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# Fermentation Profile of Millet Silage With Inclusion of Dehydrated Corn Grain, Cob and Straw

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**Abstract**

This study aimed to evaluating the fermentation characteristics of millet silage, cultivar ADR500, under the inclusion of different levels of dehydrated corn grain, cob and straw (CGCS): 0, 5, 10 and 15%, ensiled after 78 days of vegetative growth. The experimental design was completely randomized with 4 treatments and 4 replications, totaling 16 experimental units. Was evaluated pH, buffering capacity, lactic acid, acetic acid, propionic acid, butyric acid, gas and effluents losses, dry matter recovery, and soluble carbohydrates. Data were subjected to an analysis of variance and means were compared by 5% Tukey test. A regression analysis was performed for the inclusion levels. CGCS inclusion reduced ($P<0.05$) gas and effluent loss in all treatments, ranging from 6.10 to 3.48 for gases and 9.05 to 17.28 for effluents, and significantly contributed to the dry matter recovery process (DM). Buffer power values (BP), pH and ammoniacal-N were influenced ($P <0.05$) by the inclusion of different levels of CGCS. Levels of acetic, propionic, butyric, and lactic acid were influenced by treatments. Finally, soluble carbohydrate values is increased depending on the CGCS inclusion levels, proving be efficient to improve the silage fermentation profile quality.

**Keywords:** fatty acids, sewage, gas, pH and dry matter recovery

## 1. Introduction

Several technologies have been studied to minimize the seasonality effects on forage production in the tropics. Among these technologies, conservation of forage as silage is one of the most important in the current livestock production. The conservation process is complex since it involves several microorganisms from different species and genera in the conversion of soluble carbohydrates into organic acids present in the food during the fermentation process (McDonald, 1981; Herrero *et al.*,

1996; Woolford and Pahlow, 1998).

To consider a silage as good preserved, a set of variables must be evaluated together. These comprise pH, dry matter content, amount of soluble carbohydrates, ammoniacal nitrogen, and the concentration of organic acids. The variables enable us to evaluate whether the fermentation process was satisfactory and whether the ensiled matter kept its nutritional value (Bolsen *et al*., 1996; McDonald, 1982). Parameters such as loss by gases and effluents, and the index of dry matter recovery are also used to characterize the fermentative process (Vasconcelos *et al*., 2009).

Among forages, millet is commonly used for conservation as silage. The millet is an annual summer crop with satisfactory nutritive value and presents an elevated biomass production potential (Silva *et al*., 2020). However, it also presents high humidity levels, low water-soluble carbohydrate content, high buffer power, and lower lactic acid production. This makes the conservation process harder causing losses and the development of deteriorating microorganisms such as bacteria from the *Clostridium* genus (Khan *et al*., 2011; Brunette *et al*., 2014; Trevisoli *et al*., 2017).

Effluents loss constitute an important way of losing ensilaged forage nutritive value during the conservation process (McDonald, 1981). When forages have low dry matter content at the moment of silage confection, effluent losses are even greater, besides the other types of loss during the fermentation process when there is no material to sequester humidity.

Lactic bacteria use the soluble carbohydrates present in the ensiled material as substrate to produce lactic acid. The amount of carbohydrates present in millet, contributes to lactic acid production, which consequently pH decrease, thus helping the conservation process (Ávila *et al*., 2003).

Due to the high moisture content of the millet plant at the time of silage confection, the benefits of inclusion of grounded corn grain, cob, and straw (CGCS) in the silage of the millet cultivar ADR 500 were presented in this study.

## 2. Material and Methods

### *2.1 Experiment Location*

The experiment was conducted in the Departamento de Zootecnia of the Escola de Veterinária e Zootecnia (EVZ) at the Universidade Federal de Goiás (UFG) in the latitude S 16º 36’ 47.7”, longitude of W 49º 15’ 29.4”, and at an altitude of 708m. Following Koppen (1948) classification, the climate in this region is Aw, hot and semi-humid with well-defined seasons, in which the dry season occurs between May and October and the wet season ranges from November to April. The millet cultivation area has a flat topography and dystrophic red latosol typical of a clay texture with average fertility (Santos *et al*., 2006).

Meteorological data relative to the experimental period were obtained in the first class evaporimetric station of the Biosystems Engineering sector of the Escola de Agronomia (EA) of UFG, located around one kilometer from the experimental area. Data presented the following variations: temperature (ºC): 13.3 to 30.9; relative air humidity (%): 60.18 to 78.00; total precipitation (mm): 0.2 to 163.8, and insolation (h): 34.7 to 242.4.

### *2.2 Soil Preparation and Seeding*

To characterize the soil in the experimental area, samples were taken from zero to 0.2m of depth. Chemical and physical analysis were carried out yielding the following results: cmolc/$dm^3$ – Ca = 3.4; Mg = 1.1; H+Al = 2.8; CTC = 7.5; mg/$dm^3$ – P(Mehl) = 3.8; K = 69.0; pH ($CaCl_2$) 5.9; V = 62.5%; M.O. = 1.8%; sand = 35.0%; clay = 46.0%, and silt = 19.0%. Based on soil analysis results, no liming was necessary according to Martha Júnior *et al*. (2007).

Soil was prepared conventionally with a harrow plow and levelling harrow before seeding. Seeding was performed manually in spaced grooves of 0.4m, opened with a marker coupled to a tractor with a density of 40 pure and viable seeds (PVS) by linear meter of the millet cultivar ADR500 in an area of $300m^2$.

For fertilization, 80kg.$ha^{-1}$ of $P_2O_5$ (SS) and 50kg.$ha^{-1}$ (FTE BR–16) were employed. Cover fertilization was performed 17 days after emergence when plants emitted an average of four leaves. For this fertilization, 40kg.$ha^{-1}$ of $K_2O$ (KCl) (Martha Júnior *et al*., 2007) were used. In the nitrogen fertilization, 90kg.$ha^{-1}$ of N sourced from urea were employed. During the cultivation period, manual removal was used to eliminate invasive plants from the experimental area.

*2.3 Cut and Ensilage*

Harvesting of material was performed manually at 5cm above soil level 78 days after emergence, when the dry matter (DM) content reached 25.68%. Material was chopped using a stationary grinding mill coupled to a tractor, obtaining 1-2cm particles using the whole plant.

Fresh matter (FM) was divided into four equal parts and inserted in different CGCS inclusion levels based on millet fresh matter weight, comprising the following treatments: T1; millet silage without CGCS, T2; millet silage with 5% CGCS, T3; millet silage with 10% CGCS, T4, millet silage with 15% CGCS.

For experimental silos (mini silos), PVC pipes with 100mm diameter, 0.4m length, and bottom-sealed with a PVC lid were used. On to bottom of each mini silo, 0.250kg of thick sand were added followed by two layers consisting of cotton fabric and a screen to collect and measure losses by effluent. Silos were weighed before ensilage with their apparatus, bottom and lid.

In each mini silo, 1.5kg of forage were ensiled. The material was compacted up to a density of 550kg/$m^3$ of green mass. Mini silos were sealed with specific lids adapted to Bunsen valves to enable gas leak and the evaluation of gas loss during ensilage.

*2.4 Mini Silo Opening*

Mini silos were opened 60 days after closure. Inferior and superior parts were discarded, only using the central portions of each silo for analysis. Afterwards, two 0.5kg samples from each treatment were retrieved, from which one was used to collect the liquid part of the silage *"in natura*" (called juice), using a hydraulic press applying 6ton pressure force. The other sample was used for dry matter analysis by methods described by Association of Official Analytical Chemists (1990).

For the analysis of volatile fatty acids (VFA), samples of juice were preserved in phosphoric acid at a proportion of 1:5, respectively and to ammoniacal nitrogen concentration quantification ($N\text{-}NH_3$),

samples were preserved in sulfuric acid at a concentration of 1:100.

Measuring of pH was performed from the juice extracted from the "*in natura*" silage using the methods described by the Association of Official Analytical Chemists (1990). Buffer power was measured using the methods described by Playne and McDonald (1966) with the value expressed in mEq of alkali needed to change the pH from 4 to 6 in 100g of sample (DM).

The following organic and fatty acids were analyzed: lactic (LA), acetic (AcA), propionic (PA), and butyric (BA), using a High Performance Liquid Chromatographer (HPLC), SHIMADZU®, model SPD-10A VP coupled to an Ultra Violet (UV) Detector using a 210nm wave length.

Values of the loss by gases and effluents and the dry matter recovery index were calculated following (Jobim *et al*., 2009) being presented as:

Loss by gases:

$$Gas = \frac{Wcl - Wop}{FMcl - DMcl} x\ 1000$$

Where Gas are the losses by gas (% DM), Wcl is the weight of the bucked filled upon closure in kg, Wop is the weight of the bucket upon opening in kg, FMcl is the foraging mass upon silo sealing in kg, and DMcl is the forage dry matter content upon sealing in % DM.

Loss by effluents:

$$Efl = \frac{Wop - Wcl}{FMcl} \text{x } 1000$$

Where Efl is the effluent production in $kg.t^{-1}$ of green mass, Wop is the weight of the empty set (bucket, lid, sand, fabric) upon opening in kg, Wcl is the weight of the empty set (bucked, lid, sand, fabric) upon sealing in kg, and FMcl is the forage mass upon sealing in kg.

Indices of dry matter recovery:

$$DMR = \frac{FMop\ x\ DMop}{FMcl\ x\ DMcl} \text{x } 100$$

Where DMR is the dry matter recovery index in %, FMop is the forage mass upon opening in kg, DMop in the forage dry matter content upon opening in %, FMcl is the forage mass upon sealing in kg, and DMcl is the forage dry matter content upon sealing in %. Soluble carbohydrate contents were determined according to Campo et al. (2013).

The experimental design used was completely random, with each mini silo being considered as an experimental unit with one millet cultivar (ADR500) and four levels of ground ear corn (CGCS) inclusion: (0, 5, 10, and 15%) of ensiled green mass, with 4 mini silos for each treatment.

Averages for gas losses, effluents losses, dry matter recovery, carbohydrates (CHO) contents, buffer

power, pH, and ammoniacal nitrogen variables were analyzed by Shapiro-Wilk test, followed by normal distribution with the averages being submitted to a regression analysis for different additive inclusion levels. For the lactic, acetic, propionic, and butyric acid variables, variance analysis were performed with averages and a Tukey *post-hoc* test with a significance level of 5% was carried out in the software R (R Core team, 2015).

## 3. Results and Discussion

According to McDonald (1981), ensilage losses are hard to measure, while loss by gases depend on the microorganisms involved and the fermentable substrates. Therefore, procedures such as better compaction, supply of soluble carbohydrates, and reduction in humidity levels would restrict microorganism activity, thus resulting in an increase in the fermentative coefficient, in which fermentations are quicker and there is less loss by gas.

Forage millet fresh matter presented an average amount of 26.7% of dry matter (DM) during ensilage, thus being at the ideal levels preconized by McDonald (1981), which is around 25% for the out-of-pattern forages. This has minimized losses caused by effluents (Jobim *et al*., 2009).

Regarding loss by gases, values differed ($P<0.05$) between the CGCS inclusion levels with a variation of 3.48 and 6.10%, thus presenting a linear decreasing trend with increasing levels of CGCS as shown in Figure 1.

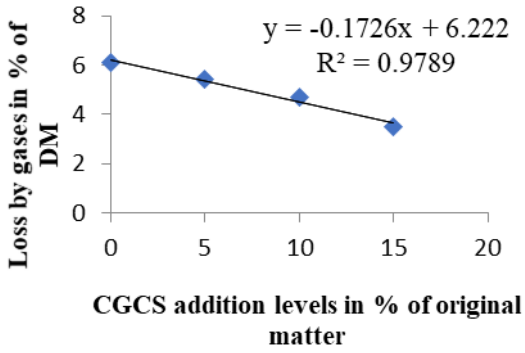


Figure 1. Losses by gas in millet silage with CGCS levels

Equally unwanted, loss by gases is associated with the type of fermentation that occurs during the process. When homofermentative bacteria perform fermentation, glucose is used as a substrate and will produce lactic acid, thus providing smaller losses. However, when fermentation is performed by heterofermentative bacteria, carbonic gas (CO2), ethanol and mannitol are produced, thus resulting in a significant loss by gas (Bolsen *et al*., 1996; Charmley, 2001; McDonald, 1982).

The largest gas productions are associated with the presence of hetero and enterofermentative bacteria, in which the butyric fermentation caused by bacteria from the genus *Clostridium* is highlighted (McDonald, 1982).

The loss by effluents presented a variation between 9.05 to 17.28% of DM at the CGCS addition levels, presenting a linear behavior according to the treatments employed as seen in Figure 2.

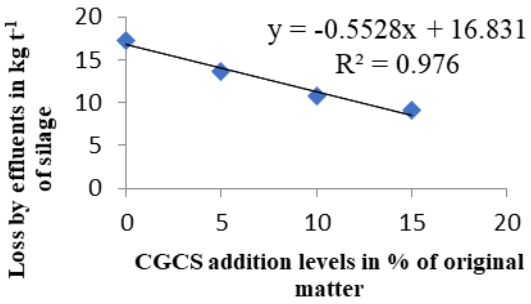


Figure 2. Loss by effluents in millet silage with CGCS levels.

Silage effluents are mostly composed of organic compounds such as proteins, sugars, organic acids, thus constituting losses in the nutritive value during silage storage. Loss by effluents is a major and undesired form, but in silages with 30% to 35% of DM this loss would be insignificant or null. Similarly, excess compaction promotes a greater loss by effluents, which is inversely proportional to dry matter contents.

Also, is valid to consider that the silage effluent carries nitrogen compounds, sugars, organic acids, and minerals (Loures *et al*., 2005; Amaral *et al*., 2007; Tavares *et al*., 2009). Therefore, including CGCS is a viable alternative as it increases the dry matter content of the ensilaged material, reducing highly digestible nutrient loss by effluents.

Reductions in losses by gases and effluents had led to a higher dry matter recovery. The dry matter recovery indices (DMRI) were influenced ($P<0.05$) by the CGCS inclusion levels, figure 3.

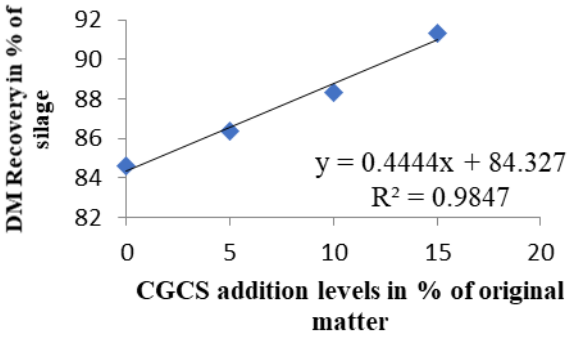


Figure 3. Dry matter recovery from millet silage with CGCS levels

Production and the consequent effluent outflow depend on several factor such as the plant dry matter content, the compaction pressure (Loures *et al*., 2005), and the shape and size of the silo (Tavares *et al*., 2009).

According to Silva and Queiroz (2006), BP evaluation together with pH provide a more precise estimate of the silage quality than the isolated pH analysis, since BP is more associated with acids, especially the lactic, while pH is influenced by the ions released by other acids.

Well-preserved silages have a pH ranging between 3.8 and 4.2. However, reports of pH levels above 5.0 have been common in tropical forage silages, especially when soluble carbohydrate limitations and a high buffer power occur (McDonald, 1981; Stella *et al*., 2016). The pH values found in the present study varied between 3.67 and 3.92 (table 2), which are thus similar to the preconized for corn and sorghum and to the 3.87 value found by Costa *et al*. (2012) for ensilage millet silages at 65 days of vegetative growth. When pH stabilizes between 3.8 and 4.2, it is adequate for the silage considered as well preserved (McDonald, 1981). Furthermore, it is considered that the pH alone cannot be a reliable criteria to evaluate silages due to its inhibitory effect over bacteria and plant enzymes is dependent upon the decline speed of the ionic concentration and the humidity degree of the environment.

Increase in soluble carbohydrates contents at end of the fermentation process can be caused by enzyme activity or by acid hydrolysis of hemicellulose, releasing soluble carbohydrates for fermentation. During the stable fermentation phase, the hemicellulose chemical lysis can still occur, releasing sugars for fermentation (Bolsen *et al*., 1996). During the initial ensilage phase, according to (Winters *et al*., 1987; Charmley, 2001), a limited amount of nutrient becomes available for fermentation. Therefore, some of these nutrients may be converted into organic acids, leading to mesophyll cellular membrane ruptures in a way similar to formic acid, further releasing nutrients for fermentation. It is also possible that vegetable cell auto-catalytic enzymes contribute to the cellular structure rupture when in anaerobic activity, releasing nutrients for fermentation. According to the same author, it is likely that these mechanisms act on the rupture of the cellular structure due to complexity of fermentation process in the ensilage (McDonald, 1981; Oliveira *et al*., 2017).

Is important to note that CHO contents presented a linear increase as inclusion levels of ground ear corn increased, varying between 5.33% and 7.38% with the addition maximum of the maximum level of inclusion and are shown in figure 4.

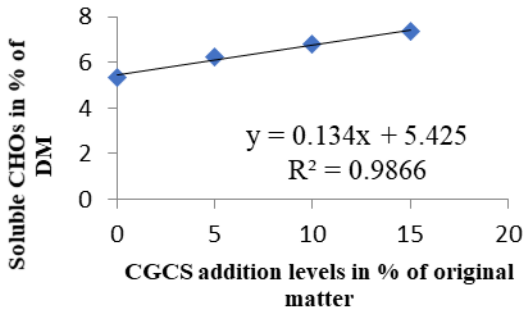


Figure 4. Soluble CHO in millet silage with CGCS levels

Therefore, CHO values determined in this research corroborate the statements by (Desta *et al*., 2016) that a level of 6% to 8% of soluble carbohydrates in the dry matter of the ensilaged material is

necessary for fermentation to take place adequately.

Buffer power refers to the capacity that the forage has to resist the pH decrease, which may prolong the fermentation process and give opportunity for bacteria such as enterobacteria and Clostridia to develop and promote undesirable fermentations, mainly with acetic and butyric acids (MCDonald, 1982; Eikmeyer *et al*., 2013; Oliveira *et al*., 2017). Significant differences ($p<0.05$) were observed for the average buffer power values between treatments determined for the millet cultivar analyzed in this research, with variations ranging between 17.60 to 22.20 meq NaOH/100g DM, figure 5.

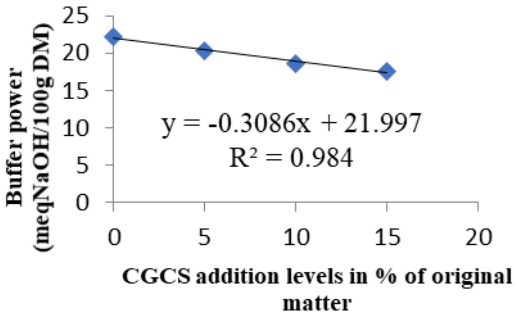


Figure 5. Buffer power in the silage depending on CGCS inclusion levels

Buffer power is directly proportional to the amount of organic acids present in the forage, mainly acids such as the malic, citric, and aspartic acids, with the oxalic acid sometimes also being present. These acids have a buffering effect, inhibiting the decrease in the ensilaged mass pH. Considering the pH values determined in this research, ranging from 3.67 to 3.92, it is possible to state that the CGCS inclusion, besides increasing ensilaged matter DM contents, also had an effective contribution in elevation of soluble carbohydrate contents, main substrate for lactic acid production, the direct causer of pH decrease (McDonald, 1981; Playne and McDonald, 1966).

Silage pH values were influenced depending on CGCS inclusion, varying between 3.67 and 3.92. pH is one of the main factors capable of influencing microorganism growth and survival, besides being used as a qualification parameter for ensilage (McDonald, 1981; Mohd-Setapar *et al*., 2012).

Studies carried out by (Jobim *et al*., 2009) demonstrated that pH is used as an important criteria to determine quality of silage fermentation, but using the concentration of dissociated organic acids is more adequate to this end, as well as titratable acidity (Silva and Queiroz, 2006).

The additive use resulted in a pH increase ($P<0.05$), but these values are within the range considered acceptable, which is between 3.8 and 4.2 and indicative of a quality silage. According to McDonald (1982), well preserved silages have a pH between 3.8 and 4.2. However, reports of pH values above 5.0 have been common for tropical forage silages, especially when soluble carbohydrate limitations exist (Jobim *et al*., 2009; Stella *et al*., 2016).

In this research, pH values between 3.67 and 3.92 were verified (Figure 6), which indicates fermentation of the ensilaged material.

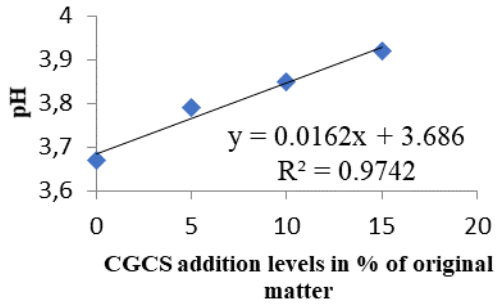


Figure 6. Millet silage pH depending on CGCS inclusion levels

Amount of N-$NH_3$/NT were positively elevated according to the CGCS inclusion levels, varying from 3.31 in the control treatment up to 3.72, thus presenting a linear quadratic behavior depending on the levels as shown in figure 7.

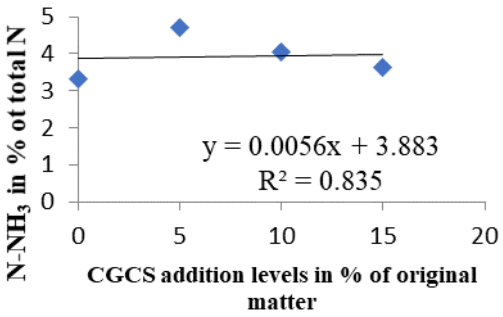


Figure 7. Ammoniacal nitrogen in the millet silage depending on CGCS inclusion levels

The N-$NH_3$/NT content is also a good indicative of silage quality, helping the fermentative process. Amounts of ammoniacal nitrogen below 10% indicate that the silage has a good quality for that parameter, since the fermentation process did not result in excessive protein breakdown into ammonia (Monteiro *et al*., 2011).

The increase in N-$NH_3$ amounts implies on the reduction of true protein. Nevertheless, these values are below the 8% threshold stated by Henderson (1993), which is the maximum acceptable value to consider a silage as being of good quality.

As observed in Table 1, CGCS inclusion in the forage millet silage promoted significant interactions ($P<0.05$) in the concentrations of lactic, acetic, butyric, and propionic acids in the silages evaluated.

Table 1. Average contents of lactic acid (La), acetic acid (Aa), butyric acid (Ba), and propionic acid (Pa), determined in the forage millet silages depending on CGCS inclusion

| Inclusion levels | Lactic acid (%) | Acetic acid (%) | Butyric acid (%) | Propionic acid (%) |
|---|---|---|---|---|
| 0 | 1.740C | 0.650B | 0.004A | 0.056A |
| 5 | 1.998A | 0.700A | 0.004AB | 0.050B |
| 10 | 2.100A | 0.620C | 0.004A | 0.048B |
| 15 | 1.963B | 0.670B | 0.003B | 0.050A |
| CV | 18.960 | 19.330 | 15.450 | 16.450 |

Averages followed by different letters in the same columns differ from each other by the Tukey test at 5% probability.

In regards to the lactic acid (La), contents presented a variation between 1.74% and 2.10%. The lactic acid exerts a great importance in fermentative process due to its greater dissociation constancy when compared to other acids. Indeed, it is the most responsible for silage pH reductions, thus presenting larger concentrations than other acids (Moisio and Heikonen, 1994).

Acetic acid amounts differed ($P<0.05$) depending on CGCS inclusion levels, varying between 0.62% and 0.70%. Acetic and butyric acid concentrations are related to smaller rates of pH decreases (Ranjit and Kung, 2000; Mohd-Setapar *et al*., 2012). This essentially related to the prolonged activity of entero and heterofermentative lactic bacteria, which are also produced by *Clostridium* in a smaller scale. Therefore, well-preserved silages must present very low amounts of acetic acid, which is in accordance with the results obtained in this research, since the average amounts found were well below the 2% threshold suggested by (Eikmeyer *et al*., 2013).

Butyric acid values were influenced ($P<0.05$) by CGCS inclusion levels in the evaluated silages, with a variation between 0.003 and 0.004. Butyric acid contents varying from 0.00 to 1.73% in grass silages were reported by Santos *et al*. (2014).

Maintenance of pH close to 4.2 is characteristic of good quality silages and, consequently, to the inhibition of proteolysis and butyric acid production (Jobim *et al*., 2009; Stella *et al*., 2016).

In a literature review study by (Zopollatto *et al*., 2009) aiming to evaluate the variation in fermentative patterns of four corn silages, four sorghum silages, six sugar cane silages, and 12 grass silages, butyric acid values on the order of 0.00, 0.1, and 0.1% were reported, respectively, which corroborates the results obtained in this research.

In regards to the propionic acid contents, interactions were observed ($P<0.05$) depending on the level of CGCS inclusion in silages, with a variation between 0.048 and 0.056%.

The presence of propionic acid above limits indicates the degradation of lactic acid by butyric bacteria (Woolford and Pahlow, 1998; Jobim *et al*., 2009), which was not observed in this study, since the highest value found for this acid was 0.056%.

## 5. Conclusion

Inclusion of grounded corn grain, cob, and straw in the forage millet silage, cultivar ADR500, is efficient to reduce losses by gas, effluents, silage buffer power, and in recovery of dry mass, improving the fermentation process and millet silage quality.

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