# Effect of urea on gas and effluent losses, microbial populations, aerobic stability and chemical composition of corn (*Zea mays* L.) *s*ilage

## Efecto de la urea en las pérdidas por gases y efluentes, poblaciones microbianas, estabilidad eróbica y composición química en el ensilaje de maíz (*Zea mays* L.)

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### ABSTRACT

We evaluated the effects of urea addition on gas and effluent losses, fermentation profile, microbial populations, aerobic stability and chemical composition of corn silages. A completely randomised design with five levels of urea (0, 0.5, 1.0, 1.5, and 2.0% based on dry matter) and five replicates was used. A decreasing linear effect of urea levels on effluent losses in corn silages was observed. In parallel, an increasing linear effect of urea levels on pH, increasing from 3.49 to 4.12 in silages without urea in relation to silages with the maximum urea level, was also observed. Urea addition improved the aerobic stability of the silages, with 62 h for the silages without urea and from 90 to >96 h for the silages with urea. Based on the results of the principal components, two groups (I and II) could be distinguished. The most discriminating variables in group I were dry matter (-0.9), pH (-1.2) and lactic acid bacteria (-0.9), while in group II, effluent losses (1.0), ethanol (1.0), acetic acid (0.8) and gas losses (0.8) were most important. The use of urea at inclusion levels of around 2% in corn silage reduced gas losses, improved the nutritive value and promote the aerobic stability of silages.

### Keywords
lactic acid • lactic acid bacteria • silage additives • yeasts • *Zea mays* L.

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**Resumen**

El objetivo del presente estudio fue evaluar el efecto de la urea en las pérdidas por gases y efluentes, poblaciones microbianas, estabilidad aeróbica y composición química en el ensilaje de maíz. Se utilizó un diseño completamente al azar con cinco niveles de urea (0, 0,5, 1,0, 1,5 y 2,0% basado en materia seca) y cinco repeticiones. Hubo un efecto lineal decreciente de los niveles de urea en las pérdidas de efluentes en los ensilajes de maíz y en los valores de pH que aumentaron de 3,49 a 4,12 en los ensilajes sin urea en relación con los ensilajes con el nivel máximo de urea. Los ensilajes de urea mejoraron la estabilidad aeróbica de los ensilajes en comparación con aquellos sin la adición de urea, siendo 62 h para los ensilajes sin urea y de 90 a> 96 h para los ensilajes con urea. El análisis de componentes principales permitió formar dos grupos (I y II). Las variables más discriminatorias en el grupo I fueron: materia seca (-0,9), pH (-1,2) y bacterias del ácido láctico (-0,9); mientras que en el grupo II: pérdidas de efluentes (1,0), etanol (1,0), ácido acético (0,8) y pérdidas de gas (0,8). El uso de urea a niveles cercanos al 2% en ensilaje de maíz reduce las pérdidas de gas, mejora el valor nutritivo y promueve una mayor estabilidad aeróbica de los ensilajes.

**Palabras clave**

ácido láctico • bacterias de ácido láctico • aditivos de ensilaje • levaduras • *Zea mays* L.

**INTRODUCTION**

Ensiling is a preservation technique for moist forage based on anaerobic fermentation, in which lactic acid bacteria metabolise soluble sugars into organic acids, especially lactic acid. Thus, the biomass becomes acidified, and the forage is preserved until the silo is opened (9, 13, 25).

Among the forage species most suitable for silage production, corn (*Zea mays* L.) is highlighted due to its high nutritive value, adequate dry matter content, high concentration of fermentable carbohydrates, low buffering power and high digestibility. However, the high concentration of water-soluble carbohydrates of many cultivars (10, 13, 20) may produce excess of lactic acid and predispose the ensiled mass to the development of yeasts, especially during aerobic exposure throughout the feeding phase (27). According to Pahlow *et al*. (2003), corn plants may have 3 to 5 log colony-forming unit (CFU)/g of yeasts, which may compromise the aerobic stability of the silage (4, 25), reducing its nutritive value (29) and precluding animal performance (6).

Yeasts are the initiating microorganisms of the aerobic deterioration of silages due to lactate assimilation (13). Aerobic instability is associated with molds and yeasts in corn silages, occurring mainly in the upper layers of the silage, which are more prone to deterioration (4, 10). To minimise this effect, additives with antifungal properties are used to reduce yeast populations and increase aerobic stability in silage (4, 25). Previous studies have focused on the reduction of losses during ensiling and after opening the silo, using chemical additives (16, 27). Urea, used as chemical additive, in silages can be converted into ammonia since it undergoes hydrolysis in the presence of moisture and under urease activity (enzyme catalyst) of plants and microorganisms, producing two molecules of ammonia and carbon dioxide (13). Thus, the addition of urea would provide temporary buffering of the ensiled mass, slightly increasing the pH values and mildly reducing the lactic acid content as a function of the heterofermentative lactic acid bacteria in the initial fermentation stage (17). This would result in silage less prone to aerobic deterioration.

Besides reducing aerobic instability, silages ensiled with non-protein nitrogen can show high contents of protein in the silage, meeting ruminant requirements (6).

In this way, the use of urea as a chemical additive in corn silages with a high concentration of water-soluble carbohydrates in experimentally defined proportions can reduce fermentative losses and the toxic effect to the population of yeasts and moulds, improving the nutritional value of silages.

In this respect, the aim was to evaluate the effect of urea on losses by gases and effluents, microbial populations, aerobic stability and chemical composition in corn silage.

## Materials and methods

### Experimental area and climatic conditions

The experiment was carried out in the Forage Farming Sector of the Department of Animal Science of the Center of Agricultural Sciences, Federal University of Paraíba - UFPB, located in the mesoregion of Agreste and the microregion of Brejo Paraibano, municipality of Areia, at the coordinates of 06°57'46" S and 35°41'31" W and an elevation of 623 m above sea level. The climate in the region, according to the Köppen classification, is As' (hot and humid), with an average annual rainfall of 1,400 mm, an average annual temperature of 24.5°C and an average relative humidity of 80%.

### Experimental design and treatments

Corn harvesting was carried out at an age of 97 days, when the grains were in the milky/pasty stage. Whole plants were manually harvested and chopped to a length of 2 cm with a stationary forage machine. Chopped corn was divided into 10-kg piles, and each pile was assigned to one of the following urea levels: 0, 0.5, 1.0, 1.5 and 2.0%, based on dry matter. All treatments were dissolved in 50 mL of deionised water and sprayed on the forages uniformly and under constant mixing.

The material was packed into five polyvinyl chloride (PVC) experimental silos, with a length of 300 mm and a diameter of 150 mm, to achieve a final packing density of 600 ± 20 kg of fresh matter/$m^3$. The PVC silos were equipped with a Bunsen's valve to allow gas exit and 1.5 kg of sand at the bottom (for effluent drainage). Subsequently, the silos were sealed, weighed and stored for 70 d in a covered area at ambient temperature (25 ± 2°C).

### Dry matter losses

The silos were weighed on the silage day and after 70 days of the fermentation process to estimate fermentation losses. To estimate gases, effluent losses and dry matter recovery, the equations described by Zanine *et al.* (2010) were used.

### Chemical analysis and aerobic stability

Before ensiling, one entire corn plant was sampled to estimate its chemical composition and microbial populations prior to ensiling (table 1).

**Table 1.** Chemical composition and microbial populations of a fresh whole corn plant before ensiling.

**Tabla 1.** Composición química y poblaciones microbianas de toda la planta de maíz antes del ensilaje.

NDFap - Neutral detergent fibre corrected for ash and protein; CFU -Colony-forming unit.
NDFap - fibra detergente neutral corregida por cenizas y proteínas; Unidad de entrenamiento de colonia de CFU.

| | |
|---|---|
| Dry matter (g kg$^{-1}$) | 262.49 |
| Crude protein (g kg$^{-1}$ DM) | 62.05 |
| Mineral matter (g kg$^{-1}$ DM) | 32.34 |
| NDFap[1] (g kg$^{-1}$ MS) | 548.34 |
| Total soluble carbohydrates (g kg$^{-1}$ DM) | 152.14 |
| Lactic acid bacteria (log cfu/g)[2] | 5.50 |
| Yeasts and moulds (log cfu/g) | 4.30 |

The pH was determined in distilled water in duplicate, using a potentiometer. Briefly, approximately 25 g of the ensiled material from each treatment were mixed with 100 mL of water. After 1h, reading was performed according to the methodology described by Bolsen *et al.* (1992).

A fraction of 10 ml was collected from these solutions, rediluted with 1 mL of distilled water, acidified with 50% $H_2SO_4$ and filtered through Whatman filter paper for organic acid and ethanol analysis (10).

Subsequently, 2 ml of the filtrate were spiked with 1 mL of 20% metaphosphoric acid and 0.2 mL of 0.1% phenolic acid. The samples were centrifuged, and organic acids (lactic acid, acetic, propionic and butyric) and ethanol were analysed by high-performance liquid chromatography (HPLC), using a SHIMADZU, model SPD detector-10 VP coupled to an ultraviolet (UV) detector at a wavelength of 210 nm.

Microbiological evaluation was performed according to Kung and Ranjit (2001); 25-g fresh silage samples, extracted according to the defined opening periods, were collected. Subsequently, we added 225 mL of sterile Ringer's solution and blended the mixture for approximately 1 min, followed by the removal of 1 mL and appropriate dilution ($10^{-1}$ to $10^{-9}$). Plating was performed in duplicate for each culture medium. In addition, the microbial populations of the forages and silages were analysed; LAB were enumerated in triplicate by pour-plating using the de Man, Rogosa and Sharpe MRS agar. Agar plates were incubated anaerobically for 48 h at 39°C, and yeasts and moulds (Y&M) were enumerated in triplicate by pour-plating with potato dextrose agar and anaerobic incubation for 7 days at room temperature. Enterobacteria were determined on violet-red bile and anaerobically incubated for 24 h at 35°C. Plates from the appropriate dilutions were counted when they contained a minimum of 30 and a maximum of 300 colonies. For data analysis, the counts were transformed into log10cfu/g.

Chemical analyses were performed at the Laboratory of Animal Nutrition and Food Evaluation of the CCA/UFPB. The samples were conditioned in paper bags, weighed and kept in an oven at 60°C for 48 h. Afterwards, they were milled in a Willey type mill with a 1-mm mesh sieve and subjected to analyses.

We analysed dry matter (DM -method no. 934·01), ash (method no. 930·05) and crude protein (CP -method no. 981·10) according to the methodologies of the AOAC (1990). Neutral detergent fibre was corrected for ash and protein (NDFap) according to Licitra *et al.* (1996) and Mertens (2002).

Water-soluble carbohydrate (WSC) values were determined via spectrophotometry according to the method described by Dubois *et al.* (1956). For extraction, 100 mg of dry and milled sample were added to 100 mL of 80% ethanol solution and placed in a water bath at 80°C for 30 min. Subsequently, the material was filtered, the solid residue discarded, the volume was completed to 500 mL with distilled water in a volumetric flask and the ethanolic extract was homogenised. We then added 1 mL of 5% phenol solution and 5 mL of concentrated sulfuric acid and removed 2-mL aliquots of ethanolic extract. A standard curve was constructed with increasing 0.01% glucose solution concentrations, and the spectrophotometer readings were conducted at 490 nm absorbance. The WSC contents were calculated in g × 100 mL$^{-1}$ based on the solution and then fitted according to the dry matter of each sample.

Corn silage aerobic stability was determined through samples of approximately 2 ± 0.03 kg silage from each silo. The samples were relocated in silos cleaned without compaction, and the internal temperature of the silage mass was verified every 1 h using a digital immersion thermometer. The ambient temperature was recorded through a thermometer suspended in the air. Aerobic stability was calculated as the number of hours before the silage mass temperature reached 2°C above room temperature (25).

### Statistical analysis
The data were analysed using the PROC GLM of the SAS 8.2 Software and subjected to analysis of variance and regression (21). The criterion adopted for choosing the regression models was the significance of parameters estimated by the models and the values of the coefficients of determination; P-values less than 0.05 were considered significant.

The significance levels of Pearson's correlation coefficients were estimated for fermentation, microbiological losses and losses of corn silage.

Principal components analysis allows grouping the largest amount of original information contained in p variables (*P*=13, in the present study) into a coordinate system covered by two principal components (1 and 2) (7), allowing to plot the variables in a two-dimensional graph. The multivariate analysis was processed using STATISTICA version 7.0 (23), and the graph was generated via the MVSP tool, trial version 3.2.

## Results

### Dry matter losses

The addition of urea to corn silage did not significantly affect ($P >0.05$) dry matter recovery (table 2).

**Table 2.** Dry matter losses of corn silages treated with urea.

**Tabla 2.** Pérdidas de materia seca de ensilajes de maíz tratados con urea.

| Item | Urea (% of DM) | | | | | SEM [1] | P-value | |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | | Lin [2] | Quad [3] |
| Gas losses (g kg⁻¹ DM) | 26.7 | 29.2 | 21.2 | 27.0 | 23.0 | 1.69 | 0.4940 | 0.0121 |
| Effluent losses (kg/ton) | 160.0 | 154.0 | 109.0 | 108.0 | 109.0 | 0.55 | <0.001 | 0.0060 |
| DMR[4] (g kg⁻¹ DM) | 946.1 | 951.0 | 961.4 | 951.0 | 948.6 | 1.55 | 0.1895 | 0.0972 |

However, there was a decreasing linear effect ($P<0.001$) of urea levels on effluent losses in corn silages, with a reduction from 160.0 to 109.0 kg/t in silages without urea in relation to silages with the maximum level of urea (2.0%), with an estimated reduction of 29.6kg/t for every 1% of urea added to the ensiled mass. The gas losses presented a positive quadratic effect ($P = 0.0121$) with regression estimated a maximum point of gas losses close to 0.52% to inclusion of urea.

### Fermentation profile and microbial populations of the silages

There was an increasing linear effect of urea level ($P<0.05$) on pH values in corn silages, increasing from 3.49 to 4.12 in silages without urea in relation to silages with the maximum urea level (table 3).

**Table 3.** Fermentative profile and microbial populations of corn silages treated with urea.

**Tabla 3.** Perfil de fermentación y poblaciones microbianas de ensilajes de maíz tratados con urea.

| Item | Urea (% of DM) | | | | | SEM[1] | P-value | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 0.5 | 1.0 | 1.5 | 2.0 | | Lin[2] | Quad[3] |
| pH | 3.49 | 3.50 | 4.08 | 4.06 | 4.12 | 0.035 | <0.001 | 0.1689 |
| Lactic acid (g kg⁻¹ DM) | 46.62 | 45.79 | 47.55 | 38.41 | 55.83 | 0.608 | 0.8010 | 0.1001 |
| Acetic acid (g kg⁻¹ DM) | 28.50 | 15.91 | 21.38 | 11.53 | 18.90 | 0.206 | 0.4506 | 0.7220 |
| Propionic acid (g kg⁻¹ DM) | 0.66 | 0.41 | 0.49 | 0.37 | 0.42 | 0.008 | 0.4770 | 0.1384 |
| Butyric acid (g kg⁻¹ DM) | 0.39 | 0.38 | 0.38 | 0.33 | 0.29 | 0.006 | 0.0131 | 0.1026 |
| Ethanol (g kg⁻¹ DM) | 21.95 | 12.63 | 5.75 | 6.01 | 7.85 | 0.566 | 0.2303 | 0.1107 |
| Lactic acid bacteria[7] (log cfu/g) | 7.52 | 8.01 | 9.01 | 9.04 | 8.58 | 0.028 | 0.1400 | 0.0004 |
| Yeasts and moulds (log cfu/g) | 4.86 | 5.01 | 3.95 | 4.25 | 4.00 | 0.013 | 0.3050 | 0.2261 |

Lactic, acetic and propionic acids did not significantly influence the corn silages ($P >0.05$). However, the butyric acid concentrations of the silages showed a linear decreasing effect ($P = 0.0131$). There was a quadratic fitting for the concentrations of populations of lactic bacteria ($P< 0.001$) (table 3), with the maximum point of LAB populations close to a urea inclusion level of 1.5%.

Average lactic acid concentration was 44.59 g/kg DM, showing that the addition of urea maintained the lactic acid contents in the corn silages ($P = 0.1001$) (table 3). A similar behaviour was found for acetic acid concentrations ($P = 0.4506$), which showed an average content of 19.24 g/kg DM in corn silages. The urea levels did not alter ($P = 0.1384$) the concentrations of propionic acid in the silages, averaging 0.47 g/kg DM. The highest concentration of butyric acid ($P = 0.0131$) was observed in silage without urea (0.39 g/kg DM) and the lowest concentration of butyric acid in silage with the highest level of urea (0.29 g/kg DM) (table 3).

Ethanol concentrations did not affect corn silage ($P$>0.05). For populations of lactic acid bacteria of the silages, the addition level of 1.5% urea resulted in 9.04 log cfu/g in the forage ($P$< 0.001) (table 3, page 313).

### Chemical composition
The addition of urea did not significantly influence the dry matter content ($P$ = 0.0522) of the silages, averaging close to 28%. There was, however, a linear effect on ash concentrations (P< 0.001) (table 4).

The crude protein content of corn silages increased linearly ($P$< 0.001), and there was a linear decrease ($P$= 0.0150) for the concentrations of NDFap in corn silages (table 4).

**Table 4.** Chemical composition of corn silage treated with urea.

**Tabla 4.** Composición química de ensilajes de maíz tratados con urea.

| Item | Urea (% DM) | | | | | SEM [1] | P-value | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 0.5 | 1.0 | 1.5 | 2.0 | | Lin [2] | Quad [3] |
| DM[4] (g kg^{-1}) | 270.80 | 311.20 | 282.46 | 296.06 | 224.93 | 1.925 | 0.5273 | 0.0522 |
| Ash (g kg^{-1} DM) | 33.37 | 33.79 | 37.86 | 39.99 | 50.53 | 1.256 | <0.001 | 0.3893 |
| CP[5] (g kg^{-1} DM) | 82.15 | 108.98 | 111.51 | 139.93 | 143.18 | 2.574 | <0.001 | 0.0017 |
| NDFap[6] (g kg^{-1} DM) | 494.80 | 502.72 | 475.12 | 444.45 | 420.60 | 7.666 | 0.0150 | 0.9514 |
| WSC[7] (g kg^{-1} DM) | 80.42 | 75.72 | 87.19 | 103.32 | 72.54 | 1.569 | 0.2170 | 0.2600 |

There was no significant effect of the urea levels in the silages on the water-soluble carbohydrate concentrations ($P$ = 0.2170), with average values of 84.89 g/kg.

### Aerobic stability of silages
The maximum temperature showed a linear decrease (P = 0.0010) after exposure to air during the 96-h period. The pH values of corn silages after exposure to air during the 96-h period were not affected ($P$= 0.1406), (table 5).

The yeast populations recorded in the corn silages after exposure to air decreased linearly ($P$< 0.001). The average values of yeasts at 96 h were higher in the silages without urea (table 5).

**Table 5.** Aerobic stability, pH and yeast populations of corn silages treated with urea.

**Tabla 5.** Estabilidad aeróbica, pH y poblaciones de levaduras de ensilajes de maíz tratados con urea.

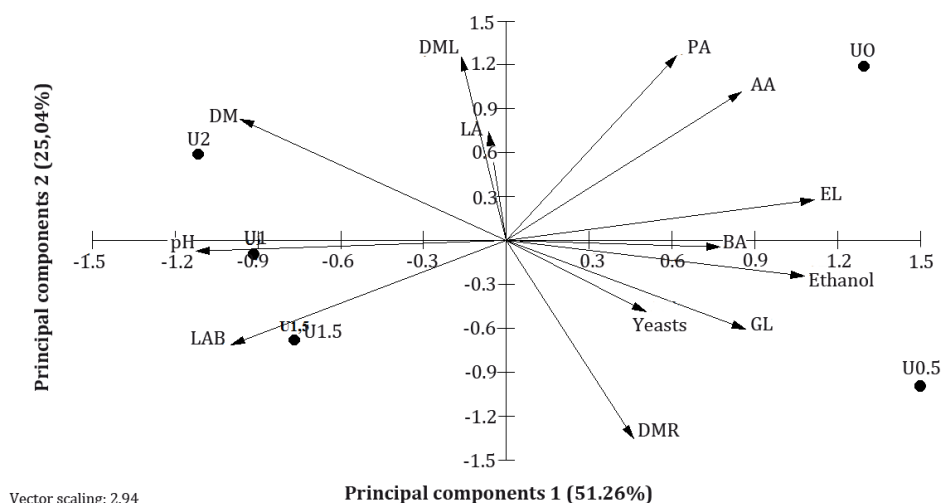| | Urea (% DM) | | | | | SEM [1] | P-value | |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | | Lin [2] | Quad [3] |
| Maximum temperature (°C) | 26.40 | 25.10 | 25.0 | 24.5 | 24.10 | 0.110 | 0.0010 | 0.12005 |
| Aerobic stability (h) | 62 | 90 | >96 | >96 | >96 | 0.365 | - | - |
| **After 96 h of air exposure** | | | | | | | | |
| pH | 4.12 | 4.49 | 4.24 | 4.22 | 4.65 | 0.072 | 0.3024 | 0.1406 |
| Yeasts and moulds (log cfu/g) | 9.69 | 8.10 | 7.92 | 7.12 | 6.84 | 0.209 | <0.001 | 0.1070 |

Urea addition improved the aerobic stability of the silages in relation to those without the addition of urea, being 62 h for the silages without urea and from 90 to >96 h for the silages with urea.

### Pearson's correlation coefficient and principal components analysis of fermentative characteristics of silages
Gas losses correlated positively with organic acid concentrations (especially butyric acid). Populations of lactic acid bacteria showed a positive correlation with pH (figure 1, page 315).

**Figure 1.** Distribution of dry matter (DM), pH, lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA), ethanol, lactic acid bacteria (LAB), yeasts and moulds (Y&M), dry matter losses (DML), gas losses (GL), effluent losses (EL) and dry matter recovery (DMR) in the coordinate system covered by principal components 1 and 2.

**Figura 1.** Distribución de materia seca (DM), pH, ácido láctico (LA), ácido acético (AA), ácido propiónico (PA), ácido butírico (BA), etanol, bacterias del ácido láctico (LAB), levaduras y mohos (L&B), pérdidas de materia seca (DML), pérdidas de gas (GL), pérdidas de efluentes (EL) y recuperación de materia seca (DMR) en el sistema de coordenadas cubierto por los componentes principales 1 y 2.

There was a positive correlation between the concentrations of acetic and propionic acids, which in turn had a strong positive correlation with dry matter losses. The ethanol content of the silages was positively correlated with the acetic, butyric and propionic acid contents and strongly correlated with effluent losses and yeast populations (table 6).

**Table 6.** Pearson's correlation coefficient of the fermentative profile, microbial populations and dry matter losses of corn silages treated with urea.

**Tabla 6.** Coeficiente de correlación de Pearson del perfil fermentativo, poblaciones microbianas y pérdidas de materia seca de ensilajes de maíz tratados con urea.

| | BA [1] | LA [2] | AA [3] | PA [4] | ET [5] | DM [6] | GL [7] | EL [8] | DMR [9] | pH | LAB [10] | Y&M [11] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BA [1] | | -0.174 | 0.442 | 0.557 | 0.510 | -0.659 | 0.597 | 0.583 | -0.072 | -0.363 | -0.432 | -0.141 |
| LA [2] | | | 0.156 | 0.156 | 0.110 | -0.359 | 0.328 | -0.004 | 0.156 | -0.083 | -0.203 | -0.432 |
| AA [3] | | | | 0.850 | 0.667 | -0.526 | 0.291 | 0.827 | -0.085 | -0.769 | -0.944 | 0.008 |
| PA [4] | | | | | 0.332 | -0.585 | 0.367 | 0.623 | 0.307 | -0.480 | -0.723 | -0.154 |
| ET [5] | | | | | | -0.410 | 0.264 | 0.909 | 0.276 | -0.842 | -0.803 | 0.403 |
| DM [6] | | | | | | | -0.738 | -0.136 | -0.120 | 0.065 | 0.327 | 0.256 |
| GL [7] | | | | | | | | 0.210 | -0.093 | -0.253 | -0.128 | -0.274 |
| EL [8] | | | | | | | | | 0.043 | -0.834 | -0.918 | 0.347 |
| DMR [9] | | | | | | | | | | -0.122 | 0.005 | 0.504 |
| pH | | | | | | | | | | | 0.873 | -0.413 |
| LAB [10] | | | | | | | | | | | | -0.299 |
| Y&M [11] | | | | | | | | | | | | |

[1] BA - butyric acid; [2] LA - lactic acid; [3] AA - acetic acid; [4] PA - propionic acid; [5] ET - ethanol; [6] DM - dry matter; [7] GL - gas losses; [8] EL - effluent losses; [9] DMR - dry matter recovery; [10] LAB - lactic acid bacteria; [11] Y&M - yeasts and moulds.

[1] BA - ácido butírico; [2] LA - ácido láctico; [3] AA - ácido acético; [4] PA - ácido propiónico; [5] ET - etanol; [6] DM - materia seca; [7] GL - pérdidas de gas; [8] EL - pérdidas de efluentes; [9] DMR - recuperación de materia seca; [10] LAB - bacterias del ácido láctico; [11] Y& M - levaduras y mohos.

Principal components analysis allowed the formation of two groups (I and II). The most discriminating variables in group I were as follows: dry matter (-0.9), pH (-1.2), and lactic acid bacteria (-0.9), while in group II, they were effluent losses (1.0), ethanol (1.0), acetic acid (0.8) and gas losses (0.8).

Group I, including silages with 1.00, 1.50 and 2.00% urea, determined by the variables pH, dry matter and lactic acid bacterial populations, can be characterised as silages with the main fermentative product (lactic acid) even at higher pH values. Additionally, silage with urea showed a negative correlation with variables such as gas losses, effluent losses and ethanol production.

## Discussion

The addition of urea to corn silage increased the pH values by the ammonia released in the hydrolysis of urea, forming a weak base, ammonium hydroxide ($NH_4OH$) (17). According to McDonald *et al.* (1991), the pH of silage should range from 3.8 - 4.2 to benefit the fermentation process; at pH levels below 3.8, yeast development is facilitated. However, acetic acid and urea may inhibit these microorganisms.

Even with the addition from 1 to 2% urea, the pH of the experimental silages was within the range verified by Weinberg *et al.* (2011), in contrast to the findings of Pinto *et al.* (2012), who verified higher pH values (3.95) in corn silages amended with urea, despite the differences between the values reported in this study. The authors verified that the use of urea in corn silage did not negatively affect the pH value. These lower pH values demonstrate that hybrids with high sugar contents are, in fact, prone to excessive acidification, making the addition of buffering additives, such as urea, a necessity.

The microbial ecosystem of corn silages is generally dominated by homofermentative lactic acid bacteria, especially *Lactobacillus plantarum* (McDonald *et al.*, 1991; Pahlow *et al.*, 2002), suggesting that the buffering allowed proliferation of heterofermentative lactic acid bacteria in a larger or smaller scale by altering the fermentative profile of silages Storm *et al.* (2010).

The lactic acid concentrations of silages in the present study were similar to those recorded by Pinto *et al.* (2012), who observed concentrations of 44.30 g/kg DM of lactic acid in corn silages. The high concentrations of acetic acid in the present study may be associated with higher activities of heterofermentative lactic acid bacteria. However, it is also possible that a significant production of acetic acid occurred in the initial period of the fermentation process when there was still oxygen in the silo. In an experiment with silages, Li *et al.* (2011) identified species of acetic bacteria in corn silages, mainly in the initial phase of the fermentation process. These acid-tolerant microorganisms convert ethanol to acetic acid under aerobic conditions and are generally associated with aerobic silage deterioration (22).

Considering the acidic pH values of all silages studied, a reduced concentration of butyric acid was expected, since the microorganisms producing this acid were developed in media with a pH close to 6.0. Thus, the low concentration of butyric acid, especially at the highest urea inclusion level, indicates that the addition of urea did not block a desirable fermentation profile of corn silages (3).

According to McDonald *et al.* (1991) and Pahlow *et al.* (2002), the number of lactic bacteria required for a significant reduction in pH of silage is about 8.0 log cfu/g. However, in the present study with the addition of urea, we observed higher numbers. Urea benefited the development of lactic acid bacteria in the silages by maintaining greater pH levels when compared to the silages with urea, besides decreasing the ethanol concentration due to the reduction of yeasts.

The reduction of yeast populations in silages treated with urea may be due to the conversion of urea to ammonia in the ensiled mass, facilitating fermentation (15). Urea is an antifungal substance and impedes the metabolism of yeasts, which are auxotrophic microorganisms and strictly depend on the availability of a specific combination between carbon sources and nitrogen sources (26).

In some studies, the authors observed a decrease in yeast populations when urea was applied to forage silage (27). Urea is a poor source of nitrogen for yeasts, which prefer other nitrogen sources (26). Thus, the addition of urea to silage increases the ammonia

concentration in the ensiled mass, delaying the development of yeasts since it unbalances the specific relationship of substrates for yeast growth. Moreover, according to Hess *et al.* (2006), high ammonia concentrations are toxic to yeasts, forcing them to modify the nitrogen metabolism, incorporating ammonium in the formation and excretion of amino acids, such as glutamine or glutamate, in the presence of adequate potassium concentrations.

The contents of dry matter in corn silages are adequate to the fermentation process with low effluent production, thus benefiting nutrient availability to the animal (13). When evaluating the effects of additives on corn silage, Pinto *et al.* (2012) found values of 35.24% DM for urea-added silages. This difference might be due to the harvest period of corn; in their study, corn was in a more advanced physiological stage.

The increase in crude protein in corn silages treated with urea occurred because urea increases the nitrogen compounds of the silages, suggesting partial incorporation of the nitrogen applied (15). Thus, the beneficial effect of the addition of urea to corn silage, besides reducing gas and effluent losses and leading to dry matter recovery due to the improved fermentation profile in the silage, can also be an increase in feed protein value; the increase in urea nitrogen fractions may also benefit rumen fermentation and promote greater synthesis of microbial proteins (15). In the present study, the silages met the protein requirements of the animals, with silage protein contents above 7.0%.

The addition of urea to corn silages resulted in a reduction of the concentrations of NDFap, most likely because urea acted on the fibrous fraction of the corn silage via ureolysis reactions (15), consisting of an enzymatic reaction in which ammonia is released through urea hydrolysis and of ammonolysis between the ammonia and the ester bonds among hemicellulose chains and between the carbohydrate groups or carbohydrate and lignin molecules, resulting in amide formation. Moreover, ammonia shows high affinity with water, thus forming a weak base, ammonium hydroxide ($NH_4OH$). The high affinity of ammonia with water promotes cell wall expansion and rupture of the fibrous tissue components of the forage ensiled with urea (16).

The degradation of lactic acid in aerobic silages, resulting from the action of acid-tolerant aerobic microorganisms (4, 11), increases the pH of the silages. In the present study, the pH value obtained with a urea inclusion level of 1.5% was ideal (13).

The smaller yeast population at 96 h in corn silages treated with urea may be associated with the ability of urea to increase pH values of the medium, reducing the potential for the development of yeast populations. On the other hand, the positive effect of urea during oxygen exposure may be associated with the lower yeast population at the time of opening, which may have been inhibited by the buffering and antifungal activity of urea during the silage fermentation phase (26). According to McDonald *et al.* (1991), Spoelstra *et al.* (1998), Storm *et al.* (2010) and Pahlow *et al.* (2002), yeasts are associated with silage deterioration and have a fermentative route (pyruvate decarboxylase acetaldehyde and subsequent reduction of acetaldehyde to ethanol), which causes high losses and can consume up to 48.9% of dry matter.

The addition of urea reduced the microbial activity and exothermic reactions in the silo after exposure to air (15). Generally, yeasts are the first microorganisms deleterious to silages exposed to air (Da Silva *et al.*, 2010), raising silage temperature and $CO_2$ production (11). However, as urea has a toxic effect on these microorganisms, it is possible that the higher resistance to heating and changes in the silages exposed to air resulted from the addition of urea to corn silage.

Principal components analysis allowed us to generate the two-dimensional biplot formed by the first two principal components. Experimental variables with positive correlations indicate an association of the variable with the samples located to the right on the horizontal axis (CP1), and those with negative correlations indicate an association with the samples located to the left on the horizontal axis (CP1). Thus, the total amount of information of the original variables retained by the two principal components was 76.3%, with 51.26% for the first principal component and 25.04% for the second principal component.

Determination of the correlation coefficients of the studied variables allowed us to verify that losses are generally associated with the production of ethanol and butyric acid and with yeast populations. Due to the higher amounts of lactic acid bacterial populations in the silages receiving more than 1.0% urea, the losses from the fermentation process were

reduced since the production of lactic acid does not generate quantitative losses because of the lack of $CO_2$ and other undesirable by-products (13).

Group II, consisting of silage without urea or with 0.5% urea, showed discriminations related to losses and the production of ethanol and acetic acid, indicating that the main fermentation of these silages was probably not only lactic fermentation, generating losses characteristic of the performance of other species of microorganisms, such as yeasts and/or acetic and propionic bacteria.

## Conclusions

The use of urea at levels close to 2% in corn silage reduces gas losses, improves chemical composition and promotes greater aerobic stability of silages.

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