Use of vinasse as an additive in corn silage

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Abstract

This paper aimed to evaluate the use of doses of vinasse as an additive in corn silage and its effect on the bromatological and mineral composition, as well as food safety aspects of the silage. This research employed a completely randomized design with five treatments (doses of 0, 2.5, 5, 7.5, and 10% of vinasse on the weight of the mass per silo) and five replications, totaling 25 experimental units. The weight of the ensiled material decreased over the fermentation period, regardless of the vinasse dose. The 2.5% dose of vinasse increased the crude protein content by 16%, as well as the mineral matter, lead, potassium, and iron content of the corn silage. Thus, adding 2.5% of vinasse to corn silage promotes greater fermentation efficiency, improves bromatology, and enhances mineralogy while preserving the silage's visual quality.

Keywords: bromatological composition; fermentation; crude protein, forage quality.

Practical Application: The application of 2.5% vinasse in corn silage demonstrates practical feasibility by enhancing nutritional value and improving silage preservation, while also promoting the sustainable use of by-products from the sugar-energy industry.

1 INTRODUCTION

Corn (*Zea mays* L.) is one of the most important crops in the world, widely used as food for both humans and animals. The corn culture holds a special position in Brazil due to its high productivity and economic importance, ranking as the second most important agricultural *commodity* in the country (Souza et al., 2017). Besides, corn has desirable attributes to ensiling, such as a high level of soluble carbohydrates and fermenting capacity, which makes it an excellent forage (Aquino et al., 2017; Rabelo et al., 2014).

Ensiling is a widely used technique for forage conservation based on anaerobic fermentation, which reduces potential hydrogen ion concentration (pH) and preserves nutrients (Cardoso & Silva, 1995). During this process, the use of additives can minimize nutritional losses and improve the quality of the final product; the additives act as a catalyst for the fermentation process (Kung Junior et al., 1993; Yitbarek & Tamir, 2014).

In this context, vinasse, a by-product of the sugar–alcohol industry, is a promising option. Rich in nutrients such as potassium, calcium, magnesium, and iron, and with a naturally low pH, vinasse can be an efficient additive to silage, improving its stability and nutritional value (Oliveira et al., 2014). Additionally, Brazil is the second-largest producer of ethanol, making vinasse a widely available resource (Instituto Brasileiro de Petróleo e Gás [IBP], 2022).

However, using vinasse in ensiling requires a careful evaluation, considering both its benefits and the associated risks. The presence of heavy metals such as chromium (Cr) and lead (Pb) in vinasse can compromise the quality and safety of the produced food (Silva et al., 2007).

Thus, this study aims to analyze the use of vinasse as an additive in corn silage, investigating its effect on the bromatological and mineral composition, as well as the food safety aspects of the silage.

1.1 Relevance of the work

The use of vinasse as an additive in corn silage represents a sustainable strategy for utilizing agro-industrial waste, promoting improvements in nutritional quality and food safety. The addition of 2.5% vinasse increased the content of crude protein and essential minerals, in addition to promoting fermentation and preservation of the silage. These results contribute to the development of more efficient and environmentally responsible agricultural practices, especially in ethanol- and food-producing regions.

2 MATERIAL AND METHODS

The experiment was conducted in Quirinópolis, Goiás, between December 2023 and June 2024, using a completely

randomized design with five treatments. The doses of 0, 2.5, 5, 7.5, and 10% vinasse on the mass weight of the silo were used, with five repetitions, totaling 25 experimental units. Each unit consisted of mini silos made of polyvinyl chloride (PVC) with a diameter of 0.1 m, a height of 0.75 m, a volume of 0.00589 m³, and a capacity for 4 kg of silage. The treatments were defined based on previous studies that indicated the potential of vinasse as a fermentative additive (Silva et al., 2018).

A chemical analysis of the soil was conducted before corn sowing (Table 1), indicating the need for liming to increase base saturation. Accordingly, 1.3 t/ha of dolomitic limestone was applied.

The corn hybrid AG 8701 PRO4 was chosen due to its high productivity and climate adaptation, and it was sown in a spacing of 0.5 m between lines with a density of 60,000 plants/ha. Sowing was conducted in December 2023 using a direct planting system in oxisol (known in Brazil as *Latossolo*), with a medium clayey texture. Given the sowing, base fertilization was carried out with 358 kg/ha of fertilizer 8–28–16 (N, P_2O_5, K_2O), according to Emgopa's (Comissão de Fertilidade de Solos de Goiás, 1988) recommendations for the crop. Fertilizing was carried out 15 days after sowing (DAS), using 100 kg/ha of urea.

The corn was harvested at the physiological maturation point, R6 reproductive stage, ideal for ensiling, 90 DAS.

After harvest, the material was crushed and mixed with the respective doses of vinasse in sterilized plastic bowls. The silage was prepared in mini silos of PVC. The vinasse was obtained through a partnership with a sugar–alcohol industry (sugarcane milling) in the region of Quirinópolis. Approximately 0.30 kg of previously dried sand was added to the bottom of each experimental silo, covered by a 20 cm diameter tissue, and later covered by a nylon net of similar dimensions. The mixture was manually compacted in the mini silos, which were sealed and stored vertically in a controlled environment for 80 days.

After the fermentation period, the mini silos were opened, and the material was homogenized. This study conducted a bromatological analysis using the following parameters: HM: Humidity; DM: Dry matter; MM: Mineral matter; CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; pH: Potential hydrogen ion concentration, according to Silva and Queiroz (2006). The mineral analysis included the elements Cr, Pb, Ca, Mg, S, K, and Fe, following the methodologies described by Malavolta et al. (1997).

After drying and milling the samples, 0.015 kg of the milled content was collected for the analysis of Cr, Pb, Ca, Mg, S, K, and Fe, following the methodology by Malavolta et al. (1997).

For the instrumental analysis of color, the evaluation was conducted using a colorimetric spectrophotometer (CR400, Minolta brand), based on the International Commission on Illumination (CIE) XYZ coordinate system (CIE, 2004). The CIE XYZ system measures the intensity of light reflected by materials in the range of red (X), green-blue (Y), and blue (Z), making it possible to quantify alterations in the silage's color related to fermentation, the presence of plant pigments, and oxidative degradation. The readings were made in an environment with controlled light.

Data were submitted to analysis of variance (ANOVA) and, when significant ($p < .05$), adjusted by linear or quadratic regression using the SISVAR 5.6 software (Ferreira, 2019).

3 RESULTS AND DISCUSSION

The vinasse doses did not affect the mass loss of the ensiled material (slurry); however, the ensiling period did (Table 2).

The weight of the ensiled material decreased over the fermentation time, regardless of the vinasse dose (Figure 1). Silage weight loss occurs naturally during anaerobic fermentation (Borreani et al., 2018; Xu et al., 2024). In the first days of fermentation, heterofermentative bacteria, yeasts, and enterobacteria consume soluble sugars that promote greater releases of leachate, organic acids, and gases (carbon dioxide, CO_2) inside the silo, resulting in losses of mass (DM) of the silage (Wróbel et al., 2023).

Although the doses of vinasse did not significantly affect weight loss, in average values, the dose of 2.5% promoted less weight loss during the fermentation time. This result may be related to better fermentation efficiency, reducing slurry production and maintaining the silage's mass (Ferreira et al., 2018).

Regarding the bromatological analysis, the vinasse doses did not affect the HM, DM, NDF, or ADF ($p > .05$). On the other hand, the vinasse doses affected the MM, CP, and pH of the silage ($p < .05$) (Table 3).Although vinasse is a liquid residue, the doses used in this study did not alter the content of HM and DM in the corn silage. The redistribution of HM during the fermentation process and dilution of the mass solids of the ensiled material might have contributed to homogenizing the values between the vinasse doses.

Naturally, ensiled corn has a high hydric content, which can limit the influence of liquid additives on HM and DM (Freitas et al., 2006). Besides, the stability of the DM content in silage, even after adding liquid additives, is associated with the mass density, the capacity to redistribute HM during fermentation, the production of effluents, and the exposure to oxygenation (Borreani et al., 2018).

Table 1. Chemical characterization in the layer of 0–0.25 m depth in oxisol used to produce corn silage.

pH	P	K	Ca²⁺	Mg²⁺	Al³⁺	H+Al	SB	CTC(T)	Mo	V
H₂O	mg/dm³				cmolc/dm³					%
4.3	7.97	0.12	1.77	0.58	0.21	3.77	2.5	6.24	1.87	39.53

pH in CaCl2, Relation 1:2,5; P resin and K – Extractor Mehlich 1; Ca2+, Mg2+ e Al³+ - Extractor KCl 1 mol L-1; H + Al – Extractor Acetate of Calcium 0,5 mol L-1 – pH 7,0; SB: sum of exchangeable bases; CTC (T): Capacity of cationic exchange (pH 7,0); Mo: C. Org x 1.724 – Walkley-Black Method; V: Index of saturation by bases.

The vinasse doses were included directly related to the ensiled mass and not the soil during cultivation. Thus, its interference with the fiber's structural composition was limited. The content of the fibers is generally determined by physiological factors of the plant, such as the maturation state and the structure of the cell wall; it is minimally influenced by additives applied after harvest (Tavares et al., 2024).

Similarly, Ferreira et al. (2018) observed that moderate applications of vinasse in corn cultivation compromised the forage's quality, maintaining the levels of fibers within the desirable patterns. Likewise, Freitas et al. (2006) observed stability in the fibrous fractions in corn silages treated with liquid additives, which confirms the results.

Regarding the MM, CP, and pH, this study identified an effect between the doses for these variables (Figure 2). The corn

Table 2. Summary of the variance analysis for the variable, WL.

		Average square
VS	LL	WL
Vinasse doses	4	0.002227^{ns}
Period	1	1.442621^{*}
Vinasse doses × time	4	0.002227^{ns}
Residue	40	0.00132
VC	–	0.95

ns: not significant; *significant; VS: variation source; LL: level of liberty, VC: variation coefficient; WL; weight loss.
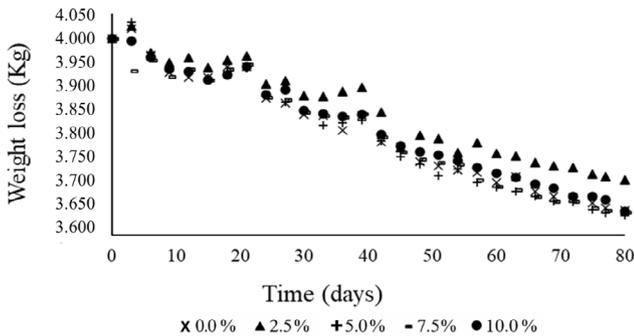

Figure 1. Weight loss of the ensiled material over 80 days of fermentation.

silage treated with 2.5% of vinasse was noted to have higher contents of MM (5.98%) and CP (8.89%). Vinasse is a liquid residue generated from alcohol fabrication and is rich in organic matter (OM). However, in addition to OM, vinasse also contains potassium and other nutrients, such as nitrogen, calcium, magnesium, zinc, copper, and others (Possignolo et al., 2015).

A meta-analysis by Zardin et al. (2017) reported values of 7.56% for CP and pH, and 3.76% for corn silage. On the other hand, García-Chávez et al. (2020) reported a value of 7.8% for CP. Corn silage treated with 2.5% of vinasse presented 8.94% for CP, above the average commonly found in corn silage. However, the supply of corn silage treated with vinasse does not meet the nutritional needs of cattle, which makes it necessary to include concentrated feed in their diets.

Adding 2.5% of vinasse to the corn silage significantly increased the contents of CP, ranging from 7.71 to 8.94%, with an increase of 16%. This parameter is essential in planning animal food due to its role in supplying amino acids for the synthesis of body proteins, which, in turn, favors the animals' growth and development. Notably, silage treated with 2.5% of vinasse had a higher CP content compared to the values reported by Maneerat et al. (2015), which were 3.80 and 6.90% in silages from pineapple and corn by-products, respectively. This is evidence of the protein potential of corn silage treated with vinasse.

According to Gerimipour et al. (2019), the chemical and mineral composition of vinasse are represented by DM (64.4%), ashes (14.8%), crude fat (0.15%), CP (20%), calcium (17.5 g kg^{-1}), phosphorus (0.8 g kg^{-1}), sodium (19.2 g kg^{-1}), potassium (64 g kg^{-1}), magnesium (2.3 g kg^{-1}), manganese (1.3 g kg^{-1}), zinc (0.028 g kg^{-1}), iron (1.4 g kg^{-1}), and copper (0.0021 g kg^{-1}). Such values confirm the nutritional potential of vinasse in animal food.

The remarkable protein content in silages in this study has significant nutritional value, as moderate intake of these foods by ruminants will increase total DM intake (Maneerat et al., 2015). Therefore, its use as an alternative source of forage in ruminant formulation will reduce the excessive dependence on conventional feeds, making it possible to reduce feed costs.

Table 3. Bromatological evaluation of corn silage treated with vinasse doses.

Variables (%/DM)	Vinasse doses (%)					Regression equation	R²	p-value
	0	**2.5**	**5**	**7.5**	**10**			
HM	68.679	67.47	65.771	66.756	67.076	$\hat{Y} = 67.151$	-	.563
DM	31.320	32.529	34.228	33.243	32.923	$\hat{Y} = 32.848$	-	.563
MM	4.318^{b}	5.944^{a}	4.874^{ab}	4.805^{ab}	4.956^{ab}	$\hat{Y} = 4.6744+0.2281x-0.0223x^{2}$	0.193	.013
CP	7.710^{b}	8.944^{a}	6.992^{b}	7.093^{b}	7.802^{b}	$\hat{Y} = 8.1853-0.1811x+0.0114x^{2}$	0.144	.000
NDF	47.897	49.136	50.559	50.518	50.080	$\hat{Y} = 49.638$	-	.904
ADF	25.273	24.379	25.635	25.318	26.685	$\hat{Y} = 25.458$	-	.768
pH	3.870^{a}	3.820^{ab}	3.770^{bc}	3.708^{c}	3.784^{b}	$\hat{Y} = 3.8823-0.0394x+0.0027x^{2}$	0.825	.000

Averages followed by different letters in the columns differ ($p < .05$) by the Tukey test: HM: humidity, DM: dry matter, MM: mineral matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, pH: Potential hydrogen ionic
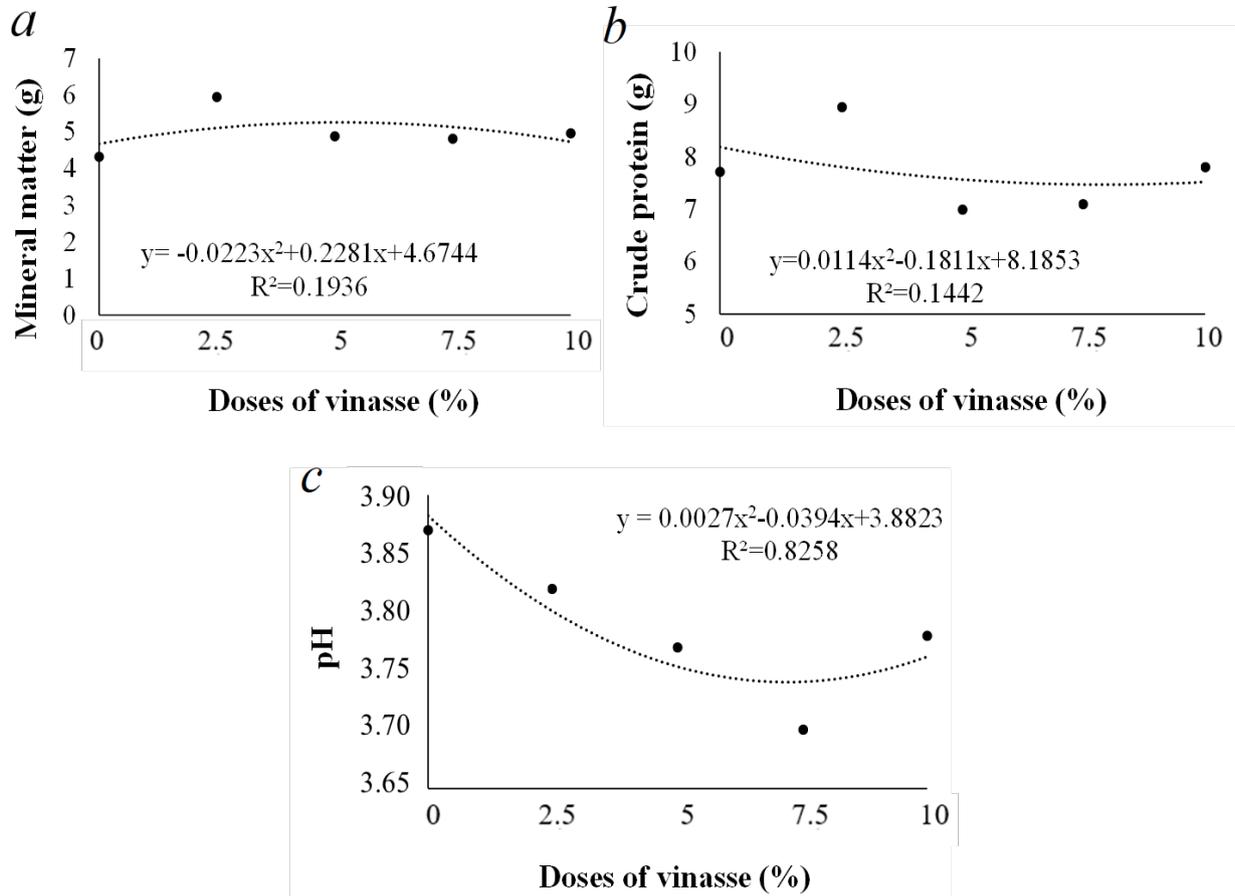
Figure 2. Mineral matter (A), crude protein (B), and pH (C) of corn silage treated with vinasse.

A trend of pH reduction was observed with the increase in vinasse doses in the silage, up to 7.5%, reaching optimal fermentation and a lower pH. This was followed by a slight increase at the 10% level, possibly due to excess OM or low microbial activity. The level of 7.5% promoted the lowest pH (3.70), indicating a more intense acidification ($p < .05$). Maneerat et al. (2015) reinforced that a well-preserved silage must have a low pH and a high lactic acid content.

The active fermentation phase begins with a decrease in oxygen concentration. At this time, the plant's respiration ceases, and the growth of aerobic microorganisms is inhibited, creating favorable conditions for lactic acid bacteria to produce lactic acid (Okoye et al., 2023). The production of lactic acid reduces the pH, inhibiting the undesired microorganisms and decreasing the proteolytic activity of enzymes present in forage (Bao et al., 2022).

On the other hand, the increase in the pH in the treatment with 10% can be related to the considerable content of CP in vinasse. As the dose of vinasse increased, the protein decomposition of the ensiled mass may have increased, increasing nitrogenous compounds capable of neutralizing lactic acid, which raised the material's pH (Breirem & Ulvesli, 1960). Thus, it justifies the possibility of the accumulation of nitrogenous compounds or the inhibition of lactic fermentation, leading to the formation of NH_4^+ or increasing the activity of proteolytic microorganisms, which in turn increases the pH.

The neutralization of lactic acid can occur due to the dissociation of H+. However, when there is a lot of free nitrogen, the "capture" of these ions occurs, forming NH^{4+}, which, depending on the amount formed, increases the medium's pH. This process is often observed in silages of legumes or of materials treated with urea (McDonald et al., 1991).

Compared to 0, adding 2.5% of vinasse increased the mineral content in the corn silage. Applying vinasse in soils also increases the content of exchangeable cations, mainly potassium and calcium, which are the mineral elements present in greater proportions in vinasse (Barros et al., 2010).

In tests conducted on Spodosol Cárbico, this behavior of potassium was attributed to its high concentration in vinasse, which increased its concentration in the soil solution, favoring it in the competition for a place in the exchange sites, resulting in greater adsorption of potassium (Brito & Rolim, 2005). In laboratory tests of vinasse percolation in the sandy soil of the Rio Claro Formation, based on the results of the retarding factor (R), the adsorption order was $K^+ > Ca^{2+} > Mg^{2+}$, corroborating the results obtained in this research (Basso et al., 2017).

On the other hand, the vinasse doses did not affect the contents of Cr, Pb, Ca, Mg, and S of corn silage ($p > .05$), while the vinasse doses did affect the concentrations of K and Fe ($p < .05$) (Table 4).

Cr is generally present in very low concentrations in vinasse, as it is a trace metal whose presence is more likely to result from specific environmental or industrial contamination. Like Cr, Pb is a heavy metal with low mobility and a high binding capacity with organic and colloidal matter, making it chemically stable and poorly soluble. These properties make its movement and absorption in the ensiled mass difficult. Pb is not described as a significant component, and when present, it is usually found at trace levels (Cunha et al. 1987; Silva et al., 2018).

The vinasse doses were not able to alter the Ca concentrations of the silage. This can be justified by the low solubility of Ca^{2+} under anaerobic conditions and the high natural availability of calcium in the corn plant, especially in the cell walls. Adding small amounts of vinasse was probably not enough to alter the total concentration of calcium in the silage. Furthermore, calcium has low mobility in plant tissue, which limits its redistribution even with liquid additives (Basso et al., 2017).

Despite being present in vinasse, Mg^{2+} is less abundant than K^+ and has a chemical behavior similar to that of calcium. The strong adsorption to cell wall components and the low leaching rate in an anaerobic medium help explain the absence of significant variation. Besides, the natural magnesium content in corn may have been enough to mask the effects of vinasse (Brito & Rolim, 2005).

This is due to its predominant chemical form in vinasse. Much of the sulfur in vinasse is not in the soluble form (sulfate), which limits its immediate availability (Possignolo et al., 2015). Potassium showed a significant difference between the treatments ($p > .05$), which can be explained by its high solubility in water and extremely mobile behavior.

K^+ is the most abundant nutrient in vinasse and is almost entirely in ionic form, which is highly soluble. Thus, when added to the ensiled mass, it tends to disperse quickly and redistribute throughout the plant matrix (Ferreira et al., 2018).

In addition, K does not bind strongly to OM like other cations, which favors its leaching by water, including through leachate. Studies have shown that potassium is the first element to be removed from soil columns irrigated with vinasse due to its high solubility and poor retention (Basso et al., 2017).

The treatment with 2.5% of vinasse presented not only the lowest weight loss but also the lowest levels of potassium loss. This behavior may be associated with low leachate production in this treatment, as potassium, being highly soluble in water and weakly retained in the plant matrix, tends to leach into systems with excess liquid (Basso et al., 2017; Ferreira et al., 2018). Thus, maintaining potassium in the silage of the 2.5% treatment reinforces the hypothesis that this level of addition generated a more balanced fermentation environment, with less effluent loss and better conservation of soluble nutrients.

Iron showed a significant difference between the treatments ($p > .05$). The reducing and anaerobic environment of the silage possibly favored the solubilization of Fe^{3+} to Fe^{2+}, increasing its bioavailability, which may have influenced this result (Fageria, 2001). Additionally, iron tends to be released from plant components and vinasse under low oxygen tension, a typical condition of well-sealed silages (Ferreira et al., 2018). This process may have influenced the higher iron levels in silage with 2.5% of vinasse, whose fermentation conditions were more stable. Therefore, the dose of 2.5% of vinasse increased the available iron content in corn silage.

For the instrumental color analysis, adding vinasse to corn silage affected the X and Z coordinates ($p < .05$), as shown in Table 5. The X and Z coordinates represent, respectively, the

Table 4. Mineral composition of corn silage treated with different levels of vinasse.

Variables (g kg⁻¹)	Vinasse doses (%)					Regression equation	R²	p-value
	0	2.5	5	7.5	10			
Cr	1.200	1.000	1.200	1.000	1.400	$\hat{Y} = 1.16$	-	.430
Pb	5.800	6.600	7.800	6.800	8.200	$\hat{Y} = 7.04$	-	.070
K	0.964[ab]	1.016[a]	0.940[ab]	0.928[b]	0.936[b]	$\hat{Y} = 0.9822 - 0.003x - 0.0003x^2$	0.414	.020
Ca	0.258	0.258	0.162	0.172	0.172	$\hat{Y} = 0.204$	-	.133
Mg	0.118	0.090	0.102	0.100	0.092	$\hat{Y} = 0.100$	-	.200
S	0.062	0.058	0.060	0.056	0.068	$\hat{Y} = 0.060$	-	.669
Fe	684.8[c]	1409[a]	923.2[bc]	1245[ab]	1096.8[abc]	$\hat{Y} = 805.87 + 133.50x - 10.71x^2$	0.336	.000

Averages followed by different letters in the columns differ ($p < .05$) according to the Tukey test. Cr: chromium; Pb: lead; K: potassium; Ca: calcium; Mg: magnesium; S: sulfur; Fe: iron.

Table 5. Color of the corn silage treated with different levels of vinasse.

Variables	Vinasse doses (%)					Regression equation	R²	p-value
	0	2.5	5	7.5	10			
X	10.28[b]	9.58[b]	13.516[a]	11.996[ab]	11.612[ab]	$\hat{Y} = 9.6917 + 0.7545x - 0.0551x^2$	0.449	.005
Z	6.31[ab]	5.942[b]	8.736[a]	7.152[ab]	6.974[ab]	$\hat{Y} = 5.9441 + 0.5584x - 0.0457x^2$	0.385	.026

Averages followed by different letters in the columns differ ($p < .05$) by the Tukey test. X: red-green; Z: blue.

contributions to visible light components in the red and blue ranges, being sensitive to chemical changes in plant biomass during the anaerobic fermentation process.

Adding 5% of vinasse increased both coordinates ($X = 13.52$; $Z = 8.74$), indicating a higher visual intensity of the silage. Possibly, such a response is associated with a higher release of plant pigments and products of phenolic compound oxidation in vinasse. The increased microbial fermentative activity, stimulated by the addition of organic liquid matter, can accelerate biochemical transformations that affect the material's color (Zhang et al., 2024).

On the other hand, the dose of 2.5% presented a lower value of X and Z, which suggests a lower degradation of natural pigments and, consequently, a more preserved color, generally associated with higher fermentative stability and lower production of darkening compounds (Andrade et al., 2010). The color variation indicated by the coordinates COORDX and COORDZ reinforces that, in addition to acting as a fermentative additive, vinasse can also affect the silage's optical and visual properties.

Light colors of silage indicate high nutritional quality for ruminants, as they reflect greater digestibility of DM, OM, NDF, and non-fructan carbohydrate (FNC) (Tahuk et al., 2020). Besides, according to Menezes et al. (2011), silage with a better visual aspect promotes higher consumption and performance in cattle. The darkened one, on the other hand, is associated with loss of nutrients and lower feeding efficiency.

In this study, the dose of 2.5% of vinasse promoted better fermentative stability, maintenance of visual attributes, and nutritional parameters compared to other concentrations. Thus, 2.5% of vinasse in corn silage contributes to silages being more efficient and more nutritionally adequate to ruminants.

4 CONCLUSIONS

Adding 2.5% of vinasse to corn silage is a viable and efficient alternative as it promotes fermentative stability, improves the nutritional contents of CP, MM, Pb, and K, and preserves the silage's visual quality.

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