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Longer ensilage time improve rumen fermentation of high moisture – High-tannin sorghum grain

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ABSTRACT

The high moisture sorghum grain silage is an important source of feed for livestock in Uruguay and some regions of the world. That is why this study aimed to evaluate the effect of ensilage time, moisture content, and variety on the *in vitro* fermentation ruminal parameters of moist sorghum grain stored in experimental silos. This study was conducted to analyze gas production *in vitro* of two sorghum grains varieties, with high and low-tannin content, ensiled with15–25%, 26–32%, and 33–42% moisture content 30, 90, and 180 d of ensilage in experimental silos. Total gas production was higher in low-tannin grains than in those with high-tannin, regardless of the ensilage time or the moisture content. Differences in the gas production rate were also observed in the interactions between tannin and moisture content, ensilage time, and moisture content as between tannin content and moisture contents for the lag time. Moreover, medium moisture content promoted the fermentability in high-tannin grains and high moisture in low-tannin grains. The fermentability of both high and low-tannin grains was enhanced with increasing ensilage time.

1. Introduction

Sorghum is the fifth most economically important cereal crop in the world, with an average production in the last two decades of 60.2 million tons, becoming one of the cereal grains most used in animal feed [1]. This is how almost all the sorghum traded in international markets is used to feed livestock. On the other hand, it constitutes an important resource for human nutrition, providing energy, proteins, vitamins, and minerals, mainly in populations that live in poverty in semi-arid or arid regions [2]. In addition to its importance as food, it provides raw material to produce starch, fiber, dextrose syrup, biofuels, and alcohol, among other products [3].

Sorghum grain is considered as lower nutritional value compared to other cereals due in part to the concentration of condensed tannins in some genotypes, the presence of kafirins in the endosperm, and its association with starch [4–6]. However, sorghum cultivars have greater resistance to climatic stress, insect attack, and fungal contamination than other grains [7,8]. Therefore, the incorporation of high moisture sorghum grain silages in livestock systems has been a good tool for intensive production systems [9]. Increasing grain moisture, rolling, and grinding are processing methods that improve the sorghum digestibility in ruminants [10]. Also, treatments such as reconstitution of dry grains with water, soaking, and germination improve the digestibility [11]. In addition, ensiling with a moisture content of over 25% can also improve the nutritional value of sorghum grains [12].

On the other hand, in a previous study, the combination of germination and ensilage of sorghum grains reduced the tannin content, increasing ruminal degradability and total digestibility [13]. However, when grains are ensiled with a moisture content over 40%, the proliferation of toxigenic fungi is increased, and a higher effluent production is observed (decreasing its nutritive value). In turn, when the moisture content is too low (under16%), air gaps may be generated inside the silo and the temperature may increase with the subsequent loss of nutrients [14]. In addition, it was found that the moisture content of sorghum grains stored in silo bags for 180 d was beneficial for increasing the fractional rate of gas production, particularly on high-tannin grains. Probably, this effect would be due to the reduction in the concentration of condensed tannins [15]. Therefore, this study aimed to evaluate the

Abbreviations: DM, dry matter; MC, moisture content; CT, condensed tannins; HT, high-tannins; LT, low-tannins; a, potential gas production; kd, fractional rate of gas production; L, gas production lag time.

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effects of ensilage time in high and low tannin sorghum grains (HT and LT) ensiled at different moisture levels on *in vitro* ruminal fermentescibility.

2. Materials and methods

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

2.1. Sample collection and silo grain treatments

Samples of sorghum grains with variety low condensed tannin content (LT, tannin concentration < 1 g/kg DM, genotypes Flash 10 and ACA 546) and variety high condensed tannin content (HT, tannin concentration > 5 g/kg DM, genotypes Morgan 108 and ACA 558) were collected immediately before the start of the ensiling process from five commercial farms located in San José, Flores, and Canelones Departments (33°33′28.3″ S 56°52′37.7″ W and 34°31′07.5″ S 56°32′21.1″ W), at the southcentral region of Uruguay. Farms were selected in a previous study according to the variety of sorghum grains used for preparing the silages [15]. At the time of harvesting, grains contained a moisture range from 26% to 32%. Samples were ensiled in experimental laboratory silos at three moisture content levels: low (15–25%), medium (26–32%), and high (33–42%). Experimental silos were opened at 30, 90, and 180 d of ensilage. Each combination was replicated 3 times, leading to a total of 135 experimental silos (5 farms × 3 moisture levels × 3 ensilage time × 3 replicates) [16].

2.2. Chemical analysis

The moisture content was determined using the method 925.09 [17]. Grains were dried in an oven at 105 °C for 1 h and placed in desiccators to cool. Tannins concentrations were determined with the butanol-HCl method described by Makkar [18].

2.3. In vitro gas production

The fermentescibility of sorghum grains before and after the ensiling process was evaluated using a cumulative *in vitro* gas production technique, according to Mauricio et al. [19]. Samples were weighed (0.5 g DM) and placed in 100 mL bottles. Then, 40.5 mL of an incubation media was added to each fermentation bottle under a continuous CO_2 stream as described by Mould et al. [20]. Afterward, bottles were sealed with butyl rubber stoppers and stored at 4 °C for 12 h to hydrate substrates. Before inoculation, bottles were pre-warmed in a water bath at 39 °C for 2 h. Then, each bottle was inoculated with 10 mL of fresh ruminal fluid from 2 cows fed with a diet composed of 2/3 pasture hay and 1/3 concentrates. Immediately after inoculation, flasks were gassed again with a CO_2 stream; butyl rubber stoppers were fastened with aluminum crimp seals and remained in the water bath throughout the measurement period. Three bottles per variety and ensilage time were incubated (48 bottles containing substrate, plus three with no substrate included as inoculum blanks, and the whole procedure was conducted in two runs.

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 and the gas was vented, according with Mauricio et al. and Mould et al. [19,20]. Gas volume in mL was predicted from pressure values using an equation obtained in a previous trial conducted under the same experimental conditions. The data of cumulative gas production were fitted to the model:

$$V = a \times (1\text{-}e^{-kd \times (t-L)}) \quad \text{(Eq. 1)}$$

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 (h^{-1}) and "*L*" is the gas production lag time (h).

2.4. Statistical analysis. The variance homogeneity of the data was assessed using the PROC UNIVARIATE statement of SAS [21]. Data of in vitro fermentation kinetics were analyzed using the MIXED procedure of SAS (version 9.0) by the model

$$Y_{ijkl} = \mu + V_i + T_j + H_k + F_l\ (V{*}T)_{ij} + (V{*}H)_{ik} + (T{*}H)_{jk} + (V{*}T{*}H)_{ijk} + \varepsilon_{ijklm} \quad \text{(Eq. 2)}$$

where Y_{ijkl} is the variable to be tested, μ is the mean, V_i the fixed effect of the grain variety (i = HT or LT), T_j is the fixed effect of ensilage time (j = 0, 30, 90, and 180 d), H_k is the fixed effect of moisture content (low, medium and high), F_l is the random effect of the farm (silo), $(V{*}T)_{ij}$ the interaction between variety i and ensilage time j, $(V{*}H)_{ik}$ the interaction between variety and moisture content, $(T{*}H)_{jk}$ the interaction between ensilage time j and moisture content k and $(V{*}T{*}H)_{ijk}$ the interaction between variety i, ensilage time j and moisture content k and ε_{ijklm} is the residual error. The means were compared using the Tukey test. Significance was considered at $P < 0.05$.

3. Results

The effect of storage time on fermentation parameters of ground sorghum grain containing high and low-tannin contents is shown in Table 1. The potential gas production was affected by the sorghum variety, the LT grains produced more gas volume than the HT grains ($P < 0.001$), independently of the ensilage time and the moisture content of the grains. A significant interaction between ensilage time and moisture content can be seen ($P = 0.03$).

In figures (1-4) the variables that presented interactions of the simple effects are shown. In the samples with medium MC the potential gas production decreased 11.5% as ensilage time increased from 30 to 180 d, while samples with high MC the potential gas production increased in the same proportion. For 30 and 90 d, the values with medium MC were also higher than those with high MC ($P < 0.05$), but at 180 d the potential gas production was higher at high MC than at medium MC ($P < 0.05$) (Fig. 1).

In the fractional rate of gas production, interactions were observed (Table 1) between the variety and the moisture content ($P = 0.007$) and between ensilage time and moisture content ($P = 0.002$). Fig. 2 shows the effect of ensilage time according to moisture content. The fractional rate of gas production was higher in samples with low and high MC at 180 d than at 30 and 90 d ($P<0.05$). The samples with medium MC did not show significant differences ($P>0.05$).

In Fig. 3, it can be observed that LT sorghum grains with high MC fermented faster than those with medium and low MC ($P <0.05$). On the other hand, HT grains with medium MC presented a higher rate of gas production than those with low MC ($P <0.05$), but with high MC was not significantly different ($P > 0.05$).

The LT grains ensiled with medium MC had a longer lag time than those ensiled with low MC ($P<0.05$) (Fig. 4). Conversely, the lag time did not change along the ensilage time, but it showed a significant interaction between the grain variety and moisture contents.

4. Discussion

The results show that the total volume of gas produced was higher in the LT grains variety compared to the HT variety. Other studies also reported superior ruminal fermentation in LT sorghum grains compared to HT grains [22–25]. Sorghum tannins form indigestible complexes with proteins and starch; hence they have a negative influence by

Table 1
Effect of storage time on fermentation parameters of ground sorghum grain containing high and low tannin contents.

Parameter	Variety				Moisture content					Storage time					P V*T	P V*MC	P T*MC
	HT	LT	SEM	P	L	M	H	SEM	P	30	90	180	SEM	P			
a	223	260	4.64	**<.001**	234	248	243	5.71	0.21	241	243	240	5.71	0.89	0.67	0.06	**0.03**
kd	0.092	0.099	0.001	0.49	0.096	0.090	0.088	0.002	**<.01**	0.087	0.089	0.098	0.002	**<.001**	0.47	**0.007**	**0.002**
L	2.81	2.75	0.04	0.36	2.73	2.76	2.71	0.04	0.73	2.72	2.73	2.75	0.04	0.82	0.54	**0.01**	0.05

HT, high tannins; LT, low tannins; SEM, Standard error of means (n = 135); P, level of significance of the V (variety), MC (moisture content) and T (storage time) and V by T, V by MC, T by MC and V by T by MC interactions; L, low moisture content; M, medium moisture content; H, high moisture content; a, potential gas production (mL of gas/g DM incubated); kd, fractional rate of gas production (h^{-1}); L, gas production lag time (h). Numbers in bold indicate significance differences.

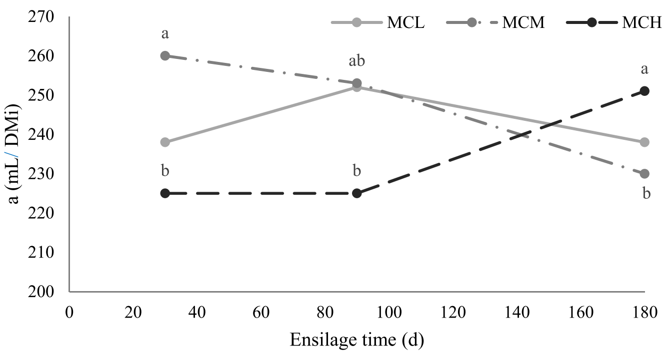

Fig. 1. Effect of moisture content (MCL: low, MCM: medium, and MCH: high) and ensilage time (30, 90, and 180 d) on potential gas production (a). Different letters indicate significant differences within the same moisture content level.

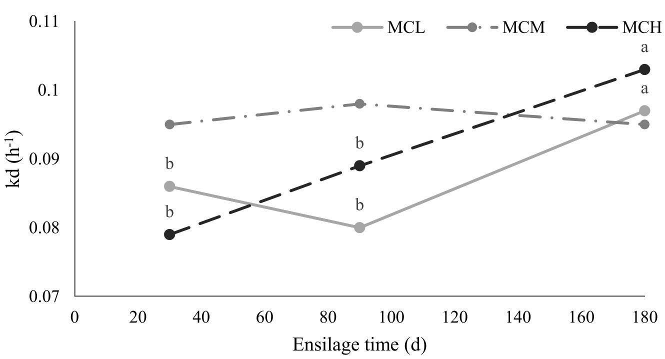

Fig. 2. Effect of moisture content (MCL: low, MCM: medium, and MCH: high) and ensilage time (30, 90, and 180 days) on the fractional rate of gas production (kd). Different letters indicate significant differences within the same humidity range.

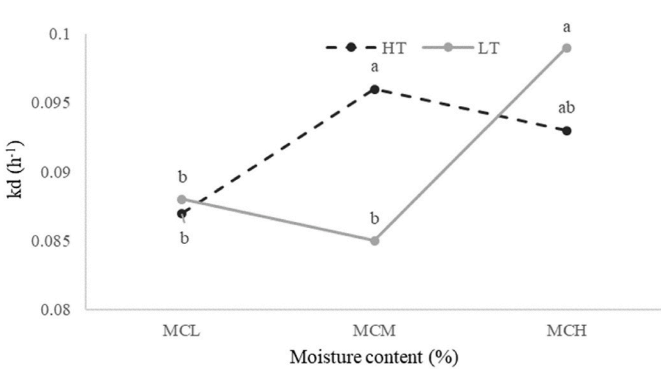

Fig. 3. Effect of moisture content (MCL: low, MCM: medium, and MCH: high) and sorghum grains varieties (HT: high tannin, and LT: low tannin) on the fractional rate of gas production (kd). Different letters indicate significant differences within the variety.

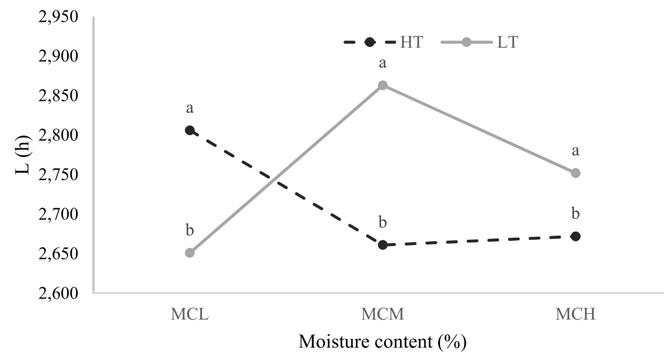

Fig. 4. Effect of moisture content (MCL: low, MCM: medium, and MCH: high) and sorghum grains varieties (HT: high tannin, and LT: low tannin) on the gas production lag time (L). Different letters indicate significant differences within the same variety.

reducing the digestibility of these grains [18]. Moreover, the arrangement and characteristics of the protein matrix that cover the starch granules influence the solubility and fermentation of these proteins [26]. The protein matrix associated with the starch granules, the presence of condensed tannins [6,27], and the higher proportion of corneal endosperm, which makes the starch less digestible. Consequently, sorghum grains are considered of lower nutritional value than other cereal grains [4].

Under the conditions in which this work was carried out, although the gas volume decreased over time in samples with medium MC, the degradation rate increased in the samples with low and medium MC. It was expected that the volume of gas would increase with more ensilage time because it has been observed that this process has a positive effect on the volume of gas produced in sorghum grains [24]. On the other hand, it was observed that with a long ensilage time (180 d) the degradation rate of sorghum grains increased, improving its fermentability. Studies comparing sorghum, wheat, barley, and corn, showed that although sorghum produced a volume of total gas like the other grains, the degradation rate was the slowest [28].

In this study, the highest degradation rate was seen in LT grains with high MC, and in HT grains with medium and high MC. Probably higher moisture content could increase the ruminal starch degradation, which causes the solubilization of the protein matrix increasing the susceptibility of starch granules to enzymatic hydrolysis [23]. On the other hand, when the material is ensiled with low MC, the compaction is not good, and air spaces are formed in the bags, which leads to a loss of nutrients due to aerobic fermentation [9]. The latter could explain the decrease in the degradation rate of grains with low MC, independently of the variety of grain used. Moisture content favors the digestibility of sorghum grains regardless of the ensilage process. This would explain why the samples with high MC showed a high degradation rate 30 d after ensilage. In contrast, samples with medium and low MC required more ensilage time to improve the gas production rate. Huck et al. [29] demonstrated that the reconstitution of dry sorghum grains (until reaching a final 25–35% MC) improved their digestibility, associated with an increase in starch digestion, suggesting that MC higher than 30% could favor the fermentative and digestibility characteristics in ruminants.

Conversely, the lag time increased in the LT varieties with medium MC, compared to the low MC. Although a statistical difference was evident, the numerical difference was less than 13 min. The expected result would have been a faster adhesion of the microorganisms in sorghum grains with a less complex structure of starch granules and protein matrix, as would occur in LT. New studies could probably be necessary to obtain a more precise explanation for this result.

Reichert et al. [30], observed that the ensilage of wet high in tannins grains under anaerobic conditions deactivates the tannins, improving the nutritional value of these grains. This effect of silage on grain tannins would help to explain the results obtained in this work.

Fermentation values were also influenced by the ensilage time. In this study, more ensilage time favored *in vitro* fermentability, an effect evidenced by increased gas production and the rate of gas production, that could be related to greater ruminal fermentation and rapid microbial growth. This result indicates that the silage process causes a higher availability of substrates than that in the unfermented grains, as has been reported in other studies where *in vitro* gas production of fermented sorghum grains was determined [13,31]. Cummins [32] reported that the increase of *in vitro* DM digestibility of sorghum grains silage was greater in high-tannin than in low-tannin hybrids, but in our study, this effect was not clear.

On the other, at 180 d of the ensiling time the abundances of toxicogenic Aspergillus and Fusarium were reduced. Conversely, the abundance of these fungi was not affected by the moisture content [16].

5. Conclusions

The gas production of grains with low-tannin concentration were favored by the ensiling process. The rate of fermentation was higher with low and high moisture content at 180 days but was similar with medium moisture content along the ensiling time.

High-tannin sorghum fermented better with medium moisture content, whereas low-tannin grains fermented more with high moisture content. The high and low-tannin content grains showed a better ruminal fermentation with a longer ensilage time.

Declaration of competing interest

The authors of the manuscript titled “Does a longer storage time improve rumen fermentation of high moisture - high tannin sorghum grain silages? They declare that there are no conflicts of interest.

Data availability

Data will be made available on request.

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