EVALUATION OF SORGHUM SILAGES OF DIFFERENT GENOTYPES WITH AND WITHOUT CONDENSED TANNINS

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ABSTRACT - A total of 10 Sorghum cultivar: two lines (CMSXS114, T) and (CMSXS165, WT), six hybrids (9953101,T; 9953130,T; BR601, WT; BR701, T; BR700, T; and AG2005, WT and two varieties (BR501, WT; and BR506, WT) with 8 replications were ensiled in PVC silos of 3 kg per silo for 60 days and then analyzed for condensed tannins (CT) (colorimetric analysis by the butanol-HCl method), CP, NDF, ADF, and in vitro (IVDMD-24 and 48hs) digestibilities. Condensed tannins (CT) from quebracho were purified using affinity chromatography with sephadex LH-20 and used as a standard. Low concentrations of CT have been defined as 10 g kg^{-1} DM or less and medium as 10 to 40 g kg^{-1} DM, and levels exceeding 40 g kg^{-1} DM as high. Hybrid 9953130 had the highest concentration of CT among the sorghum silages ($P < 0.05$), with 10.62 g kg^{-1} DM, and among the sorghum panicles, the hybrid 9953101 with 37.39 g kg^{-1} DM. Differences among sorghum lines were not detected for lignin and in vitro digestibility ($P > 0.09$). The highest CP content was noted for line CMSXS165 (97.4 g kg^{-1}; $P < 0.05$). Concentrations of CP in CMSXS114 (83.2 g kg^{-1}), BR 700 (76.4 g kg^{-1}), BR701 (80.5 g kg^{-1}), and AG2005 (79.2 g kg^{-1}) were above the overall mean (74.0 g kg^{-1}). The in vitro digestibilities (24 and 48 hours) of the two isogenics lines CMSXS114 (T) and CMSXS165 (WT) were not different. We concluded that the CT level did not affect digestibility; however, relative CT concentrations were low. Therefore, we could not suggest significant effects of CT content on DM intake, digestibility levels or performance in cattle fed with any of these sorghum silages.

Key words: sorghum, silage, feed, crude protein, NDF, ADF, lignin and IVDMD

Abbreviation Key: CT=condensed tannin; **WT**= without tannin, **T=** tannin

AVALIAÇÃO DE SILAGENS DE SORGO DE DIFERENTES GENÓTIPOS COM E SEM TANINO CONDENSADO

RESUMO - Um total de dez cultivares, sendo duas linhagens de sorgo com (T) e sem tanino condensado (WT), CMSXS114 (T) e CMSXS165 (WT), seis híbridos, 9953101(T), 9953130(T), BR601 (WT), BR701 (T) BR700 (T) e AG2005 (WT), e duas variedades, BR 501(WT) e BR506 (WT), com oito repetições foram, ensiladas em silos de PVC, com capacidade de 3 kg por silo, por 60 dias, e analisadas para tanino condensado (TC) (análises colorimétricas, pelo método de butanol-HCl), proteína bruta (CP), fibra em detergente neutro (NDF), fibra em detergente ácido (ADF) e digestibilidade *in vitro* da matéria seca(DIVMS-24 e 48h). O tanino condensado (CT), extraído de quebracho purificado, foi utilizado como padrão (cromatografia com sephadex LH-20). Baixas concentrações de TC têm sido definidas na literatura pela concentração de 10 g kg $^{-1}$MS ou menor. Valores médios são aqueles entre 10 e 40 g kg^{-1} na matéria seca e níveis acima de 40 g kg $^{-1}$ MS são considerados altos. O híbrido 9953130 apresentou a mais alta concentração de tanino condensado entre as silagens de sorgo analisadas (P < 0,05), com 10,62 g kg^{-1} MS e, entre as amostras de panículas, também revelou o mais alto valor de TC de 37,39 g kg^{1}MS. Diferenças entre silagens de sorgos não foram detectadas para lignina e DIVMS (P > 0,09). O maior conteúdo de proteína foi observado na linhagem CMSXS165 (97,4 g kg^{-1}; P < 0,05). Concentrações de proteína bruta em CMSXS114 (83,2 g kg^{-1}), BR 700 (76,4 g kg^{-1}), BR701 (80,5 g kg^{-1}) e AG2005 (79,2 g kg^{-1}) foram acima da média geral (74,0 g kg^{-1}). As duas linhagens isogênicas não foram diferentes para a DIVMS (24 e 48 h). Conclui-se que a digestibilidade *in vitro* não foi afetada pelo nível de tanino condensado encontrado; entretanto, consideram-se relativamente baixas as concentrações de tanino condensado encontradas devido, ao efeito de diluição dos grãos. Contudo, não foi possível antecipar nenhum efeito significativo do conteúdo de TC no consumo de matéria seca, digestibilidade ou performance em bovinos alimentados com esses materiais ensilados.

Palavras-chave: sorgo, silagem, tanino, proteína bruta, FDN, FDA, IVDMD

Abreviações-chaves: TC= tanino condensado; **WT**= sem tanino, **T=** tanino.

In almost every region of the world a period of abundance and another with shortage of forage always occur, even though the food demand remains almost constant. The forages with the best nutritional value for conservation as silages are corn and sorghum (Van Soest, 1994). Sorghum is a good crop choice in the period between harvests in traditional cereal-growing areas, mainly for its high tolerance to drought (Magalhães & Rodrigues, 2001). Therefore, one way to improve milk production of cattle is by developing new cultivars of sorghum forage with higher productivity and nutritional value as an alternative to overcome low availability of forage in this period.

Plant breeding programs have produced hybrids with increased grain content to improve silage production and quality. One of the biggest problems in sorghum silage production is its grain content. Since the sorghum plant is prone to bird attack, the amount of grain can fall in the silage, compromising its nutritional quality. To reduce this negative impact, researchers have been producing cultivars with a high content of condensed tannin, which can reduce the palatability to birds of the grain in the panicle. However, condensed tannins in the forage are thought to reduce the nutritional value because historically the belief has been that condensed tannins (CT) are responsible for depressing digestibility of plant material, in addition to decreasing palatability and consumption, depending on its tannin concentration.

In diets of monogastric animals, highly condensed tannin concentrations can reduce protein digestility and absorption and are responsible for low digestibility of dry matter. The use of ground sorghum with condensed tannin in the diet of monogastric animals such as young chickens has resulted in decreased consumption and caused deleterious effects such as reduction of amino acid absorption (Rodriguez *et al.,* 1999). However, for ruminants, tannins play a significant role in the nutritional value of feed, causing either adverse or beneficial effects on nutrient use, health and production. The ideal concentration of CT in forage legumes generally ranges from 20-40 g/kg DM, and at this level it may bind with dietary proteins during mastication and protect it from microbial attack in the rumen (Nguyen *et al*., 2005). The superior value of the tannin, as mentioned above, will perhaps reduce the nutritional value and the biological availability of the dietary mineral and protein intake (Barry *et al.,* 1986; Albrecht & Broderick, 1990,).

Min *et al*. (2002, 2005) demonstrated that the condensed tannin when grazing animals eat *Lotus corniculatus* or *Lespedeza cuneata* could protect proteins in the rumen, releasing them for absorption in the intestine. The hydrophobic part of protein complexes form hydrogen bridges, which interact with the tannin molecules that become unavailable in the rumen and can only be absorbed at the intestinal level. In the case of sorghum, no negative nutritional effect of the condensed tannin has been found in ruminants. In addition, tannin is the substance involved in the plant defense system against diseases and insect attack. Furthermore, condensed tannin can also be an alternative non-drug gastrointestinal parasite control. Strategies have been suggested by Min *et al*., 2002, 2005, Molan *et al*., 2000, based on using forages that contain condensed tannins (CT). CT are recognized for their property of forming complexes with soluble ruminal proteins, which reduce the degradation of protein to ammonia in the rumen and allow more dietary protein flow to the small intestine (Barry *et al*.,1986; Van Soest, 1994, Barry & McNabb, 1999). This increases the supply of digestible protein in the host (Min *et al*.**,** 2002) and has an indirect effect on gastrointestinal parasite resistance by enhancing the immune response through improved protein nutrition (MacRae *et al*., 1993; Barry & McNabb, 1999).

The objective of this study was to quantify and evaluate the effects of CT on the nutritional quality of the ensilage of sorghum for cattle.

Materials and Methods

The field experiments for production of silage were carried out at the Embrapa Corn and Sorghum Research Station in Sete Lagoas, Minas Gerais, Brazil, in 2002. Ten genotypes of sorghum were used: two isogenic lines (CMSXS114 and CMSXS165), four hybrids (BR601, BRS701, BR700 and AG2005), two experimental hybrids of high transport (9953101 and 9953130) and two varieties (BR501 and BR506). The two isogenics lines used in this work differing only by the presence of condensed tannin. The Embrapa Corn and Sorghum Research Center has developed these lines exclusively to evaluate the interference of CT in nutritional quality. The experiment was carried out in a completely randomized block design, with ten treatments (genotypes) and eight replications each (PVC silos). The data were submitted to analysis of variance and the means were grouped by the Scott & Knott, 1974 test (5%) using Ferreira's (2000) methodology. The sorghum silage was made with three middle lines from five with three meters in length for each silage genotype. When the materials reached the ensiling point (grains at drought stage) they were cut, chopped and ensiled in mini-silos made of PVC with 3kg capacity. The silos were opened 60 days afterward. For density standardization, (700kg/m^3) a press was used to compact the forage in the silos. PVC covers were made with Bunsen valves to eliminate the gases produced by fermentation. Also, samples of the panicles of all genotypes were processed to quantify the condensed tannin. In the Embrapa Dairy Cattle Center laboratories after opening the silos, two samples were taken, one left in the freezer for bromatological analyses and the other lyophilized to quantify the condensed tannin. For quantification of the condensed tannin, along with the samples of ensiled panicles, the Butanol-HCl method (Porter *et al*., 1986) was used. The quantification was carried out using chromatography with Sephadex column (Sephadex LH-20; Sigma) based on a standard TC-content curve using Quebracho 73% CT (of the tropical dicotyledon *Schinopsis balansae*) purification.

Laboratory Analyses

Some silage were frozen and others were freeze dried and ground to pass through a 1 mm (for *in vitro*) in a Wiley mill, for laboratory analyses. The Kjeldahl method was used for crude protein (CP) analysis (Helrich, 1990). Neutral detergent and acid detergent fiber (NDF and ADF) and lignin (LIG) were analyzed using the sequential methods of Van Soest *et al*. (1991). The in vitro dry matter digestibility was assessed using the methods of Tilley & Terry (1963).

Results and Discussion

The experimental accuracy of the statistical analyses, measured by the coefficient of variation (CV), was adequate for all characteristics

TABLE 1. Contents (%) of dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, in vitro dry-matter digestibility (24 and 48 hs) (IVDMD), and crude protein (CP) of some lines, hybrids and varieties of sorghum silage.

Treatments[1]	%DM	NDF	ADF	Lignin	IVDMD		CP
					24h	48h	
L-CMSXS114	32.12b	51.19a	36.22b	13.31a	56.87	60.39	83.2b
L-CMSXS165	29.95b	52.65a	33.99a	11.40a	55.19	57.88	97.4a
H-9953101	29.08b	57.90c	39.00c	17.08b	54.45	59.23	70.1c
H-9953130	26.75c	52.90a	33.94a	11.69a	52.96	58.90	72.8c
H-BR601	22.29e	58.92c	38.51c	10.28a	53.48	56.59	68.3c
H-BR701	38.73a	55.67b	38.67c	12.75a	56.49	60.84	80.5b
H-BR700	39.57a	54.31b	34.70a	11.63a	56.49	59.93	76.4b
V-BR501	25.58d	55.91b	36.90b	11.78a	53.36	58.89	62.2d
V-BR506	21.45e	62.09d	40.55c	10.65a	54.91	59.07	51.9e
H-AG2005	38.50a	53.00a	34.97a	11.19a	54.75	56.40	79.2b
CV(%)	9.91	5.34	5.22	32.31	6.05	6.06	6.38
Mean	31.23	55.35	36.55	12.12	55.22	59.01	74.2

[1] Different letters in the same column indicate significant differences between averages of the treatments, for the Scott-Knott test at 5% probability (Scott & Knott, 1974). Protein equal g/kg $^{-1}$ L = ancestry; H = hybrid; V = variety.

evaluated, with the exception of the lignin percentage (Table 1), suggesting the results obtained were trustworthy. Significant differences among the sorghum genotypes for condensed tannin concentration in the silage and panicle, percentage of DM, CP, NDF, ADF and lignin were observed, showing the existence of genetic variability in the species for these nutritional characteristics (Table 1). Rodriguez *et al*., (1999) and Gonçalves *et al*., (1999), also reported similar results. These results indicate sorghum-breeding programs aiming at improving the quality of the plants produced by these species may be successful. Average condensed tannin concentrations in silages were divided into four groups for the Scott-Knott test, varying from 1.8g CT/kg of dry matter in silage of line CMSXS165 to 10.62g CT/kg in silage of hybrid 9953130. The average concentration in panicles was 18.34 g of condensed tannin per kg of dry matter, and also formed four groups of means (Table 2). The average percentage of dry matter in silage was 31.2%, with variation amplitude of 18.13%. The genotypes were grouped in five sets for the Scott-Knott test, with hybrids BRS701, BR700 and AG2005 having higher dry matter contents.

The differences in dry matter content were related to the type of material, since lower dry matter contents were observed with sweet sorghums that have succulent culms (hybrid BR601 and varieties BR501 and BR506). Other genotypes had dry culms. Hybrids 9953101 and 9953130, as the other treatments, also had dry culms with high dry matter content. The averages of NDF, ADF and lignin were 55.3, 36.5 and 7.5%, and the genotypes divided into 4, 3 and 2 groups, respectively. CP showed five mean groups in the Scott-Knott test. The CMSXS165 line was the highest in CP percentage (9.74%) and the BR506 variety was the lowest (5.19%). The silage with more grains had higher CP, the same was observed by Moline *et al*., (2002). The in vitro digestibility averages for 24 and 48 hours varied from 55.2% to 59% and were statistically similar (P > 0,05). The results of chemical analyses agree with those on the nutritional quality of sorghum forage of Gonçalves *et al*., (1999). On the other hand, the condensed tannin in silages can form complexes with the protein fraction, reducing its degradability and consequently its availability (Van Soest, 1994). Moreover, the condensed tannin can cause cellulolytic and ureolytic bacterial enzyme inhibition (Kumar & Singh, 1984; Marinho, 1984) as well as alterations among the acids in the rumen fermentation processes (Van Hoven & Furstenburg, 1992). In fact,

TABLE 2. Percent of condensed tannin (CT) in lines, hybrids and varieties of sorghum silage and respective panicles (dry matter basis).

Treatments[1]	CT Silage[1, 2]	CT Panicle[1, 2]
L-CMSXS114	7.84 Ac	29.42 Bc
L-CMSXS165	1.80 Aa	0.26 Aa
H-9953101	10.59 Ad	37.39 Bd
H-9953130	10.62 Ab	17.11 Bb
H-BR601	1.54 Aa	1.07 Aa
H-BR701	6.29 Ac	29.19 Bc
H-BR700	9.13 Ac	28.42 Bc
V-BR501	-	-
V-BR506	-	-
H-AG2005	2.21 Aa	3.87 Aa
AVERAGE	7.59	18.34

[1] Different letters in the same line (upper case) indicate significant differences between averages of the treatments, for the Scott-Knott test at 5% probability. [2] Different letters in the column (lower case) indicate significant differences between averages of treatments for the same test.
L = ancestry; H = hybrid; V = variety.

Albrecht & Muck (1991), studying the fermentative aspects of leguminous silages, observed that condensed tannin inhibited proteolysis. The same was observed by Borges (1995) and Rodriguez *et al*. (1999). Moreover, these are sufficiently close to the results obtained for NDF, ADF and CP in corn silage by Silva *et al*., (2003). They found similar nutritional value for corn and sorghum silages. The biggest restriction to the use of sorghum for silage production is its susceptibility to bird attack when the grain is mature, reducing significantly protein percentage of the silage. The strategy to prevent this attack has been to aggregate genetic materials with higher content of condensed tannin in the grain, reducing its palatability to birds. However, doubts still exist regarding the negative effects of condensed tannin on silage's quality. In this study, we observed that the genetic materials with lower condensed tannin concentrations in the silage (L-CMSXS165, H-BR601 and H-AG2005) presented similar IVDMD in relation to the other genotypes evaluated. The L-CMSXS165 lines and H-AG2005 hybrid, for example, ranked better in the means test for almost all characteristics evaluated (Figure 1). This result, however, cannot be totally related to the effect of the condensed tannin, so the different genetic backgrounds must be involved. The only way to isolate the effect of the condensed tannin is to use isogenic lines for this characteristic, i.e. lines that only differ among themselves in relation to the presence or not of condensed tannin. Table 1 also shows that isogenics lines L-CMSXS114 and L-CMSXS165 differed greatly for condensed tannin concentrations. Lines L-CMSXS114 presented roughly four times the condensed tannin than their similar line (L-CMSXS165) in the silage and panicle respectively. For all characteristic evaluated, the two lines were statistically similar ($P < 0,05$), except for CP and ADF, showing that the process of obtaining the isogenics lines was efficient. The line with the higher condensed tannin concentration performed sufficiently close to its isogenic

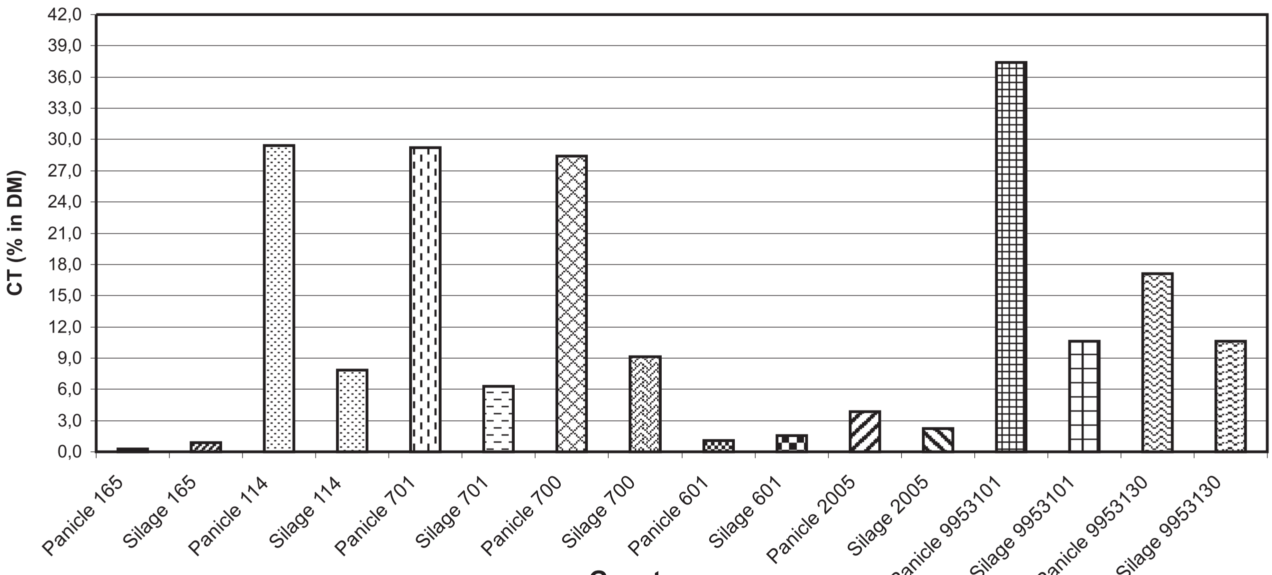

FIGURE 1. Condensed tannin concentrations (% in DM) in silage of sorghum and respective panicles of some lines, hybrids and varieties of sorghum.

line without condensed tannin, indicating that at that concentration the condensed tannin did not affect the nutritional quality of sorghum forage. These results agree with the affirmation that condensed tannin concentrations below 40 g/kg of dry matter do not interfere with the digestion, absorption and use of nutrients of silages by ruminants. Min *et al*., (2005) in their review paper, found that diets with tannin concentrations of 20 to 40 g kg^{-1} of dry matter, enhance the nutritive value, as well as the productive and sanitary performance, of grazing *Lotus pedunculatus* and *Lotus corniculatus,* improving daily weight gain and sanitary and productive aspects, probably because of higher protein availability.

The majority of genotypes available in the market has CT concentration higher than those found in the genotypes evaluated in this experiment, which are around the stipulated maximum limit, for feeding ruminants without negative effects on nutritional value. When one considers the condensed tannin concentrations in the panicle, they are still lower than the recommendation in the literature, indicating that the grain can be used as silage without great nutritional problems for ruminants. These results show that condensed tannin found at the contents was not related to lower forage digestibility. However at higher level, it is recognized as an anti-nutritional factor (Rodriguez *et al*., 1999).

To evaluate the interference of condensed tannin in the nutritional quality of sorghum silage of genotypes with high concentrations of this element, in vivo trials should be carried out with the two isogenics lines CMSXS114 and CMSXS165.

Since no interference of condensed tannin concentrations (lower than 10,62 g/kg^{-1}) was shown in the IVDMD of silages of the evaluated sorghum genotypes, they can be recommended for silage production.

Conclusions

The results show that silage of different sorghum genotypes with condensed tannin contents lower than 10,62% did not affect their in vitro dry matter digestibility and can be fed to ruminants without adverse effects as sorghum silage without condensed tannin.

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