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# Losses and nutritional value of elephant grass silage with inclusion levels of cottonseed meal

**Pablo Teixeira Viana[1*], Fábio Andrade Teixeira[2], Aureliano José Vieira Pires[2], Gleidson Giordano Pinto de Carvalho[3], Mauro Pereira de Figueiredo[4] and Hermógenes Almeida de Santana Júnior[1]**

*[1]Programa de Pós-graduação em Zootecnia, Universidade Estadual do Sudoeste da Bahia, Pç. Primavera, 40, 45700-000, Itapetinga, Bahia, Brazil. [2]Departamento de Tecnologia Rural e Animal, Universidade Estadual do Sudoeste da Bahia, Itapetinga, Bahia, Brazil. [3]Departamento de Zootecnia, Universidade Federal da Bahia, Salvador, Bahia, Brazil. [4]Departamento de Fitotecnia e Zootecnia, Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista, Bahia, Brazil. *Author for correspondence. E-mail: pabloteixeiraviana@yahoo.com.br*

**ABSTRACT.** The experiment was conducted to evaluate losses and nutritive value of elephant grass silage containing increasing levels of cottonseed meal. The experimental design was completely randomized, with five treatments: 0, 7, 14, 21 and 28% inclusion of cottonseed meal, and four replications. The material was chopped and packed in PVC silos and stored for 80 days. The pH and dry matter (DM), crude protein, lignin and ether extract of the silages increased linearly with the addition of cottonseed meal, while the levels of acid detergent insoluble nitrogen, neutral detergent fiber, acid detergent fiber, cellulose and hemicellulose decreased linearly. The addition of 28% cottonseed meal reduced the *in situ* disappearance of DM (48h) to values lower than those of silage without additives. The concentration of ammonia nitrogen and the losses from gases and effluent were reduced. The inclusion of 28% cottonseed meal in elephant grass silage containing 18.4% DM improved the fermentation characteristics of silage more efficiently by reducing the moisture content and effluent losses, starting at the 7% level of inclusion.

**Keywords:** by-product, effluent, ensiling, *Gossypium hirsutum.*

## Perdas e valor nutricional da silagem de capim-elefante com níveis de inclusão de torta de algodão

**RESUMO.** O experimento foi conduzido com o objetivo de avaliar as perdas e o valor nutritivo da silagem de capim-elefante contendo níveis crescentes de torta de algodão. O delineamento experimental utilizado foi o inteiramente casualizado, com cinco tratamentos: 0, 7, 14, 21 e 28% de inclusão de torta de algodão e quatro repetições. O material foi picado e acondicionado em silos de PVC e armazenado por 80 dias. O pH e os teores de matéria seca (MS), proteína bruta, lignina e extrato etéreo das silagens aumentaram linearmente com a adição da torta de algodão, enquanto os teores de nitrogênio insolúvel em detergente ácido, fibra em detergente neutro, fibra em detergente ácido, celulose e hemicelulose reduziram linearmente. A adição de 28% de torta de algodão reduziu o desaparecimento "in situ" da MS (em 48h) a valores inferiores à silagem não-aditivada. Foram reduzidos os teores de nitrogênio amoniacal, as perdas por gases e por efluente. A inclusão de 28% de torta de algodão na ensilagem do capim-elefante contendo 18,4% de MS melhorou as características fermentativas da silagem reduzindo de forma mais eficiente o teor de umidade e perdas por efluente a partir do nível de 7% de inclusão.

**Palavras-chave:** co-produto, efluente, ensilagem, *Gossypium hirsutum.*

## Introduction

Tropical grasses have been more and more used for silage production mainly because of their elevated dry mass productivity. One of the tropical grasses that stand out for its good characteristics for ensilage is elephant grass, which presents a high productive potential and elevated phytomass production (MACHADO et al., 1996; QUEIROZ FILHO et al., 2000); dry mass production varying between 15 and 30 ton $ha^{-1}$ year. In spite of these excellent features, at its best nutritional phase, this grass concentrates too much moisture, which can make the silage lose its quality, with high protein decomposition, in addition to big nutrient losses as effluent (FERRARI JÚNIOR; LAVEZZO, 2001).

Specifically for ensilage, the final quality of the feed is directly linked to the material that originated it (LOURES et al., 2003), so the forage to be ensiled must have a DM content above 30%. Studies involving addition of residues and by-products with elevated

DM contents to high-moisture forages at the moment of ensilage have been proposed for the production of silages of good nutritional value and adequate fermentation profile (CARVALHO et al., 2007a and b).

The cottonseed cake, a by-product of the extraction of the cottonseed oil, has a good nutritional value, mainly because of its high protein content, thereby being largely utilized in dairy and beef cattle farming. In addition to its good nutritional value, this by-product also has a high DM content, which makes it an alternative for use in ensilage of high-moisture grasses in order to reduce the effluent losses and improve the quality of the material.

Information in the literature regarding the use of cottonseed cake in the ensilage of elephant grass is very scarce. Because it is a feed of low cost and high availability in certain Brazilian regions, more research should be conducted in order to estimate the ideal levels of this material in the ensilage of tropical grasses.

This experiment was carried out to evaluate fermentation characteristics and nutritional value of elephant grass silage ensiled with crushed cottonseed cake levels.

## Material and methods

An experiment was carried out at the Forage and Pasture Laboratory of Southwest Bahia State University, Campus Itapetinga, Bahia State, Brazil, from December 5, 2009 to February 25, 2010. The grass utilized for ensilage was elephant grass *(Pennisetum purpureum* Schum. cv. Napier), originally from a previously established grassland, presenting 18.4% DM and having cottonseed cake as additive. The experimental design was completely randomized, with five treatments and four replications. The elephant grass was cut manually at 70 days of regrowth and chopped in a stationary mower to an average particle size of 2 cm. The cottonseed cake was acquired from *Indústria e Comércio de Rações e Óleos Vegetais Ltda* (ICOL), located in the municipality of Guanambi, Bahia State, Brazil. The cake was processed in a hammermill with a 5 mm screen sieve and then mixed with the freshly chopped forage at four inclusion levels (7, 14, 21 and 28%) on an as-is basis (weight weight$^{-1}$), plus a treatment without inclusion of the cake (control).

We used twenty 50 x 10 cm (height x diameter) experimental silos made of PVC, provided with a Bunsen valve, with sand and screen at the bottom. Silos were previously weighed to capture the effluent released by the material. The ensiled mass was compressed by adopting the specific mass of 600 kg natural matter cm$^{-3}$; next, silos were sealed and weighed. The nutritional composition of the elephant grass and cottonseed cake used for the ensilage is shown in Table 1.

**Table 1.** Nutritional composition of elephant grass and cottonseed cake.

| Nutrient | Elephant grass | Cottonseed cake |
|---|---|---|
| Dry matter (%) | 18.4 | 95.2 |
| Crude protein[1] | 6.9 | 25.3 |
| Neutral detergent fiber[1] | 80.4 | 54.2 |
| Acid detergent fiber[1] | 47.2 | 38.5 |
| Neutral detergent insoluble nitrogen[2] | 30.4 | 8.6 |
| Acid detergent insoluble nitrogen[2] | 14.5 | 7.6 |
| Cellulose[1] | 39.1 | 29.5 |
| Hemicellulose[1] | 33.2 | 15.7 |
| Ether extract[1] | 4.7 | 14.6 |
| Lignin[1] | 6.3 | 9.2 |
| *In situ* DM disappearance (in 48h)[1] | 45.5 | 43.4 |

[1]% of DM - dry matter. [2]% of total nitrogen.

After 10 days of storage, the material was weighed again for evaluation of gas losses, caused by fermentations occurring inside the silo. After, silos were opened for removal of silage and to weigh the sand, which was previously conditioned at the bottom of the silo for determination of effluent losses, in accordance with the methodology described by Jobim et al. (2007). The material collected in the silos was homogenized and divided in two samples: one portion, immediately after opening the silos, was used for estimation of ammonia nitrogen ($N-NH_3/TN$) and pH, according to the methodology of Bolsen et al. (1992); and the other part of the samples was pre-dried in an oven for 72 hours at 60ºC. Samples were subsequently ground in a Wiley mill of 1 mm screen sieve for analyses of dry matter (DM), crude protein (CP), neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose and hemicellulose, according to the procedures described in Silva and Queiroz (2005).

The *in situ* dry matter disappearance in 48 hours (DMD/48 hours) was estimated by incubating samples of silages in a rumen-fistulated Holstein x Zebu crossbred steer with average body weight of 250 kg. Samples were ground in 2 mm screen sieves and conditioned in TNT bags at the rate of 0.5 g bag$^{-1}$, which were inserted in the rumen with a rumen fistula, attached to a steel chain and kept for 48 hours, according to the NRC (2001). Next, bags were removed and washed in running water until it showed clear; subsequently, bags were dried in an oven at 60ºC for 72 hours to estimate the DM. *In situ* dry matter disappearance in 48 hours was obtained by the difference of weight before and after rumen incubation, expressed in percentage.

Results were interpreted by variance and regression analyses, at 5% probability, with software SAS (Statistical Analysis System) (RIBEIRO JÚNIOR, 2001).

## Results and discussion

According to the visual aspect and the odor, and because no putrefaction was observed in any of the silages, we can infer that the material ensiled was satisfactorily fermented.

Effluent losses decreased (Table 2) as the cottonseed cake was included in the elephant grass, and because effluent carries nutrients, this means reduction in the nutritional value of silages. Inclusion of 28% of the cake showed almost total absorbance of the effluent produced, compared to the non-enriched silage. The high DM content of the cake utilized at the moment of ensilage was certainly what contributed to reduction of effluent losses. These results are in accordance with those found by Teixeira et al. (2008), who, adding cocoa meal and sugarcane to elephant grass at the moment of ensilage, reduced the dry matter losses and consequently effluent losses, recommending up to 22.4% sugarcane, with 29% DM, provided it was in association with 15% cocoa meal. Bernardino et al. (2005) concluded that levels above 20% coffee husks were enough to eliminate all the production of effluent in elephant grass silages.

Regarding gas losses in the silages studied, they decreased linearly among the silages as the cottonseed cake levels were elevated. According to Pedroso et al. (2007), loss of soluble carbohydrates as gas during fermentation also results in production of water, decreasing the DM content of the forage. Ensilage of younger grasses favors the development of deteriorating microorganisms, especially clostridial bacteria, which produce ammonia nitrogen and butyric acid because of the high moisture (above 70%) and low soluble carbohydrates (lower than 5% on a DM basis) (PENTEADO et al., 2007; ZANINE et al., 2006). Thus, cottonseed cake can be an alternative for inclusion in the ensilage of elephant grass when one aims to reduce moisture and production of gases inside the silo.

Significant effect was observed on pH values. The elevation in crude protein contents might have contributed to these pH values observed. The results found in the present study were between 3.5 and 4.5, similar to the 4.1 found by Teixeira et al. (2008), who added cocoa meal, and close to the 3.8 to 4.2 reported by McDonald (1981) as ideal for a silage of good fermentation quality in the silo. Soluble carbohydrates presents in the grasses are in fact the ones, in part, responsible for the pH values in the silage; they decrease as the DM of the grass is elevated at the moment of ensilage.

**Table 2.** Mean values, coefficient of variation (CV) and regression equations for pH, gas loses (GL), effluent losses (EL), ammonia nitrogen (N-$NH_3$/TN) and dry matter recovery (DMR) of elephant grass silages with cottonseed cake inclusion levels.

| Item | Cottonseed cake levels (% as is) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | Mean | CV[1] | Equation |
| pH | 3.5 | 3.7 | 3.7 | 4.1 | 4.0 | 3.8 | 4.1 | 1 |
| EL (kg $ton^{-1}$ as is) | 142.5 | 77.7 | 31.6 | 11.1 | 1.5 | 52.8 | 12.3 | 2 |
| GL (% DM) | 3.7 | 2.1 | 1.6 | 1.8 | 1.5 | 2.1 | 25.3 | 3 |
| N-$NH_3$/TN (%) | 5.8 | 8.4 | 6.8 | 5.7 | 4.5 | 6.2 | 12.6 | 4 |
| DMR (%) | 73.2 | 80.5 | 77.1 | 77.3 | 86.1 | 78.8 | 7.3 | 5 |

[1]CV – Coefficient of variation (%). 1 – $\hat{Y}$ = 3.55150 + 0.0210357X ($r^2$ = 0.83); 2 – $\hat{Y}$ = 122.656 – 4.98195X ($r^2$ = 0.89); 3 – $\hat{Y}$ = 3.04831 – 0.647893X ($r^2$ =0.88); 4 – $\hat{Y}$ = 7.73668 – 0.541541 X ($r^2$ = 0.84); 5 – $\hat{Y}$ = 74.3600 + 0.321030X ($r^2$ =0.83).

For ammonia nitrogen (N-$NH_3$/TN), values decreased linearly as the cottonseed cake was increased, with an average 6.2%. Given that N-$NH_3$ is a product of fermentations, especially clostridial fermentations, it is worth remarking that at all cottonseed cake inclusion levels the silages had satisfactory N-$NH_3$/TN contents, below 12%, which is the upper limit to classify a silage as of good quality according to McDonald (1981), thereby demonstrating that the silages studied herein showed desirable fermentations. McDonald et al. (1991) suggested that to be considered as having good quality, silages should present an N-$NH_3$/TN content of 10% at the maximum. Higher values (12.8% N-$NH_3$/TN) were found by Santos et al. (2008) in elephant grass with inclusion of 15% jackfruit. Working with elephant grass silages with inclusion of cocoa meal, Teixeira et al. (2008) found ammonia nitrogen values within the ideal range: an average 3.9% total N, which is lower than the values found in the present study.

Inclusion of cottonseed cake linearly increased the dry matter recovery rate, indicating that the elevation in the DM content promoted by cottonseed cake prevented the material ensiled from losing more DM. The DM losses estimated at 26.8, 19.5, 22.9, 22.7 and 13.9, for treatments 0, 7, 14, 21 and 28%, respectively, can be considered high, if compared with the 4.6% of DM losses found by Teixeira et al. (2008), who included cocoa meal in elephant grass silage.

Along with ammonia nitrogen, the pH value can indicate the status of the fermentation of the material during the storage period. The reduction in ammonia nitrogen concentration with inclusion of cottonseed cake can be explained by the decrease in DM intake, which probably reduced the activity of bacteria of the genus *Clostridium*, which promote proteolysis and release of ammonia nitrogen during the ensilage process. We can infer that cottonseed cake improved the fermentation characteristics of the silage, since along with pH it kept the ammonia nitrogen within the acceptable range - between 4.5 and 8.4, respectively.

The DM, CP, NDIN, ADIN and EE contents of diets were significantly changed by the inclusion levels of cottonseed cake used at the ensilage (Table 3). Dry matter and crude protein increased linearly as the cottonseed cake levels in the ensilage were elevated; from 14% inclusion of cottonseed cake, the percentage of silage dry matter was above the threshold of 25%, considered as the minimum required to keep it at the appropriate level for ensilage of tropical grasses. The level of 14% cottonseed cake inclusion allowed us to obtain a silage with 33.3% DM, which is close to the 30% reported by McDonald (1981) as the minimum for production of good-quality silage. The high DM content reported in our study can be attributed to the elevated DM in the nutritional composition of cottonseed cake (Table 1).

The results obtained in the present study were similar to those found by Carvalho et al. (2007a), who also observed increase in the DM and CP contents of silages when they added four levels of cocoa meal (7, 14, 21 and 18%) to the elephant grass silage. Given that the additive utilized by these authors showed similar DM content (90%) to the cottonseed cake (95.2%) used in the present experiment, we can infer that the DM and CP contents of these by-products were the main responsible for the increase in these variables. These values were also similar to those found by Rezende et al. (2008), who evaluated elephant grass silage enriched with potato chips with 86.13% DM at the moment of ensilage. The CP values found in this study were higher than the ones observed by Santos et al. (2008) with inclusion levels of jackfruit (5, 10, and 15%) in a silage of the same grass. This higher increase in CP with cottonseed cake can be attributed to the higher CP content (25.3%) as compared with potato chips (12.3%).

Acid detergent insoluble nitrogen in percentage of total nitrogen decreased linearly ($p < 0.05$), presenting 18.2, 12.4, 12.6, 12.3 and 11.3% for absence and inclusion of 7, 14, 21 and 28% cottonseed cake, respectively (Table 3). From a nutritional standpoint, reduction in this chemical component indicates that in a ruminant feeding system, the protein from cottonseed cake is highly digestible. Considering that ADIN corresponds to the proteins associated with lignin, tannin-protein complexes and products derived from the Maillard reaction, it is highly resistant to microbial enzymes and indigestible through the gastrointestinal tract (LICITRA et al., 1996). Different results were found by Carvalho et al. (2007b), who, studying absence and inclusion of coffee husks (6, 12, 18 and 24%) in the ensilage of elephant grass, observed linear increase in ADIN contents of the silages produced.

**Table 3.** Mean contents of dry matter, crude protein, neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN) and ether extract of elephant grass silages with cottonseed cake inclusion levels.

| Nutrient | Cottonseed cake levels (% as is) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | Mean | CV[3] | Equation |
| Dry matter | 22.0 | 25.9 | 33.3 | 36.4 | 39.9 | 31.1 | 8.3 | 1 |
| Crude protein[1] | 6.0 | 10.7 | 13.8 | 17.0 | 19.0 | 13.3 | 5.0 | 2 |
| NDIN[2] | 25.7 | 20.1 | 23.9 | 20.7 | 17.4 | 21.6 | 13.2 | 3 |
| ADIN[2] | 18.2 | 12.4 | 12.6 | 12.3 | 11.3 | 13.3 | 12.1 | 4 |
| Ether extract[1] | 6.2 | 9.9 | 12.3 | 11.8 | 14.0 | 10.8 | 9.0 | 5 |

[1/]% of DM; [2/]% of total nitrogen; [3/]CV – coefficient of variation (%). 1 - $\hat{Y} = 22.2785 + 0.659431X$ ($r^2 = 0.98$); 2 - $\hat{Y} = 6.93379 + 0.456960X$ ($r^2 = 0.98$); 3 - $\hat{Y} = 24.4742 - 0.131292X - 0.00344211X^2$ ($R^2 = 0.85$); 4 - $\hat{Y} = 16.1809 - 0.200129X$ ($r^2 = 0.88$); 5 - $\hat{Y} = 7.40302 + 0.248176X$ ($r^2 = 0.78$).

The lowest NDF content obtained in the silage was 66.1% for the highest level of cake inclusion and the maximum, 79.6%, was found in the silage with elephant grass only (Table 4). The percentages of NDF observed in the silages are above the recommended by Van Soest (1994) not to inhibit DM intake and digestibility. The author also reports that the increase in NDF content is negatively correlated with ingestion of feed, and for the forage the limit would be close to 55 and 60%. These results were similar to those found by Rodrigues et al. (2005), who observed linear decrease in NDF as the citrus pulp was added to the elephant grass silages, and by Freitas et al. (2006), who also verified lower concentration of fiber components in the treatments with inclusion of soy residue in the silages of this grass. These results can be explained by the lower NDF content of cottonseed cake (54.2%) in relation to the elephant grass, and especially by the lower effluent production observed. The reduction in NDF content of silages can contribute to animal feeding and to increase in DM intake.

Acid detergent fiber also followed the same behavior observed for NDF, reducing linearly as the cake was added to the elephant grass silage. The increase in ADF content can interfere with digestibility of the feed, so the nutritional composition of grass silages enriched with by-products so as to improve its quality will be directly dependent, besides the grass, on the product utilized.

**Table 4.** Neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose and lignin contents of elephant grass silages with cottonseed cake inclusion levels.

| Nutrient | Cottonseed cake levels (% as is) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | Mean | CV[2] | Equation |
| NDF[1] | 79.6 | 74.6 | 73.2 | 72.1 | 66.1 | 73.1 | 3.8 | 1 |
| ADF[1] | 48.3 | 47.0 | 45.9 | 44.5 | 43.2 | 45.7 | 1.7 | 2 |
| Cellulose[1] | 39.1 | 36.5 | 35.2 | 34.6 | 33.1 | 35.7 | 1.5 | 3 |
| Hemicellulose[1] | 31.3 | 28.6 | 27.3 | 24.7 | 23.0 | 26.8 | 3.3 | 4 |
| Lignin[1] | 7.1 | 8.4 | 9.3 | 9.4 | 9.7 | 8.7 | 6.3 | 5 |

[1]% of DM; [2]CV – coefficient of variation (%). 1 - $\hat{Y} = 79.4212 - 0.434468X$ ($r^2 = 0.95$); 2 - $\hat{Y} = 48.3541 - 0.183503X$ ($r^2 = 1.00$); 3 - $\hat{Y} = 38.4844 - 0.200105X$ ($r^2 = 0.95$); 4 - $\hat{Y} = 31.0671 - 0.292834X$ ($r^2 = 0.99$); 5 - $\hat{Y} = 7.53281 + 0.0878073X$ ($r^2 = 0.85$).

On the other hand, in this study, the lignin contents increased linearly along with the cottonseed cake levels, which can be attributed to the higher lignin content in the cake (9.2%) added during ensilage in comparison with elephant grass, with 6.3% of lignin (Table 1). A factor that contributes to the high lignin content of the cottonseed cake is the presence of the cotton seed coat, raw matter for production of the cake. Cellulose and hemicellulose contents reduced linearly as the cottonseed cake levels were included in the elephant grass silage (Table 4).

Addition of cottonseed cake levels (0, 7, 14, 21 and 28% on an as-is basis) had no effect on *in situ* dry matter disappearance (DMD/48 hours), which presented similar values to the non-enriched silage (44.5, 45.3, 45.7, 46.9 and 43.3% in DM), respectively. This can be explained by the low NDF content of this ingredient, 54.2%, in relation to the 80.4% found in elephant grass. This value obtained by the authors can be considered satisfactory given the high neutral detergent fiber concentration found in the elephant grass, close to 62 and 71.4% (CARVALHO et al., 2007b).

## Conclusion

At inclusion levels starting from 7%, cottonseed cake is an efficient additive, reducing the moisture level and effluent losses in elephant grass silages.

Cottonseed cake in the ensilage of elephant grass containing 18.4% dry matter improves the fermentation quality of the silages, increasing crude protein and ether extract contents and reducing acid detergent insoluble nitrogen.

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