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In situ degradability of dry matter and fibrous fraction of sorghum silage

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ABSTRACT. This study aimed to evaluate *in situ* degradability and degradation kinetics of DM, NDF and ADF of silage, with or without tannin in the grains. Two isogenic lines of grain sorghum (CMS-XS 114 with tannin and CMS-XS 165 without tannin) and two sorghum hybrids (BR-700 dual purpose with tannin and BR-601 forage without tannin) were ensiled; dried and ground silage samples were placed in nylon bags and introduced through the fistulas. After incubation for 6, 12, 24, 48, 72 and 96 hours, bags were taken for subsequent analysis of fibrous fractions. The experimental design was completely randomized with 4 replicates and 4 treatments and means compared by Tukey's test at 5% probability. As for the DM degradation rate, silage of CMS-XS 165 without tannin was superior. Silages of genotypes BR 700 and CMS-XS 114 with tannin showed the highest values of indigestible ADF (59.54 and 43.09%). Regarding the NDF, the potential degradation of silage of CMS-XS165 line without tannin was superior. Tannin can reduce ruminal degradability of the dry matter and fibrous fractions.

Keywords: preserved food, forage, tannin, nutritional value.

Degrabilidade *in situ* da matéria seca e fração fibrosa da silagem de sorgo

RESUMO. Objetivou-se avaliar a degradabilidade *in situ* e a cinética de degradação da MS, FDN e FDA das silagens, com presença e ausência de tanino nos grãos. Foram utilizadas, duas linhagens isogênicas de sorgo granífero (CMS-XS 114 com tanino e CMS-XS 165 sem tanino) e dois híbridos de sorgo (BR-700 duplo propósito com tanino e BR-601 forrageiro sem tanino). Amostras de silagens secas e moídas foram colocadas em sacos de náilon e introduzidas nas fístulas dos bovinos. Após períodos de incubação de 6, 12, 24, 48, 72 e 96 h, os sacos foram removidos para análises posteriores das frações fibrosas. O delineamento experimental utilizado foi o inteiramente casualizado, com quatro repetições e quatro tratamentos e as médias comparadas pelo teste de Tukey em nível de 5% de significância. Quanto à taxa de degradação da MS, a silagem da linhagem CM-SXS 165 sem tanino foi superior. As silagens dos genótipos BR 700 e CMS-XS 114 com tanino apresentaram os maiores valores de fração indigestível da fração FDA (59,54 e 43,09%). Para a FDN, observou-se que o potencial de degradação da silagem da linhagem CMS-XS 165 sem tanino foi superior. A presença do tanino pode reduzir a degradabilidade ruminal, da matéria seca e das frações fibrosas.

Palavras-chave: alimento conservado, forragem, tanino, valor nutricional.

Introduction

Due to the large population growth, the need to produce increasingly good quality milk and meat has challenged producer markets. Climate adversities have making forage resources scarce in most parts of Brazil, which may be a major cause of low performance indices of livestock in our country, then forcing ranchers to seek alternatives associated with the use of breakthrough technologies. This can enhance production and meet animal requirements, thus enabling to achieve high offtake ratesin their herds.

Among the alternatives foundin Brazil, the sorghum plant stands out by a set of factors, such as its high production potential; good adaptation to mechanization, great versatility (hay, silage and direct grazing and easy adaptation to the drier regions, which makes it a safer culture that corn, with great expression in the livestock scenario. Despite such advantages, sorghum may have phenolic compounds resulting from the secondary metabolism, including phenolic acids, flavonoids and tannins. The presence of tannin in forage influences mainly the acceptability by the animals, depending on its concentration. Such compounds readily form hydrogen bonds with the mucosa and salivary proteins, reducing the digestibility of protein and fiber, reducing the voluntary intake and production indicators, in addition to lower nitrogen recovery (Vitti et al., 2005). In this sense, this study aimed to evaluate in situ degradability and the degradation kinetics of dry matter, crude protein, neutral detergent fiber and acid detergent fiber of silages of four sorghum genotypes with and without condensed tannin in the grains.

Material and methods

The experiment was conducted at Embrapa - National Center for Research on Corn and Sorghum, located at Km 65 of the MG424 highway, in the municipality of Sete Lagoas, State of Minas Gerais. The geographical coordinates are 19°28' South latitude and longitude 44°15'08" WGrW. The climate, according to Köppen is AW type (savanna climate with dry winter).

For this study, we selected four types of sorghum, two isogenic lines of grain sorghum (CMS-XS 114 with tannin and CMS-XS 165 without tannin) and two sorghum hybrids (BR-700 dual purpose with tannin and BR-601 forage without tannin). The choice of these cultivars considered the presence or absence of condensed tannins in the grains.

Sorghum genotypes weresown in the summer of 2009, in beds 5 m long, 3 m wide and 75 cm spacing between rows. Fertilization at planting was equal to 350 kg 8-28-16 (NPK) and after 40 days, 110 kg urea as top dressing, according to crop requirements. Grain materials and BR 700 were collected at pasty and hard dough stages, respectively, and BR-601, at milky stage. Plants were hand harvested, close to the ground and chopped to a particle size of approximately 2.5 cm in a stationary chopper.

They were then ensiled in laboratory silos (kg m^{-3}) made of PVC tubes, 100 mm diameter, 500 mm in length and pressed with wooden socket. Silos were sealed at the time of ensiling, with PVC caps equipped with Bunsen valves and sealed with masking tape, being weighed before and after ensiling. Four replications per treatment were made and two replicates per plot, totaling thirty-two silos.

Chemical analyses were performed at the Food Analysis Laboratory, Department of Agrarian Sciences, State University of Montes Claros, Campus of Janaúba, State of Minas Gerais. After 56 days of fermentation, silos were opened for taking silage samples, in a uniform manner, from the first, middle and final thirds of each silo, forming a composite sample for each genotype. Of this composite sample, a subsample was taken, pre-dried in forced ventilation oven at 65°C for 72 hours, ground to 1 mm particle size, and used for determination of dry matter content (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin by the sequential method of Detmann et al. (2012). Hemicellulose and cellulose were calculated from the results of NDF, ADF and lignin. In the silage juice, we measured pH (Table 1), using a pH meter, and ammonia nitrogen by distillation with magnesium oxide.

Table 1-.Chemical composition and pH of silages used in the degradability trial.

Variables	CMS-XS 165	BR-601	BR 700	CMS-XS 114
Total DM (%)	28.22	22.29	32.66	31.02
CP (%)	12.62	7.99	8.61	10.27
NDF (%)	45.63	51.22	49.86	53.03
ADF (%)	25.66	29.65	27.51	30.07
Hcel (%)	19.96	21.57	22.35	22.36
Cel(%)	20.53	25.3	19.71	22.61
Lignin (%)	5.13	4.35	7.80	7.46
pH	3.90	3.83	3.84	4.01
NH_3/NT (%)	4.28	6.02	4.01	5.89

Source: Search result.

For *in situ* evaluation, the experiment was conducted at the experimental farm of the State University of Montes Claros (UNIMONTES), in Janaúba, State of Minas Gerais and 17 further analyses in Food Analysis Laboratory of the same institution.

In situ ruminal degradation kinetics parameters of DM, NDF and ADF of sorghum silage were estimated using the nylon bag technique. There were used 10 x 20 cm nylon bags a pore size of 50 microns, dried at 65°C for 48 hours and weighed. Subsequently, they were filled with approximately 5 g silage of sorghum genotypes studied, which were previously ground to 5 mm, corresponding to a ratio sample weight/surface area of the nylon bag of 12.5 mg cm^{-2} (Nocek, 1988).

For incubation, four crossbred steers, fistulated in the rumen, with average body weight of 450 kg, were housed in pens with individual troughs and drinkers. Before the experiment, the animals were treated against endo- and ectoparasites. Each animal received daily sorghum silage (VOLUMAX), water and a commercial mineral mixture ad libitum and 2 kg concentrate based on corn and soybean meal. Three bags per incubation time were inserted into the rumen of each animal in their respective incubation times. We made 3 bags $genotype^{-1}$ $animal^{-1}$ $time^{-1}$, totaling 336 bags. Bags containing the samples were tied with a rubber band to a metal ring and attached to a restraint clip, bound to a nylon rope and an iron chain, which served as anchor, and incubated in the rumen for 6, 12, 24, 48, 72 and 96 hours following the incubation protocol.

In this case, incubations were carried out following time reversal, so that all bags were removed from the rumen at the end of fermentation period. Immediately after removal from the rumen, bags were immersed in cold water and subsequently washed manually with tap water, at room temperature, until the rinsing water was colorless.

After washing, bags were placed in trays and dried in a forced ventilation oven for 48 hours and transferred to a desiccator for 30 minutes and then weighed. Next, the remaining material in the bags, of the same genotype, animal and incubation period, was transformed into a homogeneous pool. The incubation residues were ground in a mill with 1 mm sieve and used to determine DM (AOAC, 1995), CP, NDFand ADF, according to Van Soest (1991) in a ANKOM20 Fiber Analyzer.The contents of these fractions in samples of foragesand incubation residues, together with the weights of the incubated materials and residues, 20 were used for the calculation of the disappearance of the fractions. Data were fitted to a non-linear regression by the Gauss-Newton method (Neter, Wasserman, & Kutner, 1985), using the software SAS according to the equation proposed by Orskov and McDonald (1979).

$$Y = a + b(1 - e^{-ct})$$

where:
Y = accumulated degradability of the nutritional component analyzed, after incubation time t;
a = degradability curve interval, when t = 0, corresponding to soluble fraction of the nutritional component analyzed;
b = potential degradability of insoluble fraction of the nutritional component analyzed;
a + b = potential degradability ofthe nutritional component analyzed, when time t is not a limiting factor;
c = degradation rate by fermentative action of the fraction b.

Once calculated the constants a, b and c, they are applied to the equation proposed by Orskov and McDonald (1979);

$$P = a + \frac{b.c}{c+k}$$

where:
P =effective ruminal degradability of the nutritional component analyzed;
k = ruminal passage rate of the food (0.05% h^{-1})

Effective ruminal degradability was calculated and expressed in terms of dry matter, crude protein and neutral detergent fiber effectively degraded in the rumen. The experimental design was completely randomized, with four replications and four treatments. Data, whenever significant, were subjected to analysis of variance. The results were statistically analyzed using the System Analysis of Variance (SISVAR), followed by Tukey test at 5% level for mean comparison, with the following model:

$$Yij: \mu + Gi + eij$$

where:
Yij = observed value of genotype i, in repetition j;
μ = overall mean;
Gi = effect of genotype, with i = 1, 2, 3 and 4;
eij = experimental error associated with observed values (Yij), which hypothetically has normal distribution with zero mean and variance.

Resultsand discussions

There were differences in relation to the concentrations of soluble fractions for all genotypes. Silage of CMS-XS 165 showed the highest value, followed by CMS-XS 114, BR-601 and BR 700 (Table 2). Results for the fraction A were similar to those reported by Araújo et al. (2007) who reported soluble fractions of 19.89% for the hybrid BR 700, and 20.01% for the hybrid BR-601.

Table 2. Soluble fraction (A), potentially degradable insoluble fraction (B), degradation rate (C), indigestible fraction (Fi), potential degradation (Dp), effective degradation (De) of dry matter of silages of four sorghum genotypes with and without tannin in the grains.

	Genotypes				
Parameters	CMS-XS 165	BR-601	BR-700	CMS-XS 114	EPM
A (%)	26.72a	19.06c	15.18d	20.82b	0.109
B (%)	45.89c	55.36a	53.83b	43.43d	0.141
C (% h^{-1})	7.75a	4.25c	5.75b	5.25bc	0.535
Fi (%)	27.37c	25.57d	30.98b	35.74ª	0.083
Dp (%)	72.62b	74.43a	69.01c	64.26d	0.082
De (%)	54.58a	43.52b	44.01b	43.13b	0.284

Different letters, in the same row, indicate significant differences (p > 0.05) by Tukey's test.

Source: Search result.

The potentially degradable insoluble fraction (B) of dry matter was between 43.43 and 55.36%, and the highest value was found for silage of BR-601 (without tannin), and all silages were different from each other. As to DM degradation rate, silage of CMS-XS165 without tannin was superior to all treatments. The hybrid BR700 was similar to CMS-XS 114 and superior to BR-601.

The evaluation of forage digestibility is important for comparisons between materials considering performance and economic return. In this way, the parameters of the fractions A and C are the most important in this classification. Fraction A indicates a more degradable material, while a higher value of C reflects a shorter time for disappearance of readily degradable fraction.

In the present study, we observed that all silages showed dry matter degradation rates above 2%, but the line needs longer time in the rumen to reach its maximum degradation Comparing the isogenic lines, the highest value of indigestible fraction (Fi) was registered for silage of CMS-XS 114 (35.74%) with tannin and the lowest value for CMS-XS 165 (27.37%) without tannin. This difference suggests that tanninimpairs the digestibility of silages containing it. The largest values observed for potential degradability (DP) were verified for silages without tannin (CMS-XS 165 and BR-601).

Similar behavior for genotypes without tannin was registered by Pires et al. (2009), who studied in situ degradability of sorghum silage and found potential degradability for silage of CMS-XS 165 (75.05%), followed by silages of BR-601 (68.31%), CMS-XS 114 (62.19%) and BR-700 (59.29%). These differences can be attributed to the maturity stages of sorghum in silage and the proportion of stem, leaves and grains of the ensiled material.

With respect to the effective degradation rate of dry matter, the silage of CMS-XS 165 without tannin was superior to all the other treatments and when comparing the two isogenic lines CMS-XS 165 and CMS-XS 114, it appears that the differences found can be attributed to tannin, since it may influence DM degradability. As can be seen in Figure 1, the DM degradability of CMS-XS 165 silage without tannin showed higher soluble fraction and higher degradation potential, once the indigestible fraction has stabilized after 48 hours in the rumen. The greater soluble fraction of this silage is in accordance with the highest degradation rate of this material (7.75%). The higher the soluble fraction and the degradation potential of a food, the higher the degradation rate. For silage of CMS-XS 114, the soluble fraction was similar to the soluble fraction of the genotype BR-601, but the indigestible fraction of CMS 114 silage has stabilized after 72 h of incubation. A similar trend was demonstrated for silage of BR-700, while for silage of BR601, the incubation time was not enough to stabilize DM degradation.

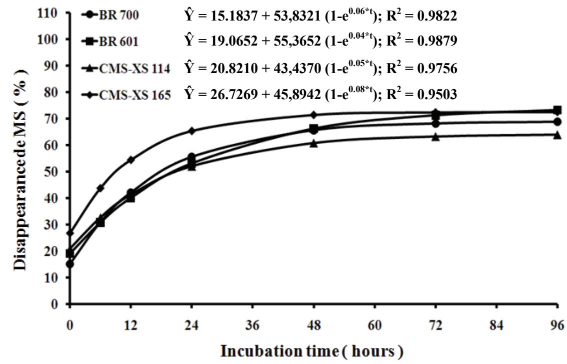

Figure 1. Dry matter (DM) disappearance of sorghum silages over incubation time (hours).

Source: Search result.

With respect to the concentrations of soluble fractions, silage of CMS-XS 165 showed the highest value. The other silages showed lower values (Table 3).

This high solubility of NDF found herein could be explained by the homogeneity in the preparation of samples for incubation, in which the milling with 5 mm mesh size sieve promoted excessive breakage of the parts of the sorghum plant, increasing the proportion of particles that escape through the nylon bag pores without being degraded, or even because the bag porosity facilitated the escape of the particles, or even failure in the confection.

Values of the parameter B, which estimate the potentially degradable insoluble fraction of NDF, were higher for the forage hybrids BR-700 and BR-601 (49.25 and 59.03%, respectively), when compared to the fractions (B) observed for sorghum grain lines CMS-XS 114 and CMS-XS 165 of 43.17 and 45.13%, respectively. These variations can be related to different agronomic parameters of lines studied, as forage cultivars are taller than 2.00 m and grain sorghum, heights from 1.00 to 1.60 m.

Table 3. Soluble fraction (A), potentially degradable insoluble fraction (B), degradation rate (C), indigestible fraction (Fi), potential degradation (Dp), effective degradation (De) of neutral detergent fiber of silages of four sorghum genotypes with and without tannin in the grains.

	Genotypes				
Parameters	CMS-XS 165	BR-601	BR-700	CMS-XS 114	EPM
A (%)	24.72a	4.54c	3.26c	6.69b	0.156
B (%)	45.13c	59.03a	49.25b	43.17c	0.488
C (% h^{-1})	12.22a	3.50b	2.50b	3.75b	0.169
Fi (%)	30.15c	36.42b	47.49a	50.13a	0.534
Dp (%)	69.85a	63.58b	52.51c	49.86c	0.533
De (%)	56.79a	27.01b	19.18c	24.52bc	0.821

Different letters, in the same row, indicate significant differences ($p > 0.05$) by Tukey's test.

Source: Search result.

Tall plants have more lignified tissue when compared to short sized plants, thereby decreasing digestibility; and in plants harvested in the milky grain stage, the fiber fraction is more digestible than the hard dough stage. Nevertheless, in the hard dough stage, dry matter accumulation in the grain can compensate for the reduction of digestible dry matter of the stem. The indigestible fraction (Fi) of NDF was between 30.15 and 50.13%. The highest values were recorded for silages of genotypes with tannin (BR-700 and CMS-XS 114). When compared to silage of isogenic lines, silage of CMS-XS 165 without tannin was 8.54 percentage points lower than of CMS-XS 114 with tannin, for indigestible fraction of NDF. This result reinforces the idea of the negative effect of tannin on the fiber digestibility. Most of this tannin in sorghum plant is in the grain, asproanthocyanidins, concentrated on the seed testa. As the cultivars are intended for grain production, it is common to find a greater presence of tannins, decreasing digestibility.

Still for NDF, the degradation potential of silage of CMS-XS 165 line without tannin was superior to all treatments. Silage of BR-700 was similar to CMSXS 114 and inferior to BR-601. Magalhães et al. (2007) examined silages of 25 sorghum hybrids and reported NDF degradation of 62% for BR601 and 60% for the BR-700. With regard to the effective NDF degradation of isogenic lines, it was observed that the tannin may have been responsible for the lower values obtained for the CMS-XS 114 line, with tannin, compared with the CMS-XS 165 line without tannin. This same effect can be verified when comparing the degradability of hybrids BR 601 (without tannin) and BR-700 (with tannin).

The parameters of ADFruminal degradation are listed in Table 4. Values of soluble fraction A of silage of BR-601 were higher compared to silages of hybrids BR-700 and CMS-XS 114 and similar to CMS-XS 165. However, higher soluble fraction than that observed herein was shown by Araújo et al. (2007), who found values of 16.24% for BR-700 and 13.07% for BR-601. According to Van Soest (1994), the soluble fraction should have values close to zero, since ADF is not soluble in water.

Table 4. Soluble fraction (A), potentially degradable insoluble fraction (B), degradation rate (C), indigestible fraction (Fi), potential degradation (Dp), effective degradation (De) of acid detergent fiber of silages of four sorghum genotypes with and without tannin in the grains.

	Genotypes				
Parameters	CMS-XS 165	BR-601	BR-700	CMS-XS 114	EPM
A (%)	5.96ab	7.06a	4.60c	5.87b	0.138
B (%)	59.48b	71.56a	35.84d	51.03c	0.962
C (% h^{-1})	2.0b	1.0c	3.75a	2.0b	0.063
Fi (%)	34.55b	21.37c	59.54a	43.09b	1.029
Dp (%)	65.44b	78.62a	40.45c	56.90b	1.029
De (%)	23.97a	17.55c	19.47b	19.99b	0.136

Different letters, in the same row, indicate significant differences (p > 0.05) by Tukey's test.

Source: Search result.

Soluble fractions obtained for evaluated silages were low and can be due to errors inherent to the *in situ* technique, such as the escape of particles through the pores of nylon bags. For potentially degradable insoluble fractions (B), we obtained values ranging from 35.84 to 71.56%. When comparing isogenic lines, it is clear that the CMS-XS 165 showed a greater potentially degradable fraction, highlighting the idea that tannin can reduce the degradability of ADF.

ADF degradation rates determined were 1.0% h^{-1} for BR601, followed by CMS-XS 114 and CMS-XS 165 with 2.0% h^{-1} and 3.75% h^{-1} for BR-700. Araújo et al. (2007) reported mean values for ADF degradation rates from1.11 to 1.58 % h^{-1}. The values found by these authors were similar or slightly lower than in this experiment. Silages of BR-700 and CMS-XS 114 genotypes with tannin presented the highest Fi values of ADF, from 59.54 to 43.09%, respectively. Nevertheless, for genotypes BR-601 and CMS-XS165 without tannin, the values were 21.37 and 34.55%, respectively.

For the ADF, the degradation potential of the silage of BR601 was superior to all treatments. Pires et al. (2009) studied the *in situ* degradability of fiber fractions of sorghum silage and observed Dp values of ADF between 25.34 and 57.62%. The greatest potential for ADF degradation of silage of BR-601 may be associated with the best quality of cellulose of this genotype and, furthermore, it does not contain tannin, and it is common to observe the high digestibility of its fiber fraction. The genotype CMS-XS 165 without tannin had the highest effective degradation of ADF, 23.97%.

The genotypes BR 700 and CMS-XS 114 (with tannin) presented a lower, not significantly different from each other. Magalhães et al. (2005) analyzed *in situ* degradability of sorghum silages and found De values of ADF for passage rates of 2, 5 and 8% h^{-1} at 34.56; 31.16 and 28.78%; 21.50; 20.22 and 19.23%, 35.50; 33.29 and 31.76% and 34.81; 34.16 and 33.61% for genotypes Volumax, CMSXS217 9929012, ATF54 9929036 and ATF53 9929036, respectively. Fiber is the fraction of structural carbohydrates in foods that is slowly or less digestible, and depending on the composition, it may reduce the intake of dry matter and energy by the animal.Thus,the adequacy of diets for ruminants requires information on the proportions of food fractions and their digestion rates. The factor with stronger effect on the extent and rate of degradation of the cell wall of plants is the presence of lignin, being observed a negative correlation between degradability of the organic matter in the rumen and lignin.

With regard to the fiber degradation potential (ADF), it can be verified, by means of Figure 2, that all genotypes had similar behavior, considering that there was no stabilization of the degradation process up to 96 h incubation. When comparing silages of isogenic lines, silage of CMS-XS 165 was superior to that of CMS-XS 114. This reinforces the idea that tannin interfere with the degradation of ADF and cellulose. This effect is attributed to the bonds of soluble tannin with cell wall components, resulting in complex indigestible or inaccessible to bacterial enzymes.

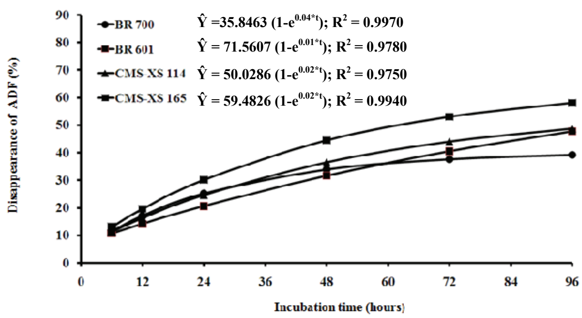

Figure 2. Acid detergent fiber (ADF) disappearance of sorghum silages over incubation time (hours).

Source: Search result.

Conclusion

The presence of tannin in sorghum genotypes can reduce ruminal degradability of DM and fiber fractions. The CMS-XS 165 line shows better ruminal degradability.

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