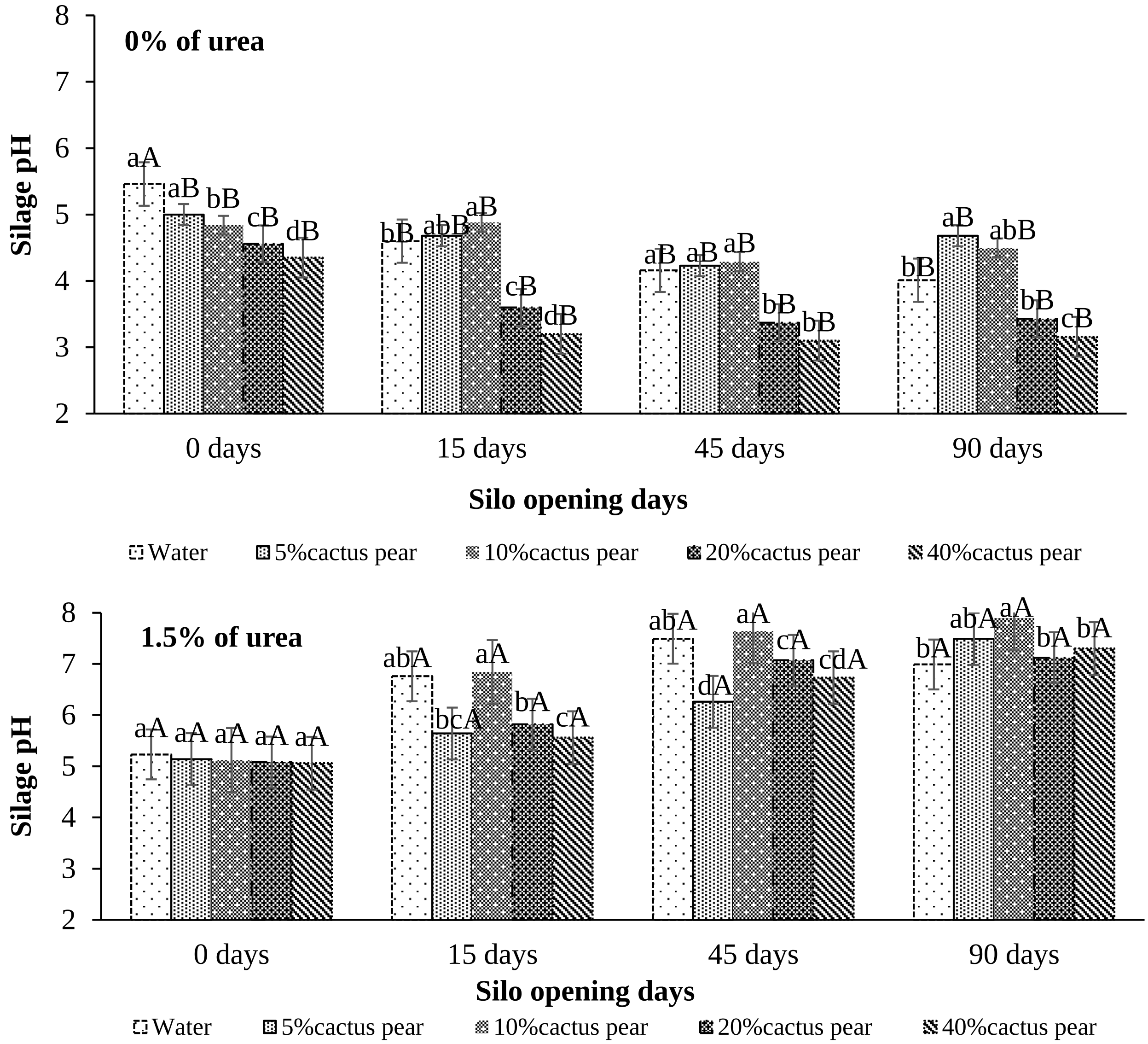


**Figure 1.** pH values of corn grain silage with different moistening additives and urea, at different silo opening periods. [Different lowercase (treatments – moistening additives) and uppercase (treatments – use of urea) letters represent significance of *P*<0.05 by the Tukey's test.]

The inclusion of the proportions of 20 and 40% of cactus pear mucilage to moisturize the corn grain silages, confirmed the efficiency of the cactus in sharply lowering the pH to satisfactory levels, maintaining the environment acidified, and inhibiting proliferation of unwanted microorganisms, thus improving the preservation of the ensiled material. Ben-Salem and Abidi (2009) stated that the lower the pH, the greater the concentration of soluble sugars in the silages. The cactuses naturally have high content of water-soluble carbohydrates, which after adequate fermentation reduce the pH to a range of preservation of silages. Miranda-Romero *et al.* (2018) pointed out that pH values around 3.8 indicate high LAB dominance, and consequently, accumulation of lactic acid, which can improve the preservation of the ensiled material and restrict the proliferation of undesirable microorganisms. Also, it should mention that silages treated with different proportions of cactus pear mucilage showed visibly better sensory characteristics such as pleasant color and odor.

The higher pH values of the silages enriched with 1.5% of urea might be due to the higher number of protein compounds through the addition of urea. Those compounds have a negative effect on the pH reduction by increasing the buffering capacity, which allowed for greater resistance in the pH drop of the silages, corroborating with Calixto Junior *et al.* (2017). In addition, among the silages treated with the different additives, the ones that contained urea had the worst noticeable aspects in all silos opening times (dark color and strong ammonia odor).

### *Volatile fatty acids*

A significant effect of interaction ($P$ <0.01) was found between the different proportions of moistening additives and the use of urea on the contents of lactic, acetic, propionic, and butyric acids in the corn grain silages (Table 3). The greatest lactic acid content was observed in the silage moistened with water and that had urea, which was 52.5 g $kg^{-1}$ DM, while for the silages moistened with cactus pear, the greatest values were observed in the treatments of 20 and 40% of cactus pear, which were 31.3 and 29.9 g $kg^{-1}$ DM, respectively. The greatest amounts of acetic acid, 19.6 and 19.8 g $kg^{-1}$ DM, were found in the corn silages with urea and that were moistened with 20 and 40% of cactus pear, respectively. The silages without urea and moistened with 5 and 10% of cactus pear, showed lower acetic acid values, which were 5.7 and 4.3 g $kg^{-1}$ DM, respectively.

**Table 3**. Volatile fatty acids in corn grain silages with different moistening additives and urea, at 90 d of ensiling.

| Urea (U) | Moistening additive (M) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Water | 5% cactus | 10% cactus | 20% cactus | 40% cactus | | |
| | Lactic Acid (g $kg^{-1}$ DM) | | | | | Mean | SEM[1] |
| Without (0%) | 6.6cB | 12.8bA | 13.1bA | 11.4bB | 28.4aA | 14.4 | 1.18 |
| With (1.5%) | 52.5aA | 12.1cA | 16.9cA | 31.3bA | 29.9bA | 27.5 | |
| Mean | 29.5 | 12.4 | 15.0 | 21.3 | 26.6 | | |
| | Acetic Acid (g $kg^{-1}$ DM) | | | | | | |
| Without (0%) | 11.9aA | 5.7bB | 4.3bB | 11.4aB | 10.9aB | 8.8 | 0.39 |
| With (1.5%) | 5.0cB | 12.9bA | 15.8bA | 19.6aA | 19.8aA | 12.6 | |
| Mean | 8.4 | 9.2 | 10.0 | 10.5 | 15.4 | | |
| | Propionic Acid (g $kg^{-1}$ DM) | | | | | | |
| Without (0%) | 9.7cA | 1.5dA | 1.3dA | 11.2bA | 15.4aA | 7.8 | 0.30 |
| With (1.5%) | 9.3aA | 1.9cA | 2.8cA | 4.2bB | 3.9bB | 4.3 | |
| Mean | 9.5 | 1.2 | 2.1 | 7.7 | 9.6 | | |
| | Butyric Acid (g $kg^{-1}$ DM) | | | | | | |
| Without (0%) | 15.4aA | 2.9dA | 1.4dA | 12.2bA | 9.6cA | 8.3 | 0.37 |
| With (1.5%) | 15.9aA | 0.4dB | 1.6dA | 13.1bA | 5.1cB | 7.2 | |
| Mean | 15.7 | 1.7 | 1.5 | 12.7 | 7.3 | | |
| | | | *P*-value | | | | |
| | | MA | U | MA x U | | | |
| Lactic Acid | | <0.01 | <0.01 | <0.01 | | | |
| Acetic Acid | | <0.01 | <0.01 | <0.01 | | | |
| Propionic Acid | | <0.01 | <0.01 | <0.01 | | | |
| Butyric Acid | | <0.01 | <0.01 | <0.01 | | | |

[1]SEM: standard error of the mean. DM=Dry matter. Different lowercase letters in the row represent significance of P<0.05 by the Tukey's test for the type of moistening of the corn grain silage. Different uppercase letters in the column represent significance of P<0.05 by the Tukey's test for the use of urea in the corn grain silage.

None of the evaluated silages had lactic acid contents greater than 60 g $kg^{-1}$ DM, which is the value indicated by Kung Jr. *et al.* (2018) as the best amount for a silage of good fermentation profile. In moistened corn grain silages, the lactic acid values are usually below 50 g $kg^{-1}$ DM (Itavo *et al.,* 2006; Jobim *et al.,* 2007). The low water activity in corn grain silage, even when moistened, may be the explanation for the low amount of lactic acid found, as according to Costa *et al.* (2018) low water concentration promotes the lack or low prevalence of heterofermentative bacteria. The high DM content of the ensiled material limits fermentation resulting in low concentrations of total organic acids (Bernard *et al.,* 2010).

The highest levels of acetic acids observed in the corn grain silages moistened with 20 and 40% of cactus pear mucilage with urea observed in all silages, were below 20 g $kg^{-1}$ DM. According to McDonald *et al.* (1991) acetic acid contents above 20 g $kg^{-1}$ DM in silages of roughages indicate the action of enterobacteria and can reduce the consumption of silage. However, acetic acid gives this silage greater control of fungi and aerobic stability, as this acid has an antifungal action according to Brito *et al.* (2020).

A low content of propionic and butyric acids was found in the silages moistened with 5 and 10% of cactus pear with and without the addition of urea, showing values lower than 2.8 g $kg^{-1}$ DM in the rehydrated corn grain silage (Table 4). The lower concentrations of propionic and butyric acids observed in the silage moistened with 5 and 10% of cactus pear mucilage, regardless of the addition of urea, gives this silage better quality due to the low concentration of these organic acids, which, according to Kononenko and Burkin (2014) can compromise the health of ruminants. According to Tomich *et al.* (2003) butyric acid concentrations lower than 3 g $kg^{-1}$ DM are indicative of silage with low clostridium fermentation, and lower losses of energy and dry matter.

### *Microbial population*

Regarding the population of microorganisms in the corn grain silage, no effect of interaction ($P$ >0.05) was found between the different proportions of moistening additives and the use of urea (Table 4) in none of the silo opening periods (0, 15, 45 and 90 d). The corn grain silages moistened with 20% of cactus pear mucilage at 15 d, and 10 and 20% at 45 d had lower lactic acid bacteria (LAB) counts ($P$ <0.01) than the other silages. However, when opening the silo at 90 d, there was no significant effect of the different moistening additives on the LAB population. The use of urea affected the LAB population only at 15 d of silo opening, providing a bigger population (6.58 logs CFU $g^{-1}$) when compared to silages without urea (6.27 logs CFU $g^{-1}$).


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**Table 4**. Microbial population in corn grain silages with different moistening additives and urea, at different silo opening periods.

| Microorganisms | Moistening additive (MA) | | | | | Urea (U) | | SEM[1] | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Water | 5% cactus | 10% cactus | 20% cactus | 40% cactus | Without (0%) | With (1.5%) | | MA | U | MA×U |
| | | | | | Silage at 0 d | | | | | | |
| LAB[2] | 6.26a | 6.78a | 6.06a | 6.69a | 6.86a | 6.79A | 6.27A | 0.30 | 0.28 | 0.06 | 0.21 |
| Enterobacteria | 4.13ab | 4.26ab | 3.68b | 4.18ab | 4.33a | 3.90B | 4.33A | 0.13 | 0.03 | <0.01 | 0.35 |
| Molds | 4.68b | 4.44b | 5.08ab | 4.82ab | 5.89a | 5.05A | 4.91A | 0.27 | 0.01 | 0.57 | 0.63 |
| Yeasts | 5.76b | 6.76a | 6.53a | 7.08a | 6.90a | 6.58A | 6.63A | 0.10 | <0.01 | 0.73 | 0.15 |
| | | | | | Silage at 15 d | | | | | | |
| LAB[2] | 6.76a | 6.59a | 6.53a | 5.61b | 6.62a | 6.27B | 6.58A | 0.16 | <0.01 | 0.04 | 0.08 |
| Enterobacteria | 4.17a | 4.15a | 3.55a | 3.59a | 3.71a | 3.81A | 3.88A | 0.15 | 0.35 | 0.66 | 0.65 |
| Molds | 5.50a | 4.39b | 5.36a | 4.57b | 5.01ab | 5.33A | 4.61B | 0.17 | <0.01 | <0.01 | 0.06 |
| Yeasts | 6.53a | 6.29a | 6.39a | 6.67a | 6.77a | 6.60A | 6.46A | 0.11 | 0.44 | 0.45 | 0.15 |
| | | | | | Silage at 45 d | | | | | | |
| LAB[2] | 6.83a | 6.90a | 6.10b | 5.59b | 6.23ab | 6.18A | 6.47A | 0.16 | <0.01 | 0.06 | 0.09 |
| Enterobacteria | 4.11a | 4.28a | 3.54b | 3.72b | 3.62b | 3.81A | 3.89A | 0.20 | 0.04 | 0.65 | 0.55 |
| Molds | 6.11a | 4.08b | 5.02b | 4.74b | 4.69b | 5.38A | 4.47B | 0.24 | <0.01 | <0.01 | 0.11 |
| Yeasts | 6.95a | 6.54a | 6.62a | 6.35a | 6.80a | 6.69A | 6.61A | 0.15 | 0.11 | 0.60 | 0.68 |
| | | | | | Silage at 90 d | | | | | | |
| LAB[2] | 7.21a | 6.55a | 7.28a | 7.01a | 7.21a | 7.12A | 6.98A | 0.17 | 0.06 | 0.40 | 0.81 |
| Enterobacteria | 4.17a | 4.38a | 3.83a | 3.85a | 4.02a | 3.94A | 4.16A | 0.20 | 0.31 | 0.24 | 0.15 |
| Molds | 5.29b | 5.54b | 5.55b | 6.43a | 5.89ab | 5.92A | 5.52B | 0.10 | <0.01 | <0.01 | 0.07 |
| Yeasts | 6.81a | 6.32ab | 5.51b | 6.32ab | 6.60a | 6.38A | 6.25A | 0.21 | <0.01 | 0.50 | 0.22 |

[1]SEM: standard error of the mean. [2]LAB: lactic acid bacteria. Different lowercase letters in the row represent significance of P<0.05 by the Tukey's test for the type of moistening of the corn grain silage. Different uppercase letters in the column represent significance of P<0.05 by the Tukey's test for the use of urea in the corn grain silage.

The lactic acid bacteria (LAB) are the main microorganisms that act in the production of organic acids, and when in high quantity, they control the growth of undesirable microorganisms (Soundharrajan *et al.* 2017). The LAB reduction observed at 15 and 45 d of ensiling in the silage moistened with 20% of cactus pear mucilage, is related to the fermentation process in each period and the early consumption of carbohydrates by LAB, which reduced the pH in these periods and treatment (Figure 1) and the silage pH is the main responsible for silage preservation (Dunière *et al.* 2013). The reduction in the population of LAB may have been triggered by aspects of fermentation inside the silo, such as temperature, availability of sugars, anaerobic state, moisture levels and pH of the medium (Borreani *et al.* 2018). An ensiling period of 28 d is recommended for LAB dominance and subsequent fermentation (Li and Nishino, 2013). At 90 d of ensiling of the moistened corn grain, the LAB population was not enough due to moistening and use of urea, presenting an average LAB population of 7.01 logs CFU $g^{-1}$, which is a value close to the one found by Carvalho *et al.* (2016) in moistened corn grain silages, after 90 d of ensiling, that was 7.24 logs CFU $g^{-1}$. According to Coelho *et al.* (2018) the main function of lactic acid in the silage is to preserve the ensiled material.

All proportions of cactus pear mucilage added in the corn grain silage proved to be efficient in reducing enterobacteria, since in large quantities they can be harmful, as many of them are toxin producers, which reduce the quality of the silage in sanitary terms and may compromise the health of animals (Kononenko and Burkin, 2014). This group of bacteria ferments water-soluble carbohydrates into acetic acid, which is a desirable acid due to its antifungal action (Brito *et al.,* 2020). However, some species of enterobacteria are capable of degrading proteins and may be undesirable during the fermentation process, and their growth occurs when environmental conditions are favorable, that is, when the pH is close to neutrality (Collins *et al.*, 2017) which was not observed in the present study when the silage was moistened by cactus pear mucilage, since the cactus showed efficacy in decreasing the pH values, that remained below 5.0.

The population of enterobacteria in the silage at 0 d was smaller ($P$ <0.05) when the silage was moistened with 10% of cactus pear mucilage (3.68 logs CFU $g^{-1}$). There were no changes in the population of these microorganisms at 15 d of ensiling, but there was reduction at 45 d in the corn grain silage moistened with 10, 20 and 40% of cactus pear mucilage, showing an average of 3.62 logs CFU $g^{-1}$ of enterobacteria, with no difference at 90 d of ensiling. The moistened corn grain silages that had no urea presented a smaller ($P$ <0.01) population of enterobacteria at 0 d (3.90 logs CFU $g^{-1}$), with no difference in the population of these microorganisms on the other days of silo opening.

The addition of water as a moistening additive without urea (control) showed reduction of enterobacteria only on the day that silos were closed and remained unchanged on the other days of storage. This reduction in the control treatment is like the results found by Frank *et al.* (2021) who evaluated rehydrated corn grain silage with and without microbial inoculant and different storage times. Muck (2010) explains that enterobacteria are the main competitors of lactic acid bacteria for the sugars of the crop, and pH values below five are usually enough to reduce the population of enterobacteria to undetectable levels in few days. Urea had an inhibitory action in the control of these microorganisms, resulting in the preservation of the material after a successful fermentation.

The use of 10% of cactus pear mucilage as a moistening additive in corn grain silage provided smaller ($P$ <0.01) yeast population (5.51 log CFU $g^{-1}$) at 90 d after ensiling. No effect was observed ($P$ >0.05) from the inclusion or not of 1.5% of urea in the ground corn grain silage on the yeast population.

One of the goals when using urea in silages is its inhibiting action on undesirable microorganisms, which favors the increase in the population of lactic acid bacteria. Kung Jr. *et al.* (2003) related the inhibitory effect of the additive on the population of these microorganisms, with the transformation of urea into ammonia, that reacts with water, consequently forming ammonium hydroxide ($NH_4OH$), thus raising the pH of the ensiled material. In the present study, the use of urea reduced the mold population at 15, 45 and 90 d after opening the silo (Table 5), due to the transformation of urea into ammonia.

**Table 5**. Losses and recovery of dry matter in corn grain silages with different moistening additives and urea, at 90 d of ensiling.

| Urea (U) | Moistening additive (MA) | | | | | Mean | SEM[1] |
|---|---|---|---|---|---|---|---|
| | Water | 5% cactus pear | 10% cactus pear | 20% cactus pear | 40% cactus pear | | |
| | Effluents (kg $t^{-1}$) | | | | | | |
| Without (0%) | 1.60 | 1.81 | 1.77 | 1.77 | 1.75 | 1.58 | 0.51 |
| With (1.5%) | 3.33 | 2.10 | 1.47 | 3.10 | 1.86 | 2.38 | |
| Mean | 2.49 | 1.53 | 1.64 | 2.43 | 1.80 | | |
| | Gases (%) | | | | | | |
| Without (0%) | 2.22 | 1.52 | 0.73 | 0.93 | 2.05 | 1.49 | 0.38 |
| With (1.5%) | 2.17 | 0.46 | 0.93 | 2.23 | 2.30 | 1.62 | |
| Mean | 2.20a | 0.99b | 0.88b | 1.62ab | 2.14a | | |
| | Silage dry matter recovery (%) | | | | | | |
| Without (0%) | 99.2aA | 98.7aA | 99.5aA | 97.3bA | 97.9bA | 98.5 | 0.10 |
| With (1.5%) | 99.3aA | 97.0bB | 98.9aA | 97.8bA | 96.9bB | 98.0 | |
| Mean | 99.3 | 97.8 | 99.2 | 95.2 | 97.4 | | |
| | *P*-value | | | | | | |
| | | | MA | U | MA × U | | |
| Effluents | | | 0.55 | 0.09 | 0.09 | | |
| Gases | | | 0.02 | 0.60 | 0.06 | | |
| Silage dry matter recovery | | | <0.01 | <0.01 | <0.01 | | |

[1]SEM: standard error of the mean. Different lowercase letters in the row represent significance of $P < 0.05$ by the Tukey's test for the type of moistening of the corn grain silage. Different uppercase letters in the column represent significance of $P < 0.05$ by the Tukey's test for the use of urea in the corn grain silage.

The development of molds in silages is mainly caused by storage deficiencies, which is possible due to the entry of oxygen into the silo, that is more likely at the top and sides of the silo (Borreani and Tabacco, 2010). The excess of moisture can also be a determining factor in the growth of these microorganisms; therefore, the result observed in the present study showed that the cactus pear mucilage did not favor the growth of undesirable microorganisms, despite its high humidity (Table 1). According to Sepúlveda *et al.* (2007) that is mainly due to the gelling aspect of the cactus pear mucilage, which has hydrophilic fraction, exhibiting high water retention capacity.

The reduction in yeast populations due to the addition of 10% of cactus pear mucilage at the opening of the silo (90 d), reflects a positive result in corn grain silages moistened with cactus pear mucilage, since according to Pedroso *et al.* (2005) the high activity of these microorganisms leads to an excessive consumption of soluble sugars and reduction in dry matter. The use of 10% of cactus pear mucilage as a moistening additive in corn grain silage provided smaller ($P$ <0.01) yeast population (5.51 log CFU $g^{-1}$) at 90 d after ensiling. No effect was observed ($P$ >0.05) from the inclusion or not of 1.5% of urea in the ground corn grain silage on the yeast population.

***Dry matter loss and recovery***

No effect of interaction was found ($P$ >0.05) between the different proportions of moistening additives and the use of urea in the corn grain silages on losses through effluents and gases (Table 5). However, there was significant effect ($P$ <0.02) of the different proportions of moistening additives on gas losses. The moistening with 5 and 10% of cactus pear mucilage caused lower values of gas losses, which were 0.99 and 0.88%, respectively. About the dry matter recovery (DMR), there was effect of interaction ($P$ <0.01) between the different proportions of moistening additives and the use of urea in the corn grain silages. The greatest DMR value, which was above 98.9%, was observed in the treatments with water and 10% of cactus pear, regardless of the use of urea.

The high dry matter recovery (DMR) of corn grain silage moistened with water, 5 and 10% of cactus pear mucilage, without urea, showed that those treatments were efficient in retaining liquids in the ensiled mass, and that characteristic directly influences reduced losses of fermentation and consequently increases DMR efficiency (Monrroy *et al.,* 2017). The silages moistened with water and 10% of cactus pear mucilage associated with urea also presented high DMR. Kung Jr. *et al.* (2003) reported the use of urea as an additive cause which increased in the silage pH, resulting in the inhibition of unwanted microorganisms. Zanine *et al.,* (2020) stated that the release of ammonia, due to the use of urea, reduces the growth of yeasts and molds, and consequently, reduces DM losses. The result of the present study was more satisfactory than those observed by Vieira *et al.* (2017) who evaluated the addition of urea in by-product of sweet corn and obtained a value of 93.3% of DMR with inclusion of the same proportion of urea.

***Aerobic stability***

Considering the different proportions of moistening additives and the use of urea in the corn grain silages, no effect of interaction was observed on the surface temperature (Figure 2). The silages moistened with cactus pear mucilage showed lower surface temperature in aerobiosis ($P$ <0.05) when compared to the silages moistened with water at 120 h, with no break in stability (Figure 2). No effect of the use of urea in the corn grain silage was found on the silage surface temperature, regarding the time of exposure of the silage to air.

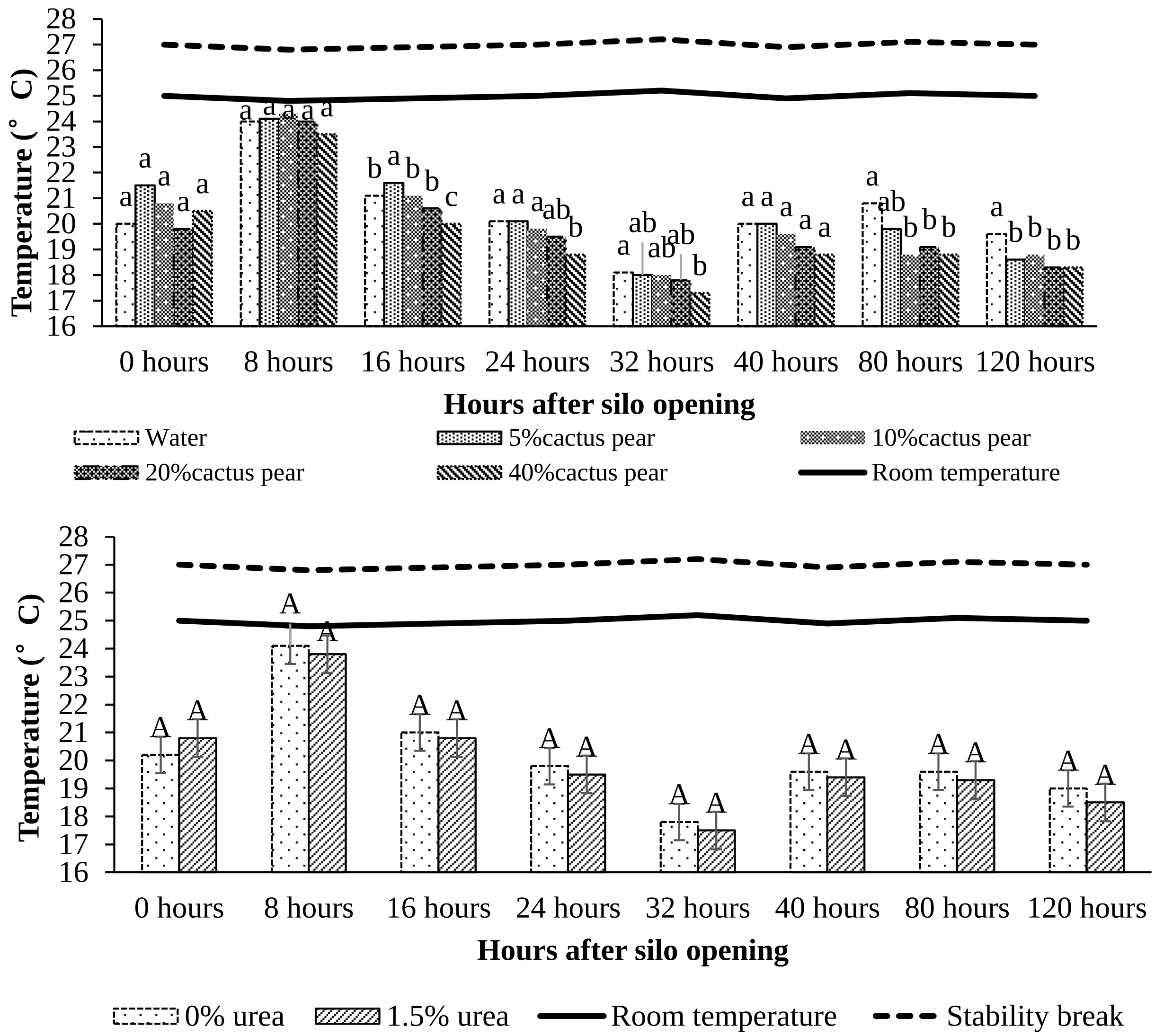


**Figure 2.** Surface temperature of the corn grain silage with different moistening additives and urea, at different periods after silo opening. [Different lowercase (treatments – moistening additives) and uppercase (treatments – use of urea) letters represent significance of *P* <0.05 by the Tukey's test.].

The temperature decreased in corn grain silages moistened with mucilage might be caused by its hydrophilic character, which delays water loss and acts as an efficient stabilizer (Allegra *et al.,* 2017) interrupting the breakdown of the ensiled mass, and exerting extension and firmness to the material. Kung *et al.* (2007) stated that moistened corn grain can cause aerobic deterioration due to its reduced fermentation capacity and high starch content when compared to other proportions of ensiled feeds, which can be used as an energy source for yeasts. However, Santos *et al.,* (2010) highlighted that better quality of the silage, the lower the stability of the ensiled material, due to the higher levels of residual soluble carbohydrates and lactic acid.

No effect of interaction was found (*P* >0.05) between the different proportions of moistening additives and the use of urea in the corn grain silages on the internal temperature of the silages (Figure 3). The silage moistened with water showed higher (*P* <0.05) internal temperature at 32, 40, 80 and 120 h of air exposure. The break in aerobic stability was observed in silages moistened with water after 40 h of air exposure, while there was no break in stability for the other treatments that used cactus pear mucilage as moisturizer in corn grain silage. There was no break in the aerobic stability of the corn grain silage regarding the use of urea, and the silages with no urea had higher temperatures in the period of 8, 16, 24, 40, 80 and 120 h of air exposure.

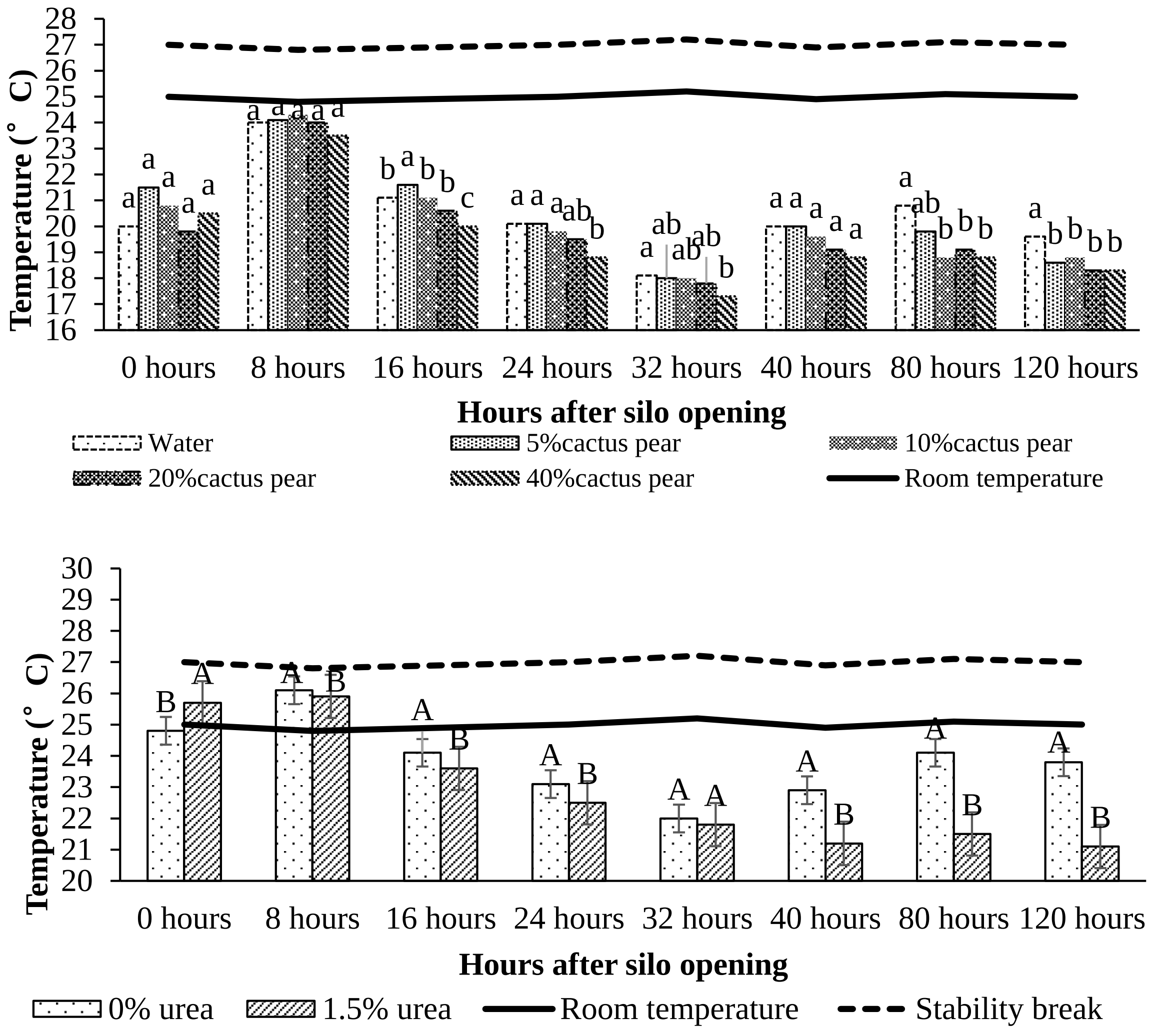


**Figure 3.** Internal temperature of the corn grain silage with different moistening additives and urea, at different periods after silo opening. [Different lowercase (treatments – moistening additives) and uppercase (treatments – use of urea) letters represent significance of $P$ <0.05 by the Tukey's test.].

No break in aerobic stability was observed up to 120 h of exposure of the silage to air, in the silages moistened with cactus pear, corroborating with the study of Pereira *et al.* (2020) who observed no aerobic deterioration in the cactus pear silage. A possible explanation for the non-deterioration of corn grain silage moistened with cactus pear mucilage is that the cactus pear has a high concentration of acetic acid, indicated as a characteristic of the cactus itself. In addition, there are buffering substances present, such as oxalic, malic, citric, malonic, succinic, and tartaric acids that result from the crassulacean acid metabolism, which can exert antifungal action, which is directly associated with the high content of acetic acid, ensuring high aerobic stability (Petera *et al.,* 2015; Isaac, 2016).

There was no break in aerobic stability of the corn grain silages in function of the use of urea, but the lowest temperature was found in silages that had urea after 8 hours of air exposure. Allen *et al.* (2003) explained that silages treated with ammonia reach lower temperature, and less DM loss than silages not treated with the additive. Santos *et al.* (2020) studied the effect of urea in corn silages, and found a positive result on aerobic stability, agreeing with the present study. Neumann *et al.* (2010) reported that after opening the silo, secondary fermentations tend to occur, but the addition of urea provides pH increase and adequate levels of lactic acid, due to the buffering power of ammonia on fermentation, in addition to delaying mold development and other unwanted microorganisms.

## Conclusions

The moistening of the ground corn grain silage with water and different proportions of cactus pear mucilage with urea, influences in different ways the chemical composition, volatile fatty acids, population of microorganisms, gas losses, dry matter recovery and aerobic stability of silages.

The cactus pear mucilage as a moistening agent for corn grain silage promotes greater aerobic stability in this silage. The use of 10% of cactus pear mucilage is recommended without the addition of urea, to promote an adequate pH, lower concentration of propionic and butyric acid, in addition to a smaller yeast population, with no break in aerobic stability up to 120 hours.

The increment of 1.5% of urea in corn grain silage reduces the mold population, and increases the content of crude protein, lactic acid, $N\text{-}NH_3$ and pH of the silage.

## ETHICS STATEMENT

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF SUPPORTING DATA

All data generated or analyzed during this study are included in this published article.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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## AUTHOR CONTRIBUTIONS

Ériton Eriberto Martins Dantas: Data curation, Formal analyses, Investigation, Writing – Original draft; Leilson Rocha Bezerra and Ricardo Loiola Edvan: Conceptualization, Supervision, Funding acquisition, Methodology; Edson Cavalcanti da Silva Filho: Funding acquisition, Methodology; Marcos Jacome Araújo, Rafael de Souza Miranda, Romilda Rodrigues do Nascimento and Keuven do Nascimento Silva: Data curation, Investigation, Formal analyses; Edson Mauro Santos: Formal analyses, Investigation, Methodology; Pedro Henrique Soares Mazza: Supervision, Investigation, Methodology, Writing – Original draft; José Morais Pereira Filho and Ronaldo Lopes Oliveira: Data curation, Supervision, Writing – Revision.

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