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Effects of calcium oxide, sugarcane molasses and corn heavy steep water on the storage and nutritional composition of corn husks

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ABSTRACT - The objective was to evaluate the effects of calcium oxide (CaO) and a mixture of corn heavy steep water and sugarcane molasses (HM) on the fermentation, aerobic stability, and ruminal disappearance of corn husk silage. Three doses of CaO (0, 20, and 40 g/kg of fresh matter (FM)) and three doses of HM (0, 100, and 200 g/kg of FM) were arranged factorially and applied to rehydrated corn husk (500 g/kg moisture) in a completely randomized design, with four replicates per treatment. Treated corn husk was placed in nylon-polyethylene bags (33 cm × 45 cm, 160 µm thick), vacuum sealed, and stored for 66 d. Significant HM × CaO interaction (P<0.05) were observed for chemical composition, including crude protein (CP) and ash-free neutral detergent fiber (aNDFom), as well as for key fermentative parameters, notably lactic, acetic, and n-butyric acids. CaO increased silage pH, with the highest dose exceeding pH 7 after 66 d of storage, indicating a high buffering capacity. Aerobic spore-forming bacteria and lactic acid bacteria (LAB) counts increased with HM and CaO, respectively, while molds decreased with CaO. Clostridia counts tended to increase with CaO, and n-butyric acid increased with both additives, suggesting reduced hygienic quality of the silage. Dry matter (DM) loss was reduced, and aerobic stability was extended by CaO, indicating inhibition of microbial metabolism. However, HM alone increased aerobic spoilage. Both additives enhanced ruminal DM disappearance, while CaO slightly improved in situ disappearance of aNDFom. Corn husk ensiled without additives already exhibited considerable ruminal DM and aNDFom disappearance, as well as good preservation. In conclusion, corn husk (500 g/kg moisture) may be ensiled without additives or, if convenient, added with up to 100 g/kg of HM.

Keywords: aerobic stability, alkalage, byproduct, fermentation, fiber digestibility

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1. Introduction

Large-scale industrial food processing generates large amounts of byproducts. These residues can have negative consequences for the environment, such as soil and water pollution when compounds are leached if disposed of incorrectly (Rosa et al., 2011). At the same time, agro-industrial byproducts have the potential to be used as animal feed and thus converted into human-edible food. Currently, most agro-industries depend on the sale of byproducts to make their final products economically viable. Additionally, the use of co-products or byproducts in animal production, especially for feeding

ruminants, reduces the demand for arable land and competition with edible foods such as corn and soybean, thereby reducing feed costs and increasing environmental sustainability (Wilkinson and Lee, 2018).

Corn husk is a bulky feed with moderate nutritional value, containing 50 to 90 g/kg of crude protein (CP) and 640 to 820 g/kg of neutral detergent fiber (NDF) on dry matter (DM) basis (Gao et al., 2019; Petzel et al., 2019; Xu et al., 2025) and can serve as a roughage source in ruminant diets. However, its conservation poses challenges due to the wide variability in moisture content (100 to 750 g/kg; Gao et al., 2019; Petzel et al., 2019; Tong et al., 2023), which may hinder proper storage and increase the risk of spoilage. Therefore, ensiling can be a feasible alternative to preserve this feedstuff and potentially improve its nutritional value.

In some agro-industries, calcium oxide (CaO) has been used as an additive for ensiling fibrous feedstuffs, aiming to improve fiber digestibility by increasing cell wall solubilization and disrupting lignocellulosic bonds (Schmidt et al., 2010; Daniel et al., 2013). However, it may negatively impact the fermentation pattern due to an increase in buffering capacity (Custódio et al., 2016; Jacovaci et al., 2017). Meanwhile, corn heavy steep water, a liquid byproduct derived from the wet extraction of starch from corn grains, is rich in non-fiber carbohydrates (~300 g/kg DM) and crude protein (~500 g/kg of DM), widely available at corn agro-industries (Silva et al., 2008). This byproduct is often mixed with sugarcane molasses and used as a ruminant feedstuff. Due to the high availability of soluble carbohydrates in the mixture of corn heavy steep water with sugarcane molasses (HM), this liquid viscous byproduct could be used to improve the fermentation of ensiled crops or byproducts with lower concentrations of soluble carbohydrates, which are essential substrates for lactic fermentation (McDonald et al., 1991). The mixture also has the potential to enhance silage fermentation and nutritive feed value through the supply of soluble nutrients, whereas corn heavy steep water may contribute to increasing the buffering capacity (Woolford, 1984). Further investigations are therefore needed to determine the optimal inclusion level of this mixture. However, no studies have evaluated the combined use of CaO and HM during the ensiling of corn husk.

Therefore, the objective of this study was to assess the impact of increased doses of HM combined with different doses of CaO on the conservation and nutritional composition of ensiled corn husks. We hypothesized that HM would enhance silage fermentation by providing readily fermentable substrates, while CaO would improve fiber digestibility. Their combined application of both additives was expected to improve both silage fermentation and ruminal disappearance.

2. Material and methods

Prior to the beginning of the study, all animal care and handling procedures were reviewed and approved by the Ethics Committee for Animal Use of Universidade Estadual de Maringá (protocol number 8208090218 – CEUA/UEM). The experiment was carried out in Maringá, PR, Brazil (23° 25' S, 51° 57' W, 550 m elevation).

2.1. Ensiling process

Chopped corn husks [Dry matter (DM) 843 g/kg as fed; ash 28.9 g/kg DM; CP 62.1 g/kg DM; ash-free neutral detergent fiber (aNDFom) 687 g/kg DM; ash-free acid detergent fiber (ADFom) 349 g/kg DM] and HM (i.e., a mixture containing 500 g/kg of corn heavy steep water and 500 g/kg of sugarcane molasses; DM 564 g/kg; ash 120 g/kg; CP 250 g/kg) were provided by an agroindustry (Cargill, Castro, PR, Brazil), which also defined the 1:1 ratio of corn heavy steep water to sugarcane molasses based on product availability and prior industrial blending practices. Corn husk was divided into nine piles (one for each treatment) and treated with three doses of HM (0, 100, and 200 g/kg on a fresh matter [FM] basis) in factorial combination with three doses of CaO (0, 20, and 40 g/kg of FM; Colombocal, Colombo, PR, Brazil). Water was added to all treatments to adjust DM content to 500 g/kg FM. CaO was applied as a powder, and HM was poured over the chopped corn husks and homogenized. Approximately 1 kg of

treated material from each pile was placed in nylon-polyethylene bags (33 cm × 45 cm, 160 µm thick) and vacuum sealed (Bench-Top Vacuum Sealer TM 250 – TecMaq, São Paulo, Brazil). Each bag (mini silo) was considered an experimental unit (n = 4 per treatment). The bags were weighted and stored within the laboratory at room temperature (18 to 27 °C).

After 66 d of storage, the silos were weighed, and samples were collected for analysis of chemical composition, aerobic stability, and ruminal in situ disappearance. The DM loss was calculated as the difference between the amount of DM ensiled and DM recovered, expressed as a proportion of DM ensiled.

2.2. Aerobic stability test

Silage samples (700 g) were collected and placed in 3.6-L plastic buckets with temperature sensors (RC-5 USB, Elitech, Niterói, Brazil; accuracy ± 0.5 °C) positioned at the center of the silage sample. Subsequently, the buckets were covered with perforated aluminum foil to prevent contamination and avoid dehydration. The buckets were stored in a temperature-controlled room (25 ± 1.8 °C). The temperature was recorded every 15 min for 10 d. Aerobic spoilage was defined as the time elapsed until silage temperature increased by 2 °C above ambient temperature (O'Kiely, 1993). Additionally, silage pH was measured daily during the aerobic exposure period. A silage sample was collected approximately 5 cm below the surface and mixed with 90 mL of distilled water (10 g of silage + 90 mL water) to prepare an aqueous extract. The mixture was blended for 2 min and filtered through four layers of sterilized gauze before pH determination.

2.3. Ruminal disappearance *in situ*

Two rumen-cannulated, non-lactating, multiparous Holstein cows (595 ± 57 kg of body weight) housed in a tie-stall barn were used for the *in situ* incubation (Nocek, 1988). Cows were fed *ad libitum* a diet containing 650 g/kg DM corn silage and 350 g/kg DM of commercial concentrate, and had free access to water.

Fresh and ensiled corn husk samples were dried and ground using a Wiley mill (model MA340, Marconi, Piracicaba, SP, Brazil) fitted with a 5-mm screen. Subsequently, 5 g of each sample was weighed into nylon bags (10 cm × 20 cm, 50 µm pore size; Ankom Technology, Macedon, NY, USA). Each treatment was incubated in triplicate per cow. Before incubation, the bags were soaked in warm water (39 °C) for 10 min to pre-hydrate the samples and simulate ruminal conditions. Two blank bags (without sample) were included to correct for bag weight loss.

One set of bags was incubated in the rumen ventral sac for 24 h to evaluate DM disappearance, whereas another set of bags was incubated for 30 h to determine aNDFom disappearance. After removal, bags were submerged in ice-cold water for 10 min. Subsenquetly, bags were rinsed under running water until the water was clear, and then dried in a forced-air oven at 55 °C for 72 h, and weighed. The dried residues were ground through a 1-mm screen and analyzed for DM and aNDFom to calculate DM disappearance at 24 h and aNDFom disappearance at 30 h, respectively.

2.4. Laboratory analysis

Samples of fresh and ensiled corn husks and residues from ruminal *in situ* incubation were dried in a forced-air oven at 55 °C for 72 h and ground in a Wiley mill (1-mm screen) for chemical composition analysis. Samples were analyzed for DM, ash, and CP (AOAC, 1990), aNDFom (assayed with heat-stable amylase, sodium sulfite, and expressed exclusive of residual ash; Mertens, 2002), and ADFom (assayed sequentially and expressed exclusive of residual ash). Organic matter (OM) was computed as 100 – ash. The CP, aNDFom and ADFom concentrations were also expressed on an OM basis (g/kg of OM) to avoid bias caused by the addition of CaO, which increases ash content and could artificially lower nutrient concentrations on a DM basis. Reporting results on an OM basis allowed a more accurate

comparison among treatments. Dry samples of treated corn husks before ensiling were also analyzed for buffering capacity (BC, g lactic acid/kg DM) according to Weissbach (1967).

Aqueous extracts of fresh silage samples were prepared by homogenizing 25 g of silage with 225 mL of distilled water for 2 min in a blender, followed by filtration through four layers of sterilized gauze for microbial counts, fermentation product analysis, and pH measurement, which was recorded (model Tec5, Tecnal®, Piracicaba, Brazil). An aliquot was diluted (10^{-1} to 10^{-7}) in sterile peptone water (1 g/L) for microbial counts using the pour-plate technique on selective media. Molds and yeasts were enumerated on malt extract agar (M137, Himedia®, Mumbai, India) acidified to pH 3.5 with lactic acid. Lactic acid bacteria (LAB) were enumerated on De Man, Rogosa, and Sharpe agar (7543A, Acumedia®, Michigan, USA) supplemented with nystatin (400,000 IU/L). All plates were prepared in duplicate and incubated aerobically at 30 °C for 2, 3, and 4 d before enumeration of LAB, yeasts, and molds, respectively. Additionally, an aliquot of the aqueous extract was pasteurized (80 °C for 10 min) and serially diluted (10^{-1} to 10^{-3}) for enumeration of bacillus and clostridia. Aerobic spore-forming bacteria were counted after pour-plating on plate count agar (M091A, Himedia®, Mumbai, India) incubated aerobically at 34 °C for 2 d. Clostridia were enumerated on reinforced clostridial agar (M154, Himedia®, Mumbai, India) supplemented with neutral red and D-cycloserine (Jonsson, 1990) incubated in anaerobic jars with an anaerobic generator (Anaerobac®, Probac®, São Paulo, Brazil) at 37 °C for 5 d. Microorganisms were counted as colony-forming units (CFU) and expressed as $\log_{10}$.

A portion of the undiluted aqueous extract was centrifuged at 10,000 × *g* for 15 min to obtain the supernatant for analysis of fermentation products. Ammonia (Chaney and Marbach, 1962) and lactic acid (Pryce, 1969) were determined by colorimetry. Ethanol, acetic acid, 2,3-butanediol, 1,2-propanediol, n-propanol, propionic acid, n-butyric acid, n-valeric acid, i-butyric acid, and i-valeric acid concentrations were determined by gas chromatography (Nexis GC-2030, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) and an autoinjector (AOC-20i Plus, Shimadzu, Kyoto, Japan), using a Stabilwax capillary column (Restek, Bellefonte, PA; 60 m, 0.25 mm ø, 0.25 µm polyethylene glycol cross bond carbowax). Identification of the compounds was performed according to their retention times and quantification was carried out using external standards (Lazzari et al., 2021). Silage DM content was adjusted to account for volatile losses during oven drying, as described by Weissbach and Strubelt (2008).

2.5. Statistical analysis

The experimental design was completely randomized in a 3 × 3 factorial arrangement, consisting of three doses of HM (0, 100, and 200 g/kg of FM) and three doses of CaO (0, 20, and 40 g/kg of FM), with four replicates per treatment. For silage characteristics (chemical composition, microbial counts, fermentation profile, losses, and aerobic stability) data were analyzed using the MIXED procedure of SAS, with a model (Eq. 1) including the fixed effects of HM, CaO, and their interaction (HM × CaO).

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \quad (1)$$

in which Y_{ijk} represents the response variable for the levels of HM (i), CaO (j), and replication (k); μ is the overall mean; α_i and β_j are the fixed effects of HM (0, 100, and 200 g/kg FM) and CaO (0, 20, and 40 g/kg FM) levels, respectively; $(\alpha\beta)_{ij}$ is the HM × CaO interaction; and ε_{ijk} is the residual error. Orthogonal polynomial contrasts were used to assess linear and quadratic effects of HM and CaO levels. Statistical significance was declared at $P \leq 0.05$. When a significant HM × CaO interaction was detected ($P \leq 0.05$), least squares means were compared using Tukey-Kramer test ($\alpha = 0.05$).

For the ruminal *in situ* disappearance, the same factorial structure was adopted, but including a fixed effect of ensiling and a random effect of cow to account for inter-animal variability. The following model was used (Eq. 2):

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \delta_l + \varepsilon_{ijklm} \quad (2)$$

in which Y_{ijklm} represents the variable response for the levels of HM (i), CaO (j), ensiling (k), cow (l), and replication (m); μ is the overall mean; α_i, β_j, and γ_k are the fixed effects of HM (0, 100, and 200 g/kg FM), CaO (0, 20, and 40 g/kg FM) levels, and ensiling (fresh or silage), respectively; $(\alpha\beta)_{ij}$ is the interaction HM × CaO; $(\alpha\gamma)_{ik}$ is the interaction HM × ensiling; $(\beta\gamma)_{jk}$ is the interaction CaO × ensiling; $(\alpha\beta\gamma)_{ijk}$ is the interaction HM × CaO × ensiling; δ_l is the random effect of cow; and ε_{ijklm} is the residual error.

3. Results

3.1. Fresh corn husks

There was an interaction HM × CaO for DM, ADFom, pH, BC (P<0.001), CP (P = 0.008), and aNDFom (P = 0.015; Table 1). At 100 g/kg of HM, DM remained similar values regardless of CaO addition. However, at 200 g/kg of HM, DM was reduced, with the lowest value observed at 40 g/kg of CaO. At 0 and 100 g/kg HM, CP fluctuated slightly with CaO addition, whereas at 200 g/kg HM, CP remained consistently higher regardless of CaO addition (Table 1). In the absence of HM, CaO addition did not affect aNDFom concentration (P = 0.12). However, at 100 g/kg and 200 g/kg HM, the addition of CaO led to a decrease in aNDFom concentration (Table 1). Without HM, the addition of 40 g/kg of CaO increased ADFom concentration. At 100 g/kg of HM, ADFom concentration increased with the addition of CaO, reaching the highest value at 40 g/kg of CaO. At 200 g/kg of HM, ADFom values were generally lower than those at 100 g/kg HM, and the effect of CaO was less pronounced (Table 1). While CaO increased pH in all treatments, the magnitude of this increase was influenced by the presence of HM, with HM slightly mitigating the pH increase caused by CaO. The presence of HM enhanced the BC, suggesting that both CaO and HM increased the resistance to pH decline (Table 1). The ash content significantly increased with the addition of both CaO and HM (P<0.001), indicating their direct contribution to increasing the mineral content (Table 1).

3.2. Silage fermentation, dry matter loss and aerobic stability

The CaO increased LAB counts (P<0.001), regardless of HM, and tended to increase *Clostridium* counts (P = 0.065), especially in the 100HM20CaO and 200HM20CaO treatments (Table 2). Aerobic spore-forming bacteria counts increased with HM addition (P = 0.015), but were not affected by CaO (P = 0.23; Table 2). The CaO increased yeast counts (P = 0.019), particularly in the 0HM40CaO and 100HM40CaO treatments (Table 2). Mold counts decreased with CaO addition (P = 0.049; Table 2).

There was an interaction HM × CaO for silage pH (P<0.001; Table 2). In silages without CaO, pH values were lower, particularly in the 0HM0CaO, 100HM0CaO, and 200HM0CaO treatments (Table 2). The inclusion of 20 g/kg CaO increased the pH to values above 4.5, regardless of the HM dose (P<0.05). However, when 40 g/kg CaO was added, pH values exceeded 8 in both 0HM40CaO and 200HM40CaO treatments, even after 66 d of fermentation (P<0.05; Table 2).

An interaction HM × CaO (P<0.001) was observed for all fermentation products (Table 2). The ammonia nitrogen (NH_3-N) was reduced with CaO and HM added separately (P<0.001); however, when both were combined, particularly in silages with 20 g/kg of CaO, NH_3-N values were increased, as observed for the 100HM20CaO treatment (P<0.05; Table 2). The lactic acid concentration was reduced by CaO, but increased by HM addition (P<0.001). The highest lactic acid content was observed for the 200HM20CaO treatment (Table 2).

Acetic acid concentration increased with HM and CaO addition (P<0.001 for interaction HM × CaO; Table 2). This effect was more pronounced at 20 g/kg CaO. The n-butyric acid concentration increased with CaO addition, especially at 20 g/kg (P<0.001), and this increase was intensified by HM inclusion (Table 2). The highest value of n-butyric acid was observed in the 100HM20CaO treatment, evidencing a synergistic interaction between HM and CaO (P<0.001). Ethanol concentration was increased by both HM and CaO (P<0.001), with the highest value observed in the 200HM0CaO treatment (Table 2).

Table 1 - Characteristics of corn husks before ensiling (n = 4)

Item	Treatment[1]									SEM	P-value[2]		
	0 HM			100 HM			200 HM						
	0 CaO	20 CaO	40 CaO	0 CaO	20 CaO	40 CaO	0 CaO	20 CaO	40 CaO		HM	CaO	HM × CaO
Dry matter (g/kg)	528ab	550a	549a	525ab	532ab	530ab	497c	514bc	467d	2.35	<0.001	<0.001	<0.001
Ash (g/kg DM)	29.1	51.0	104	36.7	65.3	109	48.6	84.0	136	4.06	<0.001	<0.001	0.29
CP (g/kg OM)	56.9de	59.4cd	51.1e	67.9b	62.1bcd	64.5bc	82.6a	82.8a	82.5a	1.55	<0.001	0.058	0.008
aNDFom[3] (g/kg OM)	645abc	655ab	663a	609bcd	598cd	567de	534ef	480f	509f	11.9	<0.001	0.12	0.015
ADFom[4] (g/kg OM)	229de	228e	295abc	283abc	290abc	312a	264c	279abc	273bc	7.47	<0.001	<0.001	<0.001
pH	6.12g	10.4d	11.5a	4.83h	10.2e	10.9c	4.46i	10.0f	11.2b	0.365	<0.001	<0.001	<0.001
BC (g lactic acid/kg DM)	24.9f	84.1e	150c	24.6f	155c	269b	24.8f	122d	370a	2.70	<0.001	<0.001	<0.001

CP - crude protein; BC - buffering capacity; SEM - standard error of the mean.
[1] HM - mixture (1:1, fresh matter basis) of corn heavy steep water + sugarcane molasses, applied at 0, 100, and 200 g/kg fresh matter; CaO - calcium oxide, applied at 0, 20, and 40 g/kg fresh matter.
[2] HM - effect of the addition of corn heavy steep water + sugarcane molasses; CaO - effect of the addition of calcium oxide; HM × CaO - interaction effect.
[3] aNDFom - neutral detergent fiber assayed with sodium sulfite, heat-stable amylase and expressed excluded of residual ash.
[4] ADFom - acid detergent fiber assayed sequentially and expressed excluded of residual ash.
Means with different letters in the rows differ statistically (Tukey test α = 0.05).

Table 2 - Microbial counts, fermentation profile, aerobic stability and DM loss of corn husk silages treated with calcium oxide (CaO) and a mixture of corn heavy steep water and sugarcane molasses (HM) stored for 66 days (n = 4)

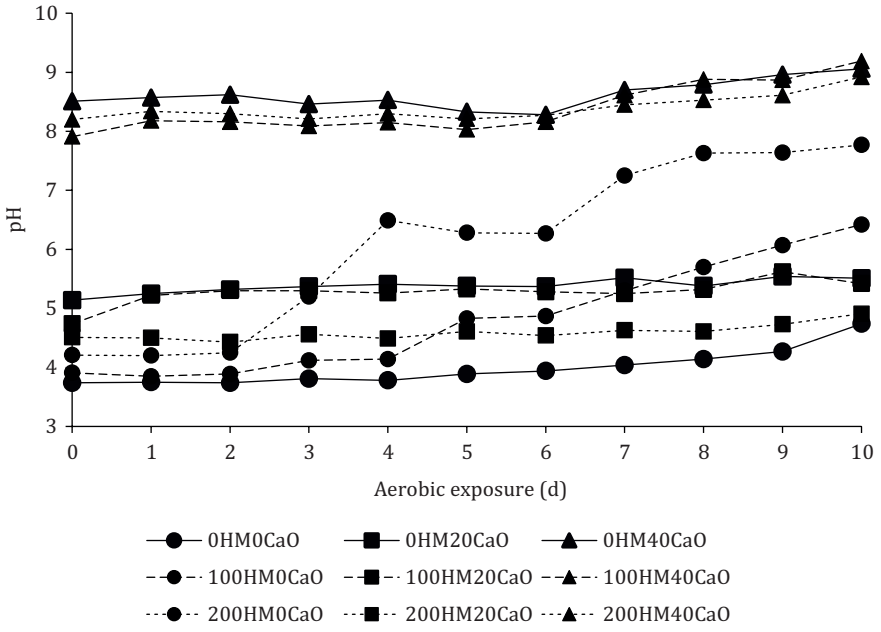
Item	Treatment[1]									SEM	P-value[2]		
	0 HM			100 HM			200 HM						
	0 CaO	20 CaO	40 CaO	0 CaO	20 CaO	40 CaO	0 CaO	20 CaO	40 CaO		HM	CaO	HM × CaO
LAB (log cfu/g)	6.19	6.72	7.96	6.28	5.22	8.17	6.61	5.26	7.88	0.417	0.44	<0.001	0.14
Clostridia (log cfu/g)	3.12	3.06	2.90	3.37	3.60	3.09	3.14	3.77	3.15	0.213	0.11	0.065	0.57
Aerobic spore-forming bacteria (log cfu/g)	3.25	3.68	3.55	3.68	3.86	3.83	3.57	3.54	3.37	0.133	0.015	0.23	0.33
Yeasts (log cfu/g)	4.60	4.61	5.52	5.18	3.00	5.49	4.73	3.33	3.00	0.549	0.54	0.019	0.32
Molds (log cfu/g)	5.62	4.77	3.50	4.51	3.00	3.00	4.03	4.00	3.00	0.703	0.11	0.049	0.80
pH	3.74d	5.14b	8.51a	3.91d	4.74bc	7.91a	4.21cd	4.51c	8.20a	0.151	0.09	<0.001	<0.001
NH_3-N (g/kg N)	260a	206abc	141cd	177bcd	221ab	126d	142cd	172bcd	150bcd	17.4	<0.001	<0.001	<0.001
Lactic acid (g/kg DM)	22.9c	21.8c	18.8d	25.4b	26.7b	21.9c	26.5b	40.4a	21.5c	0.55	<0.001	<0.001	<0.001
Acetic acid (g/kg DM)	4.65d	8.61ab	5.05d	10.2a	7.53bc	5.55cd	8.72ab	10.8a	4.42d	0.052	<0.001	<0.001	<0.001
n-Butyric acid (g/kg DM)	0.62f	6.73b	1.85ef	3.19def	11.0a	4.59bcd	5.90bc	3.86cde	6.27bc	0.57	<0.001	<0.001	<0.001
Ethanol (g/kg DM)	0.385c	1.24bc	1.67bc	4.15b	0.58c	2.24bc	17.9a	0.62c	1.37bc	0.073	<0.001	<0.001	<0.001
2,3-butanediol (g/kg DM)	0.205e	3.93a	0.853de	1.63bcd	1.71bc	1.28bcd	1.27bcd	0.913cde	1.92b	0.018	0.15	<0.001	<0.001
Propionic acid (g/kg DM)	0.090e	3.49a	0.733cd	1.18bc	1.40b	0.540de	0.685cd	0.368de	1.30b	0.012	<0.001	<0.001	<0.001
Aerobic stability (h)	240a	240a	240a	73.9b	240a	240a	46.3b	240a	240a	9.06	<0.001	<0.001	<0.001
DM loss (g/kg DM)	123b	174a	68.1c	125b	113b	89.4c	152a	124b	37.7d	4.95	<0.001	<0.001	<0.001

LAB - lactic acid bacteria; cfu - colony-forming unit; SEM - standard error of the mean.
[1] HM - mixture (1:1, fresh matter basis) of corn heavy steep water + sugarcane molasses, applied at 0, 100, and 200 g/kg fresh matter; CaO - calcium oxide, applied at 0, 20, and 40 g/kg fresh matter.
[2] HM - effect of the addition of corn heavy steep water + sugarcane molasses; CaO - effect of the addition of calcium oxide; HM × CaO - interaction effect.
i-Butyric, i-valeric and n-valeric acids, n-propanol and 1,2-propanediol concentrations were <0.1 g/kg DM.
Means with different letters in the rows differ statistically (Tukey test α = 0.05).

Both CaO and HM increased the 2,3-butanediol and propionic acid concentrations, but the effect size was dependent on the specific combination (P<0.001 for the interaction HM × CaO). Without HM, the highest concentrations of 2,3-butanediol and propionic acid were observed for 20 g/kg of CaO. At 100 g/kg HM, the propionic acid decreased with 40 g/kg CaO. At 200 g/kg HM the greatest concentrations of 2,3-butanediol and propionic acid were observed in silage treated with 40 g/kg CaO (Table 2).

The inclusion of 40 g/kg of CaO reduced the DM loss during fermentation (P<0.001; Table 2). When HM was added, the DM loss was also reduced (P<0.001). Then the combination of CaO and HM resulted in the lowest DM loss among treatments (P<0.001; Table 2).

Only 100HM0CaO and 200HM0CaO silages heated up during the aerobic stability test, after 74 and 46 h, respectively (P<0.01). Parallelly, an increase of pH occurred for those treatments after 2 and 3 d of aerobic exposure, respectively (P<0.001; Figure 1).



Effect of HM (P<0.001); effect of CaO (P<0.001); effect of day of exposure (P<0.001); effect of interaction HM × CaO (P<0.001); HM × day (P<0.001); CaO × day (P<0.001), and HM × CaO × day (P<0.001). Standard error of the mean = 0.135.

Figure 1 - pH of corn husk silages treated with calcium oxide (CaO) and a mixture of corn heavy steep water and sugarcane molasses (HM) throughout the aerobic exposure period.

3.3. Chemical composition of corn husks silages

There was an interaction HM × CaO (P<0.001) for DM, ash, CP, aNDFom, and ADFom concentrations (Table 3). Overall, DM decreased with HM (P<0.001) but increased with CaO addition (P<0.001). Ash concentration increased with both CaO and HM, with the highest ash concentration observed in the 200HM40CaO treatment (Table 3). Overall, CP decreased with CaO, but increased with HM (P<0.001), reaching the highest values in the 200 HM treatments and the lowest in the 0HM treatments with 20 or 40 g/kg of CaO (Table 3). The aNDFom concentration decreased with both CaO and HM, with the lowest value observed for 200HM40CaO treatment (Table 3). The ADFom concentration varied with CaO addition depending on the HM level (P<0.001). At 100 g/kg HM, CaO increased ADFom, whereas CaO did not affect ADFom concentration at 200 g/kg HM (Table 3).

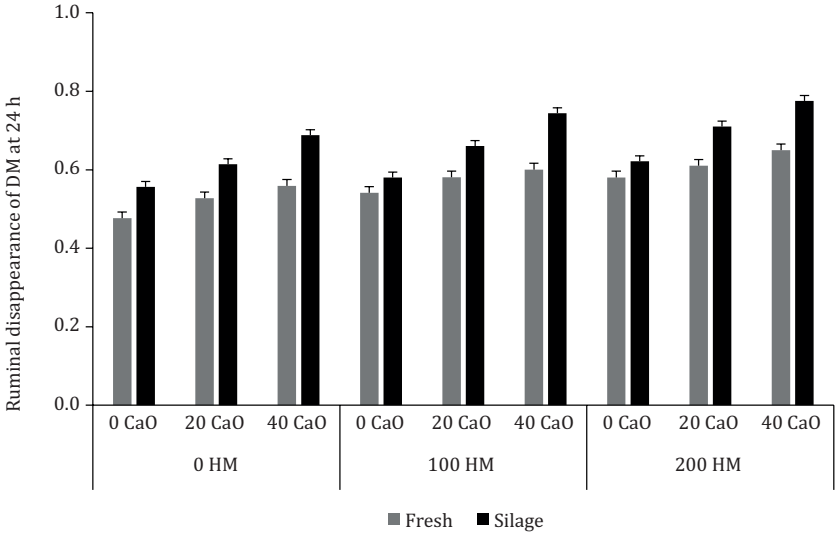
Table 3 - Chemical composition of corn husk silages treated with calcium oxide (CaO) and a mixture of corn heavy steep water and sugarcane molasses (HM) (n = 4)

Item	Treatment[1]									SEM	P-value[2]		
	0 HM			100 HM			200 HM						
	0 CaO	20 CaO	40 CaO	0 CaO	20 CaO	40 CaO	0 CaO	20 CaO	40 CaO		HM	CaO	HM × CaO
Dry matter (g/kg)	456ab	448bc	469a	437cd	432d	449bc	403e	439cd	426d	3.81	<0.001	<0.001	<0.001
Ash (g/kg DM)	27.9h	71.4e	108c	38.0g	90.8d	124b	54.9f	93.1d	145a	2.19	<0.001	<0.001	<0.001
CP (g/kg OM)	50.8d	40.2e	41.2e	68.9c	71.4c	65.5c	96.9a	81.3b	81.7b	1.49	<0.001	<0.001	<0.001
aNDFom[3] (g/kg OM)	615ab	636a	566c	578bc	539c	476d	552c	491d	421e	10.4	<0.001	<0.001	<0.001
ADFom[4] (g/kg OM)	248d	248d	342a	264cd	339a	337a	291bc	298b	283bc	6.08	<0.001	<0.001	<0.001

CP - crude protein; SEM - standard error of the mean.
[1] HM - mixture (1:1, fresh matter basis) of corn heavy steep water + sugarcane molasses, applied at 0, 100, and 200 g/kg fresh matter; CaO - calcium oxide, applied at 0, 20, and 40 g/kg fresh matter.
[2] HM - effect of the addition of corn heavy steep water + sugarcane molasses; CaO - effect of the addition of calcium oxide; HM × CaO - interaction effect.
[3] aNDFom - neutral detergent fiber assayed with sodium sulfite, heat-stable amylase and expressed excluded of residual ash.
[4] ADFom - acid detergent fiber expressed excluded of residual ash.
Means with different letters in the rows differ statistically (Tukey test α = 0.05).

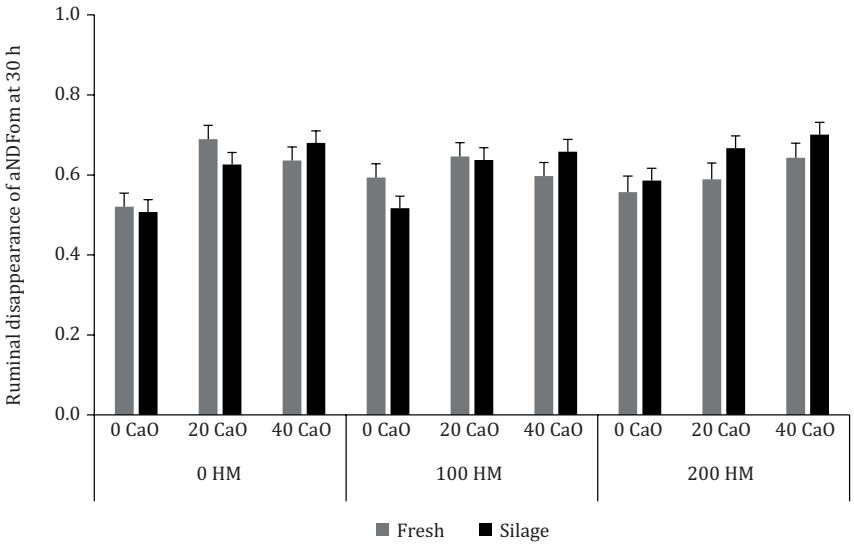
3.4. *In situ* ruminal disappearance

Both CaO and HM induced an increase of DM disappearance (P<0.001; Figure 2). Furthermore, ensiling induced an increase of DM disappearance in comparison with fresh husks (P<0.001). The addition of HM (P = 0.64) and the ensiling (P = 0.40) did not change the ruminal disappearance of aNDFom at 30 h of incubation. The addition of CaO, however, had a positive linear effect (P<0.001) effect on the ruminal degradability of aNDFom (Figure 3).



HM effect (P<0.001); CaO effect (P<0.001); HM × CaO interaction (P = 0.99); ensiling effect (P<0.001); ensiling × HM interaction (P = 0.78); ensiling × CaO interaction (P<0.001); ensiling × CaO × HM interaction (P = 0.59); linear contrast for HM effect (P<0.001); quadratic contrast for HM effect (P = 0.65); linear contrast for CaO effect (P<0.001); quadratic contrast for CaO effect (P = 0.75). SEM_{Fresh} = 0.016; SEM_{Silage} = 0.014.

Figure 2 - Ruminal disappearance of dry matter (DM) at 24 h of *in situ* incubation in corn husks (fresh and silage) treated with calcium oxide (CaO) and a mixture of corn heavy steep water and sugarcane molasses (HM).



HM effect (P = 0.64); CaO effect (P<0.001); HM × CaO interaction (P = 0.19); ensiling effect (P = 0.40); ensiling × HM interaction (P = 0.12); ensiling × CaO interaction (P = 0.089); ensiling × CaO × HM interaction (P = 0.37); linear contrast for HM effect (P = 0.43); quadratic contrast for HM effect (P = 0.56); linear contrast for CaO effect (P<0.001); quadratic contrast for CaO effect (P = 0.005). SEM_{Fresh} = 0.036; SEM_{Silage} = 0.030.

Figure 3 - Ruminal disappearance of ash-free neutral detergent fiber (aNDFom) at 30 h of *in situ* incubation in corn husks (fresh and ensiled) treated with calcium oxide (CaO) and a mixture of corn heavy steep water and sugarcane molasses (HM).

4. Discussion

The conservation and feeding value are the main issues driving the efficiency of utilization of wet fibrous byproducts. This study demonstrated for the first time that wet corn husks can be conserved efficiently by ensiling, while presenting a reasonable aNDFom and DM digestibility. This experiment also provides insights into the positive and negative effects of CaO and HM additives on the fermentation pattern, aerobic stability, composition, and digestibility of corn husk silage.

As an indication of successful acidification, pH values of the 0HM0CaO, 100HM0CaO, and 200HM0CaO silages were 3.74, 3.91, and 4.21, respectively. The critical pH to avoid clostridia fermentation is directly linked to DM content, as the susceptibility of clostridia to organic acids increases with decreasing water activity. Weissbach (1968) reported that silages with DM content close to 500 g/kg must have a pH below 5.0 to stabilize anaerobically and guarantee good conservation. In contrast, in silages treated with 40 g/kg of CaO, the pH was higher than 7.9, whereas in silages treated with 20 g/kg of CaO, the pH was higher than 4.5 even after 66 d of ensiling. The addition of CaO increased the buffering capacity, thus preventing an efficient acidification and conservation of the corn husks. Even with a high concentration of soluble sugars resulting from the addition of HM, the excessive release of hydroxyls ions from the hydration of CaO and the formation of calcium hydroxide [$Ca(OH)_2$] neutralized, nullified the protons from the acids produced during fermentation. This higher pH environment likely favored the growth of undesirable microorganisms such as *Clostridium*, particularly in silages treated with CaO alone, as also evidenced by the higher concentration of n-butyric acid detected in these treatments.

The increase in LAB count due to the presence of CaO may be associated with the increase in buffering capacity, which prolongs the period during which the pH remains high without inhibiting the growth of these microorganisms. Cavali et al. (2006) also reported an increase in LAB population in sugarcane silage treated with 15 g/kg of CaO. However, CaO may have decreased lactic acid formation or, more likely, induced its degradation. The reduction in mold counts by CaO may have been caused by hydroxyl ions released by calcium hydroxide, which can cause damage to cytoplasmic membranes (Siqueira and Lopes, 1999; Jacovaci et al., 2017).

The increase in yeasts observed in the 0HM40CaO and 100HM40CaO treatments indicates that the CaO doses may not have been sufficient to inhibit yeast growth, which is typically suppressed by the hydroxyl ions released during $Ca(OH)_2$ formation, which can damage cytoplasmic membranes, as discussed previously. The small increase in aerobic spore-forming bacteria counts in the presence of HM may have occurred due to the corn heavy steep water containing considerable amounts of these microorganisms (i.e., *Bacillus* spp.), due to spontaneous fermentation in the agroindustry (Vecino et al., 2014). The increase of *Clostridium* counts in CaO treatments may have been due to the high pH of these silages, as these microorganisms frequently develop in crops with high buffering capacity (Woolford, 1984). Custódio et al. (2016) also observed *Clostridium* development in sugarcane silages treated with 15 g/kg of calcium oxide, regardless the high fermentability coefficient (>45) of sugarcane crop. The authors reported that the amount of lactic acid necessary to reduce the pH to inhibit clostridia development increased by 7.7-fold when the crop was treated with CaO.

The NH_3-N content was reduced with the addition of CaO, probably due to the restriction of microbial activity. The lactic acid content also decreased with the addition of CaO, suggesting a reduction in the metabolism of LAB during fermentation or, alternatively, a degradation of lactic acid by clostridia, as those silages had higher concentration of n-butyric acid compared with the control (0HM0CaO). The increased acetic acid concentration in silages treated with CaO and HM addition also suggest greater activity of undesirable bacteria in these silages (McDonald et al., 1991; Pahlow et al., 2003). n-Butyric, propionic, and acetic acids are typically associated with the development of undesirable microorganisms, such as clostridia and bacilli (McDonald et al., 1991). The higher buffering capacity caused by CaO addition may have improved the conditions for the greater activity of these microorganisms (Woolford, 1984). Custódio et al. (2016) found higher concentrations of n-butyric acid in sugarcane silages treated with CaO, indicating that the higher buffering capacity due to CaO addition

prolongs the time in during which the silage pH remains high, allowing the development of clostridia. Kaiser et al. (2002) established a threshold for butyric acid concentration of 3 g/kg DM, silages with concentration greater than this threshold can be consider their fermentation dominated by clostridia. Even though the treatment with 40 g/kg of CaO had low n-butyric acid concentration, the high pH value of 8.51 indicates that CaO might have strongly restricted microbial activity, preventing good conservation. High ethanol concentrations are often associated with yeast activity (Kung et al., 2018); however, other microorganisms can also contribute to its production. Heterofermentative bacteria can produce small amounts of ethanol, but its accumulation may also be associated with *Clostridium* development, as certain species are able to metabolize carbohydrates and amino acids into ethanol and other undesirable compounds, as previously discussed (Pahlow et al., 2003).

Beyond the negative impacts of CaO on the silage fermentation pattern, such as impaired acidification, increased pH, and proliferation of undesirable microorganisms, the treatments with CaO, especially at 40 g/kg, resulted in lower values of DM loss. This outcome suggests that the strong alkalizing effect of CaO may have limited overall fermentative activity by inhibiting certain microbial groups, but other undesirable microorganisms, such as *Clostridium*, were less affected, particularly at the doses used. Moreover, the greater ash content for CaO-treated silages contributes mathematically to lower DM loss, as the mineral fraction is not degraded during fermentation. Similar findings were reported by Santos et al. (2008) and Xavier et al. (2015), who also attributed the reduced DM loss to CaO-treated silages.

Aerobic stability was lower in silages that did not receive any dose of CaO and received doses of HM (100 and 200 g/kg). This was likely due to the higher availability of sugars and fermentation products, which served as substrates for microorganisms associated with aerobic spoilage. As expected, well-preserved silages are more susceptible to aerobic spoilage (McDonald et al., 1991), as molds and, in particular, yeasts consume sugars and lactic acid, leading to a rise in pH. This was observed in the current study, where only the treatments with HM addition but without CaO (100HM0CaO; 200HM0CaO) had lower aerobic stability. On the other hand, silages treated with CaO showed greater resistance to aerobic deterioration. This may be related to their fermentative pattern as a result of the buffering action of CaO, which promoted the production of weak organic acids such as acetic acid and n-butyric acid, resulting in greater aerobic stability (Amaral et al., 2009). Additionally, the CaO addition promoted the inhibition of molds (Jacovaci et al., 2017), due to the dissociation of calcium hydroxide into hydroxyl ions, which may damage the cell structures (Siqueira and Lopes, 1999). Other aerobic spoilage microorganisms, such as acetic acid bacteria and *Bacillus* spp., may have been involved in aerobic deterioration. Although silages with high acetic acid concentrations are generally more aerobically stable due to inhibition of yeasts and molds, the presence of acetic acid bacteria can oxidize ethanol under aerobic conditions, generating heat and promoting spoilage (Spoelstra et al., 1988; Nishino et al., 2009). Furthermore, *Bacillus* spp., as facultative anaerobic bacteria, could have contributed to aerobic deterioration (McDonald et al., 1991). Considering that CaO induced poorer fermentation in corn husk silage (as observed previously in other silages treated with alkali), other silage additives would be more appropriate to curtail aerobic deterioration. For instance, *Lentilactobacillus buchneri* and chemical additives based on the salts of weak acids (e.g., sorbate, benzoate, propionate) have consistently been reported to improve the aerobic stability of various silages, without negative effects on the fermentation profile (Kung et al., 2003; Arriola et al., 2021).

Both CaO and HM caused changes in the chemical composition of corn husks. The increase of ash concentration with HM and CaO in fresh and ensiled husks was due to the relatively high mineral content in both ingredients, especially CaO, which contains a high proportion of ash in its composition (Oliveira et al., 2008). Corn heavy steep water is also rich in nitrogen, with approximately 480 g/kg of CP (DM basis), therefore, the CP content in treatments containing HM was higher. On the other hand, there was a decrease in the percentage of CP concentration with the addition of CaO to the silages.

The aNDFom content was not changed in fresh husks treated with CaO, indicating that the fiber hydrolysis does not occur instantly. On the other hand, a reduction of aNDFom concentration in silages confirmed the hydrolytic effect of CaO on the cell wall, with the dose of 40 g/kg of CaO

being more efficient. Swelling and hydrolysis of the cell wall may have occurred due to the alkaline nature of CaO, resulting in its expansion and disruption of the intermolecular hydrogen bonds (Jackson, 1977). Additionally, Coombre et al. (1979) reported that alkaline treatment causes partial solubilization of hemicellulose and delignification of cellulose, making it more accessible to rumen fibrolytic microorganisms. However, the alkaline nature of CaO might have also affected the results of the neutral detergent fiber analysis, as CaO may have changed the pH of the neutral detergent solution, causing partial solubilization of the hemicellulose and lignin fractions. On the other hand, the ADFom value increased in some treatments in which HM and CaO were combined, suggesting that HM may have reduced the CaO hydrolysis efficiency. Additionally, the CaO likely increased the pH of the acid detergent solution, reducing its efficiency in removing hemicellulose during the ADF analysis.

Ruminal DM disappearance in situ at 24 h was increased by both HM and CaO. Corn husk silage treated with CaO also showed greater aNDFom disappearance at 30 h of ruminal incubation. Merchen and Bourquin (1994) reported that treating forage with hydrolytic additives, such as calcium hydroxide, resulted in changes in the cell wall, such as hemicellulose solubilization and increased fiber degradation. However, increased DM degradability may not necessarily be due to hemicellulose solubilization. Jacovaci et al. (2017), who conducted a meta-analysis of sugarcane silages treated with CaO, observed an increase in fiber digestibility, attributed to the hydroxyl groups promoting fiber swelling and facilitating the action of fibrolytic bacteria in the rumen. Furthermore, the additives used are soluble, especially HM, which may also have contributed to this increase in DM degradation.

Ensiling provided greater DM degradability when compared with fresh husks. However, the hydration of corn husks particles caused by ensiling may also have accelerated the process of colonization and degradation by ruminal microorganisms. The linear effect observed with the addition of CaO on DM degradability was likely due to the alkalizing effect of the additive (Jackson, 1977).

5. Conclusions

Although silages treated with CaO showed lower DM loss, higher aerobic stability and slightly greater *in situ* disappearance of DM and aNDFom, silage treated with CaO had poorer hygienic quality, as indicated by greater *Clostridium* counts and n-butyric acid concentration. Therefore, under practical conditions, corn husks might be ensiled without additives or, if convenient, added with up to 100 g/kg of HM as they showed good conservation pattern and satisfactory ruminal disappearance of DM and aNDFom.

Data availability

The datasets supporting the results of this study is available upon request to João Daniel (jlpdaniel@uem.br).

Author contributions

Conceptualization: Silva, M. C. and Daniel, J. L. P. **Data curation:** Carvalho, M. G. M. and Daniel, J. L. P. **Formal analysis:** Carvalho, M. G. M.; Oliveira, B. C. S.; Gomes, A. L. M. and Daniel, J. L. P. **Funding acquisition:** Daniel, J. L. P. **Investigation:** Carvalho, M. G. M.; Oliveira, B. C. S. and Gomes, A. L. M. **Methodology:** Carvalho, M. G. M. and Daniel, J. L. P. **Project administration:** Daniel, J. L. P. **Resources:** Daniel, J. L. P. **Software:** Daniel, J. L. P. **Supervision:** Daniel, J. L. P. **Validation:** Carvalho, M. G. M. and Daniel, J. L. P. **Visualization:** Carvalho, M. G. M.; Bragatto, J. M. and Daniel, J. L. P. **Writing – original draft:** Carvalho, M. G. M. **Writing – review & editing:** Carvalho, M. G. M.; Bragatto, J. M. and Daniel, J. L. P.

Conflict of interest

The authors declare no conflict of interest.

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