



## Short Communication

## Storage time and condensed tannin content of high-moisture sorghum grains: Effects on in vitro fermentation and mold populations

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## ABSTRACT

Silage of high moisture sorghum grains is a highly relevant source of energy in cattle production systems in South America. There is little information related to the chemical characteristics, the kinetics of fermentation and the toxic fungal populations of these feedstuffs. The aim of this study was to evaluate the effect of storage time and condensed tannins content of the grain on chemical composition, in vitro fermentation parameters, and toxicogenic fungal populations of moist sorghum grain stored in silo-bags. Samples of 2 varieties of sorghum grains (high-tannin [HT] and low-tannin [LT]), were obtained during the grain harvest before silage making and after 30, 90, and 180 d of storage ( $n = 16$ ). High-tannin grains had higher acid detergent fiber, tannins, gas production ( $P < 0.05$ ) and lower starch and rate of gas production ( $P < 0.01$ ). Interaction variety  $\times$  storage time were observed for all chemical parameters ( $P < 0.01$ ), except for neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash (aNDF) and pH. Starch and protein content increased in both varieties, tannins decreased in HT and LT, and organic matter (OM) increased in HT and declined in LT ( $P < 0.05$ ). The rate of gas production increased with the storage time for HT and LT ( $P < 0.01$ ). A linear reduction in the *Aspergillus* number of colonies in the HT varieties was observed ( $P < 0.01$ ), whereas a linear increase in *Penicillium* isolation was detected in the LT sorghums ( $P < 0.01$ ). The storage time was beneficial in terms of decreasing the condensed tannins, increasing fractional rate of gas production and minimizing fungal contamination, particularly on HT grains.

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## 1. Introduction

The nutritional value of sorghum grain is considered lower than that of other cereal grains owing to different factors inherent to the grain (Offner et al., 2003). The concentration of phenolic compounds (tannins) in some genotypes, the nature of endosperm proteins (kafirins), and the association of these proteins with the

starch, would be responsible for the lower nutritive value of sorghum grain (Huntington, 1997; Schofield et al., 2001; Offner et al., 2003). Nevertheless, sorghum has some advantages over other grains, such as resistance to climatic difficulties, insect attack, and fungal contamination (Waniska, 2000). This resistance to mold contamination is strongly associated with high concentrations of phenolic compounds (i.e. tannins, flavan-4-ols) and kernel hardness (Menkir et al., 1996; Waniska, 2000). To minimize negative consequences of these compounds on nutritive value, measured in vitro or in vivo, sorghum grains can be subjected to different processing methods. Rolling and grinding (Owens et al., 1997), soaking, germination and reconstitution of dry grains with water (Balogun et al., 2005). Moreover, anaerobic fermentation with a moisture content of at least 25% (Lopes et al., 2017) has been used, along with other techniques. In fact, a previous study from our group (Aguerre et al., 2015) showed that a combination of germination and ensiling sorghum grains reduced the tannin content and

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increased the ruminal degradability and total digestibility of the grains. Given these results, it is expected that the ensiling of early-harvested sorghum would improve the nutritive value of the grain.

Otherwise, during the silage process, grains can be contaminated with toxicogenic fungi, which is related to the level of humidity and temperature of the silages (González-Pereyra et al., 2011). Most of the toxicogenic fungi generally isolated from grain silages correspond to the *Fusarium*, *Aspergillus*, and *Penicillium* genera (Alonso et al., 2013; Cheli et al., 2013). These fungi produce secondary metabolites (mycotoxins) that cause mycotoxicosis in livestock, domestic animals and humans, and their consumption is a significant public health risk (Richard, 2007). The production of mycotoxins is favored by environmental factors (weather conditions, agricultural methods, etc.) and the susceptibility of some grain genotypes to fungal contamination during processing and storage periods (Jonathan and Esho, 2010). Given the relevance of this type of food in our production systems and the scarce information related to chemical characteristics, fermentation kinetics, and toxicogenic fungal populations in moist sorghum grain silages, we proposed to carry out a study to investigate all these aspects.

Therefore, the main goal of this work was to evaluate the effect of grain variety and storage time on chemical composition, in vitro fermentation parameters, and populations of total fungi and of toxicogenic genera in sorghum grain silages.

## 2. Materials and methods

This study was conducted following the guidelines recommended by the Bioethics Committee of Animal Experimentation of the Veterinary Faculty, University of the Republic, Montevideo, Uruguay.

### 2.1. Sample collection

Samples of high-moisture sorghum grain silages were collected from commercial dairy farms located in San José and Flores Departments (33°33'28.3" S 56°52'37.7" W and 34°31'07.5" S 56°32'21.1" W), in the south-central area of Uruguay. The silos were representative of the size and storage method mostly used in dairy farms in the region and selected according to the variety of sorghum grain used for preparing the silages. In this way, silage samples were obtained from 4 farms: 2 made with sorghum varieties high in tannins (HT, genotypes Morgan 108 and ACA 558) and 2 made with varieties with low tannin content (LT, genotypes Flash 10 and ACA 546). The sample from each genotype was considered replicate for the variety (HT or LT). For high-moisture grain silage, grains were harvested at approximately 30% moisture content, mechanically chopped, and enclosed within polystyrene bags of 250 µm thickness. The silo bags were 60 m in length, 1.7 m in height, and 2.5 m in diameter, and were each filled with about 180 t of grain. Grain samples were collected during the ensiling process (d 0), and the silos were sampled on d 30, 90, and 180 after storage. Sorghum grains subsamples were manually taken with a cutting machine at 6 equidistant points along silos at 50-cm horizontal depth in transects at 3 levels (upper, middle, and lower). Grains subsamples were mixed and homogenized to obtain 1 kg of sample from all points and stored at –20 °C until analysis.

### 2.2. Chemical analysis

The dry matter (DM), ashes, and crude protein (CP) contents of sorghum samples were determined according to AOAC official methods as described by procedures 934.01, 923.93, and 984.13, respectively (Association of Official Analytical Chemists (AOAC), 1997). Organic matter (OM) content was calculated as the

difference between DM and ashes. The neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash (aNDF) analysis was based on the procedures described by Mertens (2002), using heat-stable α-amylase and expressed inclusive of residual ash. Acid detergent fiber (ADF) levels were determined according to Van Soest et al. (1991) in an ANKOM 220 fiber analyzer (Ankom Technology Corp., Fairport, NY, USA). Total starch content was determined using a total starch assay kit (K-TSTA 06/17, Megazyme Int., Wicklow, Ireland). Condensed tannins (CT) were analyzed with the butanol–HCl method described by Makkar (2000). The pH was measured on-site immediately after obtaining the samples using a digital pH meter (EW-05991-36, Cole Parmer, Vernon Hills, IL, USA) and diluting 10 g of grain with 100 mL of distilled water. Chemical composition, in vitro fermentation parameters, and total and toxicogenic fungal population of sorghum grains before the ensiling process are summarized in Table 1.

### 2.3. In vitro gas production

To evaluate the ruminal fermentation of sorghum grains before and after the ensiling process, fermentation kinetics of the grains were assessed using a cumulative in vitro gas production technique, according to Mauricio et al. (1999). Samples were weighed (0.5 g DM) and placed in 100 mL bottles. Then, 18 mL of a buffer solution containing Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, KH<sub>2</sub>PO<sub>4</sub> and MgCl<sub>2</sub>·6H<sub>2</sub>O, 18 mL of a buffer solution composed of NaHCO<sub>3</sub> and NH<sub>4</sub>HCO<sub>3</sub> and 4 mL of a reducing solution containing NaOH and cysteine HCl, were added to each fermentation bottle under continuous CO<sub>2</sub> stream as described by Mould et al. (2005). Afterward, bottles were sealed with butyl rubber stoppers and stored at 4 °C for 12 h to hydrate substrates. Prior to inoculation, bottles were pre-warmed in a water bath at 39 °C for 2 h. Then, each bottle was inoculated with 10 mL of a mixture of single fluids taken from 2 ruminally cannulated Holstein cows, fed a diet composed by oats hay *ad libitum*, 3.3 kg of sorghum grain, 1.7 kg of soybean meal, 50 g of urea

**Table 1**

Chemical composition, in vitro fermentation parameters, and fungal populations of sorghum grains before ensiling.

| Item                                   | Genotypes <sup>1</sup> |       |       |       |
|----------------------------------------|------------------------|-------|-------|-------|
|                                        | 1                      | 2     | 3     | 4     |
| DM, g/kg                               | 763.7                  | 696.3 | 727.5 | 845.1 |
| Chemical composition, g/kg DM          |                        |       |       |       |
| OM                                     | 975.2                  | 978.9 | 980.7 | 981.6 |
| aNDF                                   | 278.6                  | 201.6 | 151.5 | 172.9 |
| ADF                                    | 86.0                   | 112.1 | 37.8  | 71.9  |
| Starch                                 | 587.2                  | 695.2 | 701.6 | 684.5 |
| CP                                     | 83.5                   | 73.3  | 76.0  | 99.8  |
| CT                                     | 12.3                   | 7.8   | 0.9   | 0.7   |
| pH                                     | 4.89                   | 5.95  | 4.53  | 6.53  |
| Fermentation parameters <sup>2</sup>   |                        |       |       |       |
| a, mL of gas/g OM                      | 331.2                  | 317.4 | 299.5 | 296.6 |
| kd, per h                              | 0.074                  | 0.058 | 0.052 | 0.057 |
| L, h                                   | 2.62                   | 2.93  | 2.91  | 3.01  |
| Fungal population (number of isolates) |                        |       |       |       |
| Total molds                            | 22                     | 90    | 10    | 171   |
| <i>Fusarium</i>                        | 0                      | 6     | 0     | 25    |
| <i>Aspergillus</i>                     | 15                     | 56    | 1     | 1     |
| <i>Penicillium</i>                     | 2                      | 23    | 3     | 4     |

DM = dry matter; OM = organic matter; aNDF = neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash; ADF = acid detergent fiber expressed inclusive of residual ash; CP = crude protein; CT = condensed tannins.

<sup>1</sup> Genotypes 1, 2, 3, and 4 are samples of the genotypes Morgan 108, ACA 558, Flash 10, and ACA 546, respectively.

<sup>2</sup> a, potential gas production; kd, fractional rate of gas production; L, gas production lag time.

and 30 g of a mineral premix. Immediately after inoculation, flasks were gassed again with a CO<sub>2</sub> stream; butyl stoppers were fastened with aluminum crimp seals and remained in the water bath throughout the measurement period. Three bottles per genotype and storage time were incubated (48 bottles containing substrate, plus 3 with no substrate included as inoculum blanks; in total: 51 bottles), and the whole procedure was conducted in 2 runs. All the bottles incubated per variety and storage time were considered analytical repetitions.

Gas production was measured in the bottles at 2, 4, 6, 8, 10, 12, 18, 24, 48, 72, and 96 h after inoculation using a transducer fixed to a pressure meter (840065, Sper Scientific, Scottsdale, AZ, USA) and registered in psi units. Gas volume in milliliters was predicted from psi values using an equation obtained in a previous trial conducted under the same experimental conditions. The data for cumulative gas production was fitted to the model:

$$V = a \times [1 - e^{-kd \times (t - L)}],$$

where  $V$  is the cumulative gas production at time  $t$  (mL/g DM incubated),  $a$  is the potential gas production (mL/g DM incubated),  $kd$  is the fractional rate of gas production (per h) and  $L$  is the gas production lag time (h).

#### 2.4. Mycological analysis

For fungi isolation, 100 grains of sorghum from each sample were randomly selected, and surface sterilized in a 0.4% solution of NaOCl for 1 min, rinsed 3 times with sterile distilled water, and dried with sterile absorbent paper. Seeds were subsequently placed in Petri dishes containing potato dextrose agar (Sigma–Aldrich, 70139). Plates were incubated at 25 °C and alternating cycles of light and dark for 7 to 10 d. At the end of this period, the resulting colonies were transferred to fresh media to allow identification, following conventional mycological methods as previously described (Nelson et al., 1983; Klich, 2002; Leslie and Summerell, 2006). Black light was used to induce sporulation in some cultures. Those that failed to sporulate after 1 month were considered as sterile mycelia.

#### 2.5. Statistical analysis

The variance homogeneity of the data was assessed using the PROC UNIVARIATE statement of SAS (version 9.0). Data of chemical composition of silages, in vitro fermentation kinetics, and total and toxicogenic fungal populations were analyzed using the MIXED procedure of SAS (version 9.0) by the model:

$$Y_{ijk} = \mu + G_i + T_j + (G \times T)_{ij} + \varepsilon_{ijk},$$

where  $Y_{ijk}$  is the variable to be tested,  $\mu$  is the mean,  $G_i$  the fixed effect of the grain variety ( $i$  = HT or LT, each one with 2 genotype replicates),  $T_j$  is the fixed effect of storage time ( $j$  = 0, 30, 90, and 180 d),  $(G \times T)_{ij}$  the interaction between genotype and storage time, and  $\varepsilon_{ijk}$  is the residual error. The farm (silo) was included as the subject for the repeated measurement, and the run was included as a random effect. Linear and quadratic effects for increasing storage time on high and low tannin-containing sorghum genotypes were also tested. The PROC GLM procedure of SAS (version 9.0) was used to compare chemical composition, in vitro fermentation, and toxicogenic fungal populations between fermented and non-fermented materials independently of storage time. Additionally, Pearson correlation and PROC CORR statement of SAS (version 9.0)

analysis were used to determine significant relationships among chemical composition and in vitro fermentation parameters with total and toxicogenic fungal populations. Significance was declared at  $P < 0.05$ , and tendencies at  $P < 0.10$ .

### 3. Results

The chemical composition of sorghum grains was affected differently by the storage time depending on whether the grains were high or low in tannins (grain variety  $\times$  time interaction;  $P < 0.01$ ; Table 2). For the HT variety, DM and OM content quadratically increased up to 90 d of ensiling and decreased towards 180 d ( $P < 0.01$ ), while for the LT variety, DM and OM decreased linearly ( $P < 0.01$ ) at an increasing rate towards 180 d of silage. Starch content increased linearly and quadratically in HT grains ( $P < 0.05$ ). Crude protein content increased linearly in both varieties of sorghum ( $P < 0.01$ ), while tannin content decreased linearly, representing a decrease of 70% in HT grains and 50% in LT grains.

Overall, fermented grains had lower CT concentrations than those non-fermented (3.00 vs. 5.43 g/kg, SEM = 0.527,  $P = 0.05$ ). A linear and quadratic response of pH to storage time was observed for both HT and LT sorghums, revealing a reduction of pH at a decreasing rate as storage time increased ( $P < 0.05$ ).

Regarding in vitro fermentation parameters, the potential gas production was affected by the sorghum variety ( $P = 0.03$ ), with HT sorghums producing more gas than the LT sorghums (Table 2). Both varieties and storage time altered the fractional rate of gas production ( $kd$ ) ( $P < 0.01$ ), and this parameter increased linearly with storage time for both varieties ( $P < 0.05$ ). There were no differences between fermented and non-fermented grains (0 d vs. 30, 90 and 180 d, not shown in Table 2) in the volume of gas produced, but fermented ones produced gas more rapidly ( $kd$  0.06 vs. 0.07 per h, SEM = 0.002,  $P = 0.04$ ) and with a shorter lag time (2.85 vs. 2.62 h, SEM = 0.044,  $P = 0.02$ ).

Fermented grains showed lower number of colonies of total molds (73.3 vs. 44.9, SEM = 5.849,  $P = 0.03$ ), *Aspergillus* (18.2 vs. 6.6, SEM = 2.189,  $P = 0.02$ ), and *Fusarium* (7.7 vs. 1.7, SEM = 0.927,  $P < 0.01$ ) for fermented and non-fermented grains, respectively (data not shown in Table 2). The total populations of fungi and particularly those from the 3 toxicogenic genera evaluated were affected by storage time ( $P < 0.01$ ), and there was a variety  $\times$  time interaction for *Aspergillus* and *Penicillium* number of colonies ( $P < 0.01$ ). A linear reduction in the *Aspergillus* number of colonies in the HT variety was observed ( $P < 0.01$ ) at a decreasing rate ( $P < 0.01$ ), whereas a linear increase in *Penicillium* isolation was detected in the LT sorghums ( $P < 0.01$ ). Quadratic effects were also observed for all the fungal populations in the HT genotypes ( $P < 0.05$ ) and in the total and *Fusarium* isolations in the LT genotypes ( $P < 0.05$ ; Table 2).

Total molds and *Fusarium* populations were positively correlated with the DM ( $P < 0.01$ ) and CP content of silages ( $P < 0.01$ ), while a negative correlation was found between *Aspergillus* number and CP content of silages ( $P < 0.05$ ; Table 3). The ADF content was positively correlated with *Aspergillus* number of colonies ( $P < 0.05$ ), but this correlation was negative for *Penicillium* number of colonies ( $P < 0.05$ ). Starch content was positively correlated with total molds ( $P < 0.05$ ) and *Penicillium* number of colonies ( $P < 0.01$ ). The CT content was negatively correlated with *Fusarium* and *Penicillium* number of colonies ( $P < 0.05$ ) and tended to be negatively correlated with total mold number of colonies ( $P = 0.08$ ), but this correlation was positive for *Aspergillus* number of colonies ( $P < 0.01$ ). Positive correlations were also found between silage pH and total

**Table 2**

Effect of storage time on chemical composition, in vitro fermentation parameters, and total and toxicogenic fungal populations of ground sorghum grain containing high and low tannin contents.

| Item                                 | HT    |       |       |       | LT    |       |       |       | SEM <sup>1</sup> | P-value <sup>2</sup> |       |       | HT P-value <sup>3</sup> |       | LT P-value <sup>4</sup> |       |
|--------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|------------------|----------------------|-------|-------|-------------------------|-------|-------------------------|-------|
|                                      | 0 d   | 30 d  | 90 d  | 180 d | 0 d   | 30 d  | 90 d  | 180 d |                  | G                    | T     | G × T | L                       | Q     | L                       | Q     |
| DM, g/kg                             | 730   | 773   | 808   | 757   | 786   | 772   | 785   | 736   | 47.2             | 0.97                 | <0.01 | <0.01 | 0.28                    | <0.01 | <0.01                   | 0.06  |
| Chemical composition, g/kg DM        |       |       |       |       |       |       |       |       |                  |                      |       |       |                         |       |                         |       |
| OM                                   | 977   | 983   | 983   | 982   | 981   | 981   | 981   | 975   | 1.9              | 0.50                 | <0.01 | <0.01 | 0.08                    | <0.01 | <0.01                   | 0.05  |
| aNDF                                 | 215   | 215   | 205   | 194   | 187   | 168   | 224   | 198   | 2.0              | 0.37                 | 0.69  | 0.37  | 0.50                    | 0.97  | 0.23                    | 0.21  |
| ADF                                  | 99.0  | 64.1  | 109.4 | 83.4  | 54.8  | 71.3  | 75.1  | 63.8  | 7.01             | <0.01                | <0.01 | <0.01 | 0.94                    | 0.34  | 0.70                    | 0.13  |
| Starch                               | 641   | 629   | 628   | 697   | 693   | 671   | 740   | 698   | 13.1             | <0.01                | <0.01 | <0.01 | <0.01                   | 0.02  | 0.32                    | 0.07  |
| CP                                   | 78.6  | 79.0  | 82.5  | 84.2  | 87.7  | 90.6  | 91.9  | 99.9  | 7.26             | 0.27                 | <0.01 | <0.01 | <0.01                   | 0.38  | <0.01                   | 0.36  |
| CT                                   | 10.05 | 7.35  | 5.75  | 3.00  | 0.80  | 0.80  | 0.70  | 0.40  | 0.540            | <0.01                | <0.01 | <0.01 | <0.01                   | 0.23  | <0.01                   | 0.26  |
| pH                                   | 5.42  | 4.43  | 4.50  | 4.54  | 5.53  | 4.44  | 4.04  | 4.48  | 0.380            | 0.85                 | <0.01 | 0.22  | 0.02                    | <0.01 | 0.03                    | <0.01 |
| In vitro parameters <sup>5</sup>     |       |       |       |       |       |       |       |       |                  |                      |       |       |                         |       |                         |       |
| a, mL/g DM                           | 333   | 325   | 334   | 329   | 310   | 313   | 319   | 294   | 13.6             | 0.03                 | 0.70  | 0.84  | 0.92                    | 0.88  | 0.42                    | 0.39  |
| kd, per h                            | 0.065 | 0.079 | 0.073 | 0.082 | 0.054 | 0.047 | 0.067 | 0.071 | 0.0005           | <0.01                | <0.01 | 0.07  | 0.01                    | 0.71  | 0.01                    | 0.74  |
| L, h                                 | 2.63  | 2.40  | 2.46  | 2.51  | 2.43  | 2.71  | 2.59  | 2.49  | 0.373            | 0.70                 | 0.10  | 0.66  | 0.61                    | 0.15  | 0.93                    | 0.61  |
| Fungal populations (Number isolated) |       |       |       |       |       |       |       |       |                  |                      |       |       |                         |       |                         |       |
| Total molds                          | 56.0  | 25.5  | 34.5  | 64.0  | 90.5  | 38.0  | 40.0  | 67.0  | 28.02            | 0.71                 | <0.01 | 0.42  | 0.15                    | <0.01 | 0.68                    | 0.01  |
| <i>Fusarium</i>                      | 3.0   | 0.0   | 0.0   | 1.5   | 12.5  | 3.0   | 0.5   | 5.0   | 3.82             | 0.41                 | <0.01 | 0.06  | 0.58                    | 0.01  | 0.16                    | <0.01 |
| <i>Aspergillus</i>                   | 35.5  | 14.0  | 3.0   | 4.5   | 1.0   | 17.0  | 1.0   | 0.0   | 6.43             | 0.239                | <0.01 | <0.01 | <0.01                   | <0.01 | 0.14                    | 0.44  |
| <i>Penicillium</i>                   | 12.5  | 4.0   | 6.0   | 14.5  | 3.5   | 14.0  | 15.0  | 28.5  | 4.39             | 0.220                | <0.01 | <0.01 | 0.17                    | <0.01 | <0.01                   | 0.94  |

HT = high-tannin; LT = low-tannin; DM = dry matter; OM = organic matter; aNDF = neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash; ADF = acid detergent fiber expressed inclusive of residual ash; CP = crude protein; CT = condensed tannins.

<sup>1</sup> SEM, standard error of means ( $n = 16$ ).

<sup>2</sup> Level of significance of the genotypes (G), the silage time (T) and genotypes by silage time interaction.

<sup>3</sup> Level of significance of the lineal (L) and quadratic (Q) effect of HT genotypes.

<sup>4</sup> Level of significance of the lineal and quadratic effect of LT genotypes.

<sup>5</sup> a, potential gas production; kd, fractional rate of gas production; L, gas production lag time.

**Table 3**

Correlations between chemical composition, fermentation parameters, and fungal population.

| Item   | SP       | Total molds | <i>Fusarium</i> | <i>Aspergillus</i> | <i>Penicillium</i> |
|--------|----------|-------------|-----------------|--------------------|--------------------|
| DM     | <i>r</i> | 0.375**     | 0.429**         | −0.151             | −0.031             |
| ADF    | <i>r</i> | −0.026      | −0.116          | 0.348*             | −0.309*            |
| Starch | <i>r</i> | 0.286*      | 0.200           | −0.218             | 0.682***           |
| CP     | <i>r</i> | 0.432**     | 0.469***        | −0.336*            | 0.209              |
| CT     | <i>r</i> | −0.259      | −0.287*         | 0.390**            | −0.347*            |
| pH     | <i>r</i> | 0.775***    | 0.792***        | 0.489***           | −0.119             |
| a      | <i>r</i> | −0.163      | −0.096          | 0.268              | −0.119             |
| kd     | <i>r</i> | −0.182      | −0.400*         | −0.213             | 0.037              |
| L      | <i>r</i> | 0.080       | 0.245           | −0.081             | −0.250             |

SP = statistical parameters; DM = dry matter; ADF = acid detergent fiber; CP = crude protein; CT = condensed tannins; a = potential gas production (mL of gas/g DM incubated); kd = fractional rate of gas production (per h); L = gas production lag time (h).

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

molds, *Fusarium*, and *Aspergillus* number of colonies ( $P < 0.01$ ). Regarding the fermentation parameters, a negative correlation between the rate of gas production and *Fusarium* number of colonies was observed ( $P < 0.05$ ).

#### 4. Discussion

While the effect of the variety was evident only for some chemical components, the length of the storage period affected all the chemical composition parameters in the silos, except for the aNDF content. It is notable that the effects of fermentation and storage time led to a decrease in CT concentrations, which was more evident in HT than in LT sorghums. The effect of silage fermentation in reducing sorghum tannin content has been previously reported in other studies (Patrício et al., 2006; Gollcher et al., 2010; Aguerre et al., 2015). This decrease in tannin content can be related to the acidic environment and the anaerobic microbial activity within the silo, inactivating the condensed tannins, which are

depolymerized to low molecular weight compounds in acidic media (Lopes et al., 2017). This is a beneficial effect since the inactivation of tannins improves the digestive utilization of sorghum grains (Mitaru et al., 1984; Aguerre et al., 2015). In fact, in the present study, fermented grains had a lower lag time and produced gas faster, in comparison with the grains at harvest.

The variety of sorghum affected the volume of gas production. According to results of other studies, the fermentability of sorghum grains of LT varieties is higher than that of HT varieties (Hibberd et al., 1982; Gameda and Hassen, 2015), as tannins decrease the fermentability of feeds. Surprisingly, in our study, the volume and rate of gas production were higher in the HT variety than in the LT, even before ensiling (d 0). This effect was not expected, considering that HT variety had a lower starch content and a higher fiber content than LT grains. Probably, characteristics of components not evaluated in this study, such as the amylose amylopectin ratio, could explain these differences.

The rate of fermentation was also influenced by storage time. A longer storage time favored the rate of gas production observed, which may be related to higher speeds of ruminal fermentation and microbial growth. These results suggest that the silage process could produce a higher availability of substrates than that observed in the unfermented grains. Similar reports were proposed in other studies where in vitro gas production of fermented sorghum grains was measured (Aguerre et al., 2015). Cummins (1971) reported that the increase in the in vitro DM digestibility of sorghum silages was greater in the high-tannin (HT) hybrids than in the low-tannin ones. In our study, digestibility of grains was not evaluated, but different responses on in vitro fermentation between LT and HT grains were not detected (no interaction). This result could indicate that the difference observed in the rate of fermentation derived from ensiling is not explained by tannins.

Total fungal and toxicogenic populations were affected by fermentation and storage time. *Penicillium* colonies increased with storage time, being more pronounced in LT grains. However,

*Aspergillus* colonies decrease with storage in both varieties. During storage, *Aspergillus* and *Penicillium* are the main contaminants and producers of mycotoxins. In addition, some *Aspergillus* species may be found before harvest (Scudamore and Livesey, 1998). As storage time increased, a decrease in *Fusarium* populations was observed. Considering the fermentation characteristics and the storage time of the grains, it would seem that the optimal storage time would be 180 d, but if we consider the fungal populations measured, the optimal moment is at 90 d.

Populations of total fungi and *Fusarium* were positively correlated with DM, starch, CP, and pH. In general, a higher level of moisture in the grains favors the silage process, with a higher production of organic acids that decrease fungal growth within the silo (Baron et al., 1986). The pH values decreased and maintained in a range expected for wet silage sorghum grains. Rowe et al. (1999) claim that the lactic acid produced in stored grains under anaerobiosis silage conditions reduced the pH, and thus helps to preserve the quality of the grains. The fact that *Fusarium* and *Penicillium* were negatively correlated with CT concentrations would indicate that higher tannin levels in sorghum grains reduce fungal contamination. This was not observed for *Aspergillus*, probably because of the high contamination prior to silage. In this sense, one of the agro-nomic advantages of HT sorghum is given by the protective effect of these compounds against fungal contamination (Harris and Burns, 1973; Waniska, 2000). The presence of condensed tannins in combination with phenolic acids and flavan-4-ols would be responsible for the resistance to fungi in mature grains (Jambunatham et al., 1992; Menkir et al., 1996). However, in other studies, the resistance to fungal contamination in sorghum grains is considered to be related with both HT and LT varieties (Bandyopadhyay et al., 1988; Jambunatham et al., 1992).

## 5. Conclusions

This work confirms a beneficial effect of storage time for 180 d of moist sorghum grains in silo bags, reducing the concentration of condensed tannins, increasing the fermentation speed of the grains and minimizing fungal contamination, both in high and low tannin varieties. It seems that 180 d of storage would be the ideal to improve the ruminal utilization of the grains, although 90 d of storage seems to be the optimal time to minimize contamination by the species of fungi evaluated.

## Conflicts of interest

The authors declare that there are no conflicts of interest.

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