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Characteristics and aerobic stability of sugarcane silages, treated with urea, NaOH and corn

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ABSTRACT: The objective of this study was to determine the effect of different additives on the aerobic stability of sugarcane, preserved as silage in laboratory silos. The dry matter (DM), ethereal extract (EE), mineral matter (MM), crude protein (CP), pH and aerobic stability of the silage treated with urea, NaOH and ground corn, were determined. A completely randomized design, with four repetitions, was used to evaluate the following treatments: sugarcane without additives, sugarcane + 4 % ground corn, sugarcane + 1 % urea, sugarcane + 1% urea + 4 % ground corn, sugarcane + 1 % NaOH, and sugarcane + 1 % NaOH + 4 % ground corn. The silages treated with urea had a higher percentage of EE. In the presence of urea and NaOH, the addition of ground corn significantly increased the CP. The NaOH increased the MM percentage. The DM losses caused by gases were significantly higher in the silages that did not receive chemical additives. There were significant differences in the production of CO_2 at different times of exposure to air. It is concluded that the additives improved the chemical characteristics, except the DM content, and reduced the loss by gases in anaerobic fermentation.

Key words: additives, gases, silage

INTRODUCTION

Ensilage is a forage conservation method, based on spontaneous lactic fermentation, under anaerobic conditions. The epiphyte lactic bacteria ferment the carbohydrates of the forage to lactic acid and, to a lesser extent, to acetic acid. Due to the presence of those acids, the pH of the ensiled material decreases and the undesirable microorganisms are inhibited (Santos and Zanine, 2006).

Sugarcane is a forage that enhances animal productivity in many tropical regions, due to some of its characteristics: high capacity to produce dry matter (DM) and energy per surface unit in only one annual cutting, during the dry season; maintenance of its digestibility for a relatively long time; possibility to obtain a low cost per ton of DM; and low content of neutral detergent fiber (NDF), which constitutes a synonym of the high content of non-fibrous carbohydrates (NFC) (Miranda, 2006).

Nevertheless, sugarcane silages show alcoholic fermentation and lose up to 30 % of DM during storage, which causes a high reduction of the nutritional value of the forage (Pedroso *et al*., 2005; Freitas *et al*., 2006b). In this sense, several products have been evaluated as additives, which modify the main fermentative route and reduce the losses of nutritional value of the sugarcane silage, by inhibiting the yeast growth and/or blocking the fermentative production route of alcohols, which improves the standard fermentation (Santos *et al*., 2009; Pedroso *et al*., 2011).

Urea, sodium benzoate, and sodium hydroxide are among the studied additives, but the results of their application have been variable (Siqueira *et al*., 2007; Mari, 2008; Pedroso *et al*., 2008). For such reason, different application doses have been tested, as well as the combination of additives, searching for higher conservation efficiency (Siqueira *et al*., 2010).

The studies with sugarcane treated with urea proved that its use requires an additional source of true protein. According to Pinto *et al*. (2003), cotton bran and rice are useful to complement this type of diet, because the increase in the yield of the animals occurs due to the concentrate source of energy (starch) and/or protein, which escape the ruminal microbial digestion and which are absorbed as glucose and aminoacids in the small intestine. Likewise, the corn ear or the ground corn is an energy source recommended to be added to the sugarcane forage in feeding, when urea is used as crude protein (Andrade *et al*., 2001).

The objective of this research was to determine the effect of different additives in the aerobic stability of sugarcane, preserved as silage in laboratory silos.

MATERIALS AND METHODS

The trials were conducted at the Center of Agricultural Sciences of the Federal University of Alagoas –Brazil–, in the laboratory of agricultural microbiology of Río Largo AL, located at 9º 29' 45" South latitude and 35º 49º 54" West longitude, at 165 m of altitude and an average annual rainfall of 1842,5 mm.

For producing the silage the industrial sugarcane variety RB4764 was used, whose stems were ground in a Nogueira DPM 12 chopping machine. In the case of ground corn the Nogueira Junior chopping machine was used. Urea, NaOH and ground corn were applied as additives, and the treatments were the following: sugarcane (T_1, control), sugarcane + 4 % of ground corn (T_2), sugarcane +1,0 % of urea (T_3), sugarcane + 1,0 % of NaOH (T_4), sugarcane +1,0 % urea + 4 % ground corn (T_5), sugarcane + 1,0 % NaOH + 4 % ground corn (T_6).

The forage, cut and chopped, was deposited on a plastic canvas. Afterwards, the urea and NaOH, in the form of aqueous solution, as well as the ground corn, were uniformly applied, according to the established treatments. In addition, a sample was preserved to determine the dry matter of the forage.

Plastic buckets of 20-L capacity, with a height of 35 cm and a diameter of 30 cm, containing a Bunsen valve for the escape of gases and effluents, were used as minisilos. The sugarcane was manually compressed to achieve the highest possible density. After being filled, the silos were closed, sealed with plastic adhesive tape and were stored at ambient temperature, during 65 days. For the evaluation of total losses, the silos were weighed when empty and full, as well as at the end of the conservation period.

After 65 days, the silos were opened and the content of the top and lower portion of the silage was discarded. The material of the central part was homogenized on a plastic tray and two samples were taken. The first one (250 g) was introduced in paper bags and put in a forced-air oven at 65 ºC, during 72 h, for determining the DM; afterwards, it was ground and placed in a glass recipient with cap –identified and stored– to determine the crude protein (CP), ethereal extract (EE) and mineral matter (MM), according to the methodology described by Silva and Queiroz (2002). The second sample (25 g) was used to analyze the pH, for which 100 mL of water were added. Then, it was left to rest during 2 h and the pH was determined with a potentiometer.

The dry matter loss caused by gases was quantified by means of the weight difference, through the following equation (Schmidt, 2006):

$$PG = \frac{Pse - Psa}{Me} \times 1\,000$$

where:

PG: loss by gases (% DM)
Pse: weight after opening the silo
Psa: weight before opening the silo
Me: ensiled material (kg of DM)
Me: *MFi* x *MSi*
MFi: forage mass at closure (kg)
MSi: DM content of the forage at closure

The following equation was used to estimate the recovery of DM:

$$RMS = (MFP\ MSF\ x) / (x\ MSi\ MFi) \times 100$$

RMS: rate of DM recovery (100)
MFi: forage mass at closure (kg)
MSi: DM content of the forage at closure (100)
MFP: forage mass at opening (kg)
MSF: DM content of the forage at opening (100)

For the evaluation of the aerobic stability the methodology proposed by Ashbell *et al*. (1991), called PET system, was used. Each unit of this system was prepared using two bottles of polyethylene (PET) of two liters, with perforated bottom and cap, which allowed air circulation. For such purpose, one of the bottles was cut in half and it was refilled with 450 g of silage; then, the top part was adjusted and sealed with a tape. The other bottle was cut in half and 100 mL of KOH at 20 % were put in it, which served as support for the bottle that contained the silage sample. After setting this system, the materials were exposed to air during 4, 8 and 12 days. Then, it was opened and 10 mL of KOH solution at 20 % were withdrawn for the titration with HCl 2N. Subsequently, the pH of the solution was lowered to 9, to release the CO_2. Likewise, a second evaluation was made with HCl 1N and the pH was decreased from 9 to 3,6; to reduce the pH in the interval from 8,1 to 3,6 the quantity (mL) of acid used was observed. During this process, the electrode of the potentiometer was kept in contact with the solution of KOH at 20 %, according to the technique recommended by Ashbell *et al*. (1991).

The quantity of CO_2 (g kg^{-1} DM) was calculated through the following formula:

$$CO_2 = \frac{0{,}044 \times T \times V}{(A \times S \times DM)}$$

T: volume of HCl 1N consumed in the titration (mL)

V: total volume of KOH at 20 % (100 mL)

A: volume of 20 % KOH used in the determination (10 mL)

S: quantity (kg) of fresh silage put in the bottles

DM: dry matter of the silage

When multiplying the quantity of CO_2 by the factor 0,68 –proportion of the nutrients released in the form of CO_2–, the dry matter loss could be estimated (Guim *et al*. (2002).

The aerobic stability (expressed in hours) was evaluated by controlling the temperature of the silage exposed to air, according to the method adapted from Kung Jr. *et al*. (2000). The silage of each treatment was maintained on a surface. The temperature was measured every 12 h with a thermometer that was placed in the geometric center of the forage mass of the silos. When the temperature exceeded in 2ºC the initial temperature, it was considered the beginning of the silage deterioration.

The experimental design was completely randomized, with four repetitions. For determining the CO_2 production a factorial design was used. The data were subject to a variance analysis (F = $p < 0{,}05$) and the means were compared by Scott Knott ($p < 0{,}05$).

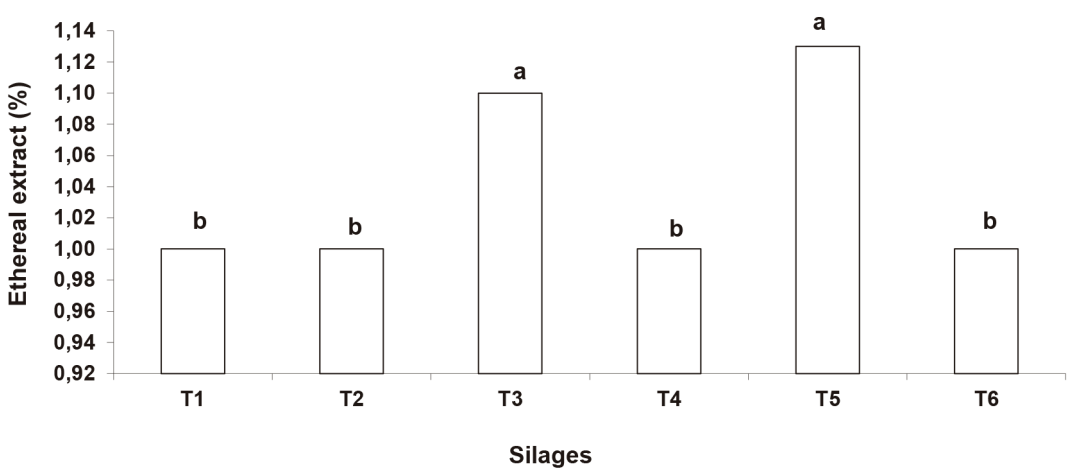
RESULTS AND DISCUSSION

The variance analyses for the CP, EE, MM and pH showed statistical significance ($p < 0{,}01$) in the evaluated characteristics, except for the DM, whose content varied from 25,60 to 28,96 % and it was within the interval (25-35 %) proposed by Sousa *et al*. (2008) as optimal for obtaining good-quality silage.

Schmidt (2010) reported that the EE in the sugarcane silage is non-significant. In that sense, in this study a significantly higher ($p < 0{,}05$) EE concentration was obtained in the treatments that contained urea (fig. 1). These results coincide with those reported by Pinto *et al*. (2003), in silages of two sugarcane varieties (RB806043 and RB72454) in which additives with urea were used (1,47 and 1,17 %, respectively). Such values were below the limit (8 % of EE) recommended by McGuffey and Schingoethe (1980) to prevent feed ingestion from decreasing, which propitiates the reduction in the animal yield.

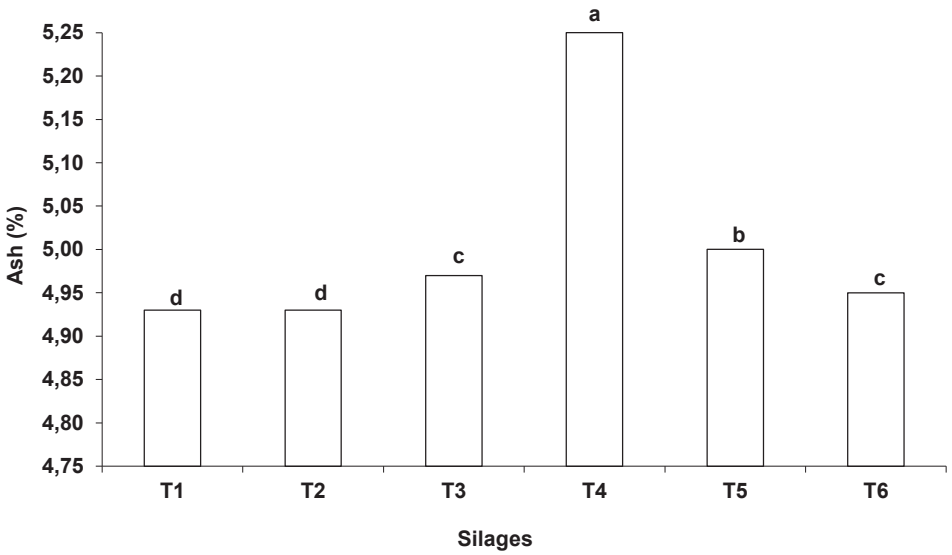
The ash quantity increased significantly ($p < 0{,}05$) with the addition of urea and NaOH (fig. 2). The highest values were shown in T_4; this result can be explained by the addition of mineral Na, through NaOH. Independently from the presence of additives, in all the treatments the ash percentage was higher than the one recommended by Schmidt (2010) for the sugarcane silage (> 3 %).

The increase of the mineral fraction in the silages treated with chemical additives is reported in literature by several authors. Simkins *et al*. (cited by Santos *et al*., 2008) found an ash content of 6,9 % in a corn silage treated with calcium carbonate (0,5 % of the green matter) as compared with 5,2 % in the control silage.



Means with different letters statistically differ by the Scott-Knott test ($p < 0{,}05$).

Figure 1. Ethereal extract of the sugarcane silage treated with urea, NaOH and ground corn.



Legend: means with different letters statistically differ by the Scott-Knott test ($p < 0,05$).

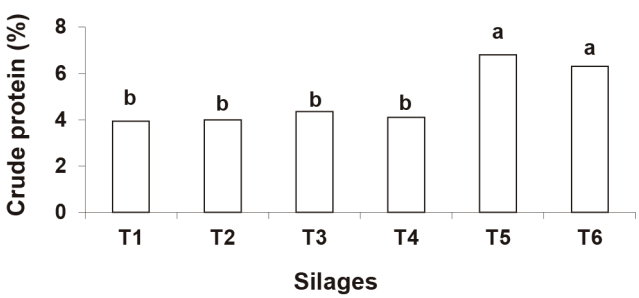
Figure 2. Ash of the sugarcane silage treated with urea, NaOH and ground corn.

Alcántara *et al*. (1989), when using 3 % of sodium hydroxide in the sugarcane silage, obtained significant increases in the ash content (7,03 vs. 4,60 % DM for the silage without additive).

Sugarcane shows, among its limitations, low crude protein content. However, when it is preserved as silage, the addition of urea can reduce this deficiency. In this study, the presence of ground corn and the addition of urea and NaOH increased the CP content of the silage (fig. 3).

Andrade and Lavezo (1998) stated that it is possible to achieve an increase of crude protein with the addition of bran and corn meal. According to Santos and Zanine (2006), this meal can increase the respiration and fermentation processes, which causes the forage to have a higher content of cell components and an increase of protein in the silage DM.

The pH value indicates the acidity of silage as a result of the action of acid-lactic bacteria. Its decrease inhibits the development of undesirable microorganisms, with which the quality of fermentation is ensured. In inadequate acidification, the bacteria that produce acetic and butyric acid are developed, and under those conditions, the proteolytic activity is stimulated, for which low- to moderate-quality silage is produced.



Means with different letters statistically differ by the Scott-Knott test ($p < 0,05$).

Figure 3. Crude protein of the sugarcane silage treated with urea, sodium hydroxide and ground corn.

Regarding this indicator (fig. 4) there was significant difference ($p < 0,05$) among the treatments, except between T_1 and T_3. The highest values were observed in T_4 and T_6.

According to the reports by Siqueira *et al*. (2010), the NaOH has a high capacity to increase pH, which, combined with the increase of the buffering capacity, provides the highest pH values after opening. Castrillón *et al*. (1978) treated sugarcane with NaOH at 4 % and observed that, in spite of obtaining a high production of lactic acid (12,2 %), the treated silages had a pH of 4,41 and the untreated silages, 4,12. On the other hand, Siqueira *et al*. (2007) obtained pH values between 4,3 and 5,8 in sugarcane silages treated with NaOH, which are similar to the ones reached in this work; although they differ from the results reported by other authors (Andrade *et al*., 2001; Freitas *et al*., 2006a, 2006b), who found pH values between 3,12 and 3,96 in treated –or untreated– silages.

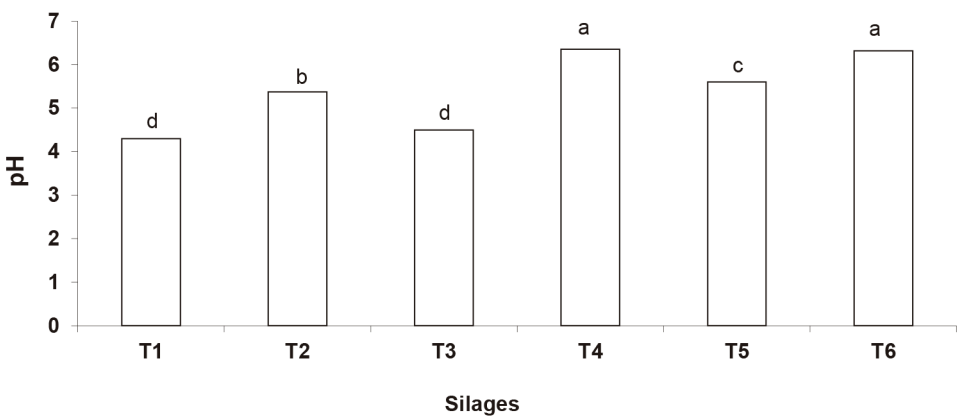
The DM losses by gases (fig. 5) were significantly higher ($p < 0,05$) in the silages that did not receive chemical additives. They were associated to the type of fermentation which occurs in the silage, because when alcohol is used fermentation is promoted due to the heterofermentative bacteria, yeasts and enterobacteria, and in this process the rate of heat produced by the microbial activity and the heat losses by conduction, radiation, convection and evaporation are directly related to the oxidation of DM, which causes losses in the form of carbon dioxide (Hill and Leaver, 2002). This does not occur when fermentation is produced through the homofermentative –which utilize glucose as substratum to produce lactate–, process in which the loss of DM is much lower.

The moment of opening the silo is considered a critical stage of the ensiling process. In this phase, the ensiled material enters in contact with oxygen again, and sugar oxidation and degradation of the lactic acid produced during fermentation begin, which causes its deterioration and reduction of its nutritional value. The resistance of the ensiled forage mass to deterioration, after opening the silo and being exposed to air, is defined as aerobic stability (Jobim *et al*., 2007).

There were significant differences ($p < 0,05$) in the quantity of hours needed to increase the temperature of the silages in 2ºC over the initial temperature (fig. 6). The best results were obtained in treatments T_2 and T_5 (44 and 48 h, respectively), while in the silage without additive this was reached at 28 h.

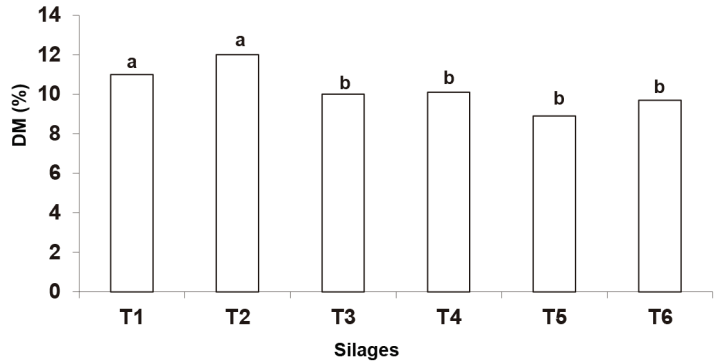
These results do not coincide with those reported by Britt and Huber (cited by Sousa *et al*., 2008), who used corn silage, treated with 0,5 and 1,0 % of urea. In this case 11 and 12 days were needed, respectively, for the appearance of visible signs of fungal growth in the silage exposed to air.

The determination of the CO_2 production of the silage exposed to air can be useful in the characterization of the deterioration rate (Ashbell



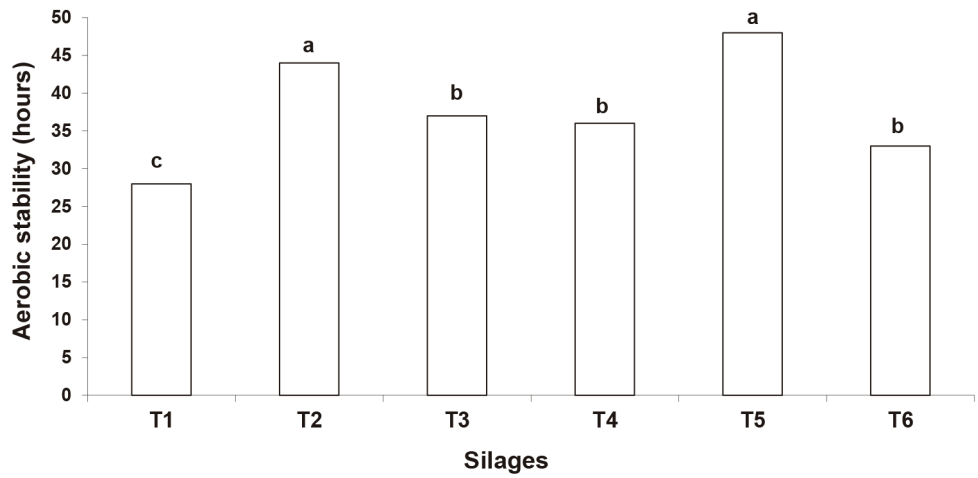
Means with different letter statistically differ through the Scott-Knott test ($p < 0,05$).

Figure 4. pH of the sugarcane silage treated with urea, sodium hydroxide and ground corn.



Means with different letter statistically differ by the Scott-Knott test ($p < 0,05$).

Figure 5. DM losses by gases in the sugarcane silage treated with urea, NaOH and ground corn.



Means with different letter statistically differ by the Scott-Knott test ($p < 0,05$).

Figure 6. Temperature variation associated to the aerobic stability of the sugarcane silage treated with urea, NaOH and ground corn.

et al., 1991; Moura *et al*., 2001), which implies the follow-up of the complete production of this gas in the process, because when multiplying the quantity of CO_2 by the factor 0,68 (relation of nutrients released as CO_2) it is possible to estimate the DM losses (Honing and Woolford, 1979).

Regarding the CO_2 production, there was a significant effect on the silage and the time of exposure, as well as on the interaction of both factors (table 1).

The interaction showed that the effect of time was significant ($p < 0,05$) only in T_3, with the highest values 8 days after exposure to air (table 2),

Table 1. Mean squares and variation coefficients obtained from the variance analysis of the CO_2 production.

Variation source	GL	Mean square
Silage (E)	5	85,228**
Time (T)	2	14,280**
E x T	10	11,738**
Repetition	3	11,738
Residue	50	0,858
VC %	15,16	

Legend: * significant for the F test ($p < 0,05$); ** significant for the F test ($p < 0,01$)

which did not occur in the other treatments. This silage was the least stable and produced a higher quantity of CO_2. It should be emphasized that in the other treatments, in spite of existing significant differences among them in the first 8 days, the variation that could be observed was minimal and the CO_2 production was similar at 12 days.

The CO_2 produced in the treatments was low, if it is compared with the reports in literature for low-quality silages. Ashbell *et al*. (1991), when evaluating the aerobic stability of grass silages exposed to air during 4, 8 and 10 days, obtained 31,2; 45,8 and 139,2 g of CO_2 kg^{-1} DM, respectively. On the other hand, Guim *et al*. (2002), when studying the aerobic stability of a wilted elephant grass silage, without inoculation, observed a gradual increase of the CO_2 production, with values of 0,72; 5,97; 12,54 and 20,63 g of CO_2 kg^{-1} DM, at 2, 4, 6 and 8 days, respectively. Likewise, Marques *et al*. (2002) found high values (46,54 and 43,65 g of CO_2 kg^{-1} DM, after 6 days of exposure) in a wilted sunflower silage, treated with inoculant.

Table 2. Mean values of CO_2 production, associated to the aerobic stability of the sugarcane silage.

	CO_2 production (g kg^{-1} DM)		
Silage	4 days	8 days	12 days
T_1	$4,00^{Ca}$	$3,67^{Ca}$	$4,00^{Ba}$
T_2	$5,69^{Ba}$	$6,60^{Ba}$	$6,00^{Ba}$
T_3	$7,51^{Ac}$	$15,59^{Aa}$	$10,45^{Ab}$
T_4	$5,00^{Ba}$	$5,87^{Ba}$	$5,00^{Ba}$
T_5	$4,06^{Ca}$	$3,63^{Ca}$	$4,63^{Ba}$
T_6	$5,75^{Aa}$	$6,00^{Ba}$	$4,40^{Ba}$

Means with different letter statistically differ by the Scott-Knott test ($p < 0,05$).

Capital letters compare the means vertically, and the small-case letters, horizontally.

The results of this study allow to conclude that the additives used improved the chemical characteristics of the silages and reduced the DM losses caused by gases in the anaerobic fermentation, although the treatment with urea at 1 % increased the CO_2 production after opening the silo.

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