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Fermentative losses and chemical composition of elephant grass silage added with castor bean hull[1]

Perdas fermentativas e composição química de silagens de capim-elefante aditivadas com casca de mamona

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ABSTRACT - Fermentation and chemical composition of elephant grass silage was evaluated with 0, 10, 20 and 30% castor been hull based on the natural matter. The experimental design was completely randomized with five replications. Silos were weighed out during manufacture, and reweighed before and after the opening after storage for 30 days. We determined the chemical composition, specific mass and effluent and gas losses, dry matter recovery, pH and ammonia nitrogen of the silage. The specific mass, pH, dry matter content, crude protein, acid detergent fiber, total carbohydrates and cellulose increased linearly (P<0.05) with the inclusion of castor bean hull. Quadratic effect was observed for effluent and gas losses, ammonia nitrogen and non-fibrous carbohydrates with minimal point when the castor bean hull was included at 25.63; 31.19; 28.07 and 11.7%, respectively. Dry matter recovery and neutral detergent fiber adjusted to a quadratic equation with maximum point when the castor bean hull was added at 24.20 and 20.62%, respectively. The inclusion of castor bean hull in elephant grass silage reduces losses during fermentation without compromising the chemical composition when included in the ratio of up to 25%, based on the natural matter.

Key words: Biodiesel. Byproduct. Forage conservation. Moisture absorbent. *Ricinus communis* L..

RESUMO - A fermentação e a composição química da silagem de capim-elefante foram avaliadas com 0; 10; 20 e 30% de casca de mamona com base na matéria natural.O delineamento experimental foi inteiramente casualizado com cinco repetições. Os silos foram pesados durante a confecção dos mesmos, sendo novamente pesados antes e após a abertura, depois de serem armazenados por 30 dias. Determinou-se a composição química, massa específica, perdas por efluente e gases, recuperação da matéria seca, pH e nitrogênio amoniacal da silagem. A massa específica, pH, teor de matéria seca, proteína bruta, fibra em detergente ácido, carboidratos totais e celulose aumentaram linearmente (P<0,05) com a inclusão da casca de mamona. Efeito quadrático foi observado para as perdas por gases e efluentes, nitrogênio amoniacal e carboidratos não fibrosos com ponto de mínima quando a casca de mamona foi incluída em 25,63; 31,19; 28,07 e 11,7%, respectivamente. Já a recuperação de matéria seca e teor de fibra em detergente neutro ajustaram-se à equação quadrática com ponto de máxima quando a casca de mamona foi incluída em 24,20 e 20,62%, respectivamente. A adição de casca de mamona na ensilagem de capim-elefante reduz as perdas durante a fermentação sem comprometer a composição química quando incluída na proporção de aproximadamente 25%, com base na matéria natural.

Palavras-chave: Biodiesel. Subproduto. Conservação de forragem. Absorvente de umidade. *Ricinus communis* L..

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INTRODUCTION

Animal production in semi-arid regions is strongly influenced by rainfall regime because of its irregularity and when associated with years of low rainfall, restricts the supply of forage food. Thus, the conservation of the surplus forage production in the rainy season becomes a fundamental strategy for the adequate feeding planning of herds. Ensiling is one of the alternatives for forage conservation, in which elephant grass (*Pennisetum purpureum* Schum.) is among the most commonly used grasses for this purpose.

As with most tropical grasses, elephant grass presents high moisture (dry matter less than 30%) at the phenological stage most suitable for use in the ensiling process (FERREIRA *et al*., 2010). This causes increased losses during the fermentation process, inhibits adequate fermentation, as well as produces a high amount of effluents, which carry the nutrients, reducing its nutritive value (MCDONALD; HENDERSON; HERON, 1991).

One of the solutions to inhibit fermentative losses in the ensiling of grasses with high moisture content is the use of additives with high proportion of dry matter (SILVA *et al*., 2011). Several additives have already been tested, including dried passion fruit peel (CRUZ *et al*., 2010), rice bran and soybean hull (MONTEIRO *et al*., 2011), coffee hull, cocoa meal or cassava meal (PIRES *et al*., 2009). In this sense, the use of byproducts from the biodiesel industry, which has already been evaluated in animal feed, can contribute to improving the fermentation process of grass silage, besides adding value and increasing the competitiveness of these oilseed plants.

Among the crops studied, stands out the castor bean (*Ricinus communis*) with good results the use of the cake (GOMES *et al*., 2011) and castor bean meal (VIEIRA *et al*., 2010) in ruminant feed. In the case of castor bean hull, its nutritional limitations have restricted its percentage of use in feed (SANTOS *et al*., 2011).

The castor bean hull represents on average 25% of the weight of the fruit and the remaining 75% correspond to the weight of the seed (POMPEU *et al*., 2013). Often the process of seed extraction happens in the farm generating large amount of hull that has been used basically as fertilizer. Its use in animal feed is limited by its chemical composition, with high fiber content (72%), low crude protein content (5.0 to 8.0%) and presence of ricinoleic acid that reduces intake (POMPEU *et al*., 2013; SANTOS *et al*., 2011). Although its composition varies according to the presence of seed fragments in the hull.

Reductions in intake and performance in sheep fed diets where Tifton 85 grass hay has been replaced by different proportions of castor bean hull were reported by several authors (ANDRADE *et al*., 2013; SANTOS *et al*., 2011). On the other hand, Pompeu *et al*. (2013) recommended the substitution of Tifton 85 grass hay for castor bean hull up to 33%, showing a depression in carcass and non-carcass traits from this value.

The use of castor bean hull as a moisture-absorbing additive in elephant grass silage may be an alternative to make this byproduct more attractive in animal nutrition. Thus, this study was conducted with the objective of evaluating the effects of including different proportions of castor bean hull on the fermentative characteristics and chemical composition of elephant grass silage.

MATERIAL AND METHODS

The research was conducted from April to September 2015 in the Forage Sector of the Department of Animal Science, CCA/UFC, Fortaleza, State of Ceará. The municipality of Fortaleza is located in the coastal zone at 15.49 m altitude, 3º43’02” South latitude, and 38º32’35” West longitude with Aw, tropical rainy climate, according to Köeppen classification.

This was completely randomized experimental design with four treatments, corresponding to four proportions of castor bean hull (0, 10, 20 or 30%) included in the silage of elephant grass cv. Roxo (*Pennisetum purpureum* Schum) based on the natural matter of the ensiled mass, with five replicates (mini silos).

The elephant grass came from an existing an area on the campus of the Federal University of Ceará and was cut close to the ground after 70 days of regrowth. The plants were processed in a forage harvester adjusted to produce particles from 2.0 to 3.0 cm in size.

The castor bean hull was obtained from a castor bean plantation of the UFC in an area adjacent to the elephant grass area. During the separation of the seed from the hull, all the fragments of seeds were removed, guaranteeing 100% purity of the hull. For the preparation of silages, the castor bean hull was crushed in a hammer mill with a 5.0 cm sieve.

Twenty experimental silos with a diameter of 19.4 cm and a height of 15.0 cm were used, which were sealed with adapted cap with a Bunsen valve to allow the escape of gases from the fermentation. Prior to forage placement, 1000 g of inert absorbent material (sifted sand) were added to each silo, separated from the forage by a cotton cloth, thus allowing the absorption of the produced effluent. The elephant grass was weighed and homogenized with the castor bean hull and later compacted inside the silo with wooden sticks until reaching a specific mass of 600 kg m^{-3}.

Silos were opened 30 days after sealing, and the upper and lower thirds of the ensiled mass were discarded. Samples of elephant grass and castor bean hull during the ensiling process (Table 1) and two samples of the silages after the opening were collected. A sample of each elephant grass silage and another of the castor bean hull were weighed and dried in a forced ventilation oven at 55 ºC to constant weight. The samples were weighed for determination of the pre-dried matter, processed in a 1.0 mm mesh mill and stored for analysis of dry matter (DM), ether extract (EE), total nitrogen (multiplying by 6.25 to obtain the crude protein content, CP), neutral and acid detergent fiber (NDF, ADF), hemicellulose, cellulose and lignin according to the methodologies compiled by Silva and Queiroz (2002). Total carbohydrates (TC) and non-fibrous carbohydrates (NFC) were calculated according to Sniffen *et al.* (1992) and Weiss (1999), respectively. The second sample of each silage was used to measure pH and ammonia nitrogen content (NH_3-N), according to the methodologies described in Silva and Queiroz (2002).

The specific mass (SM), gas (GL) and effluent (EL) losses, DM recovery index (DMR) were quantified by weight difference, according to the methodologies of Jobim *et al.* (2007). SM, GL, EL and DMR were obtained by equations 1, 2, 3 and 4, respectively.

$$SM\ (kg\ DM\ m^{-3}) = (GMf \times \%DMf/100)/Vs \quad (1)$$

$$GL\ (\%\ DM) = [(WSSc - WSSo)/(GMf \times \%DMf/100)] \times 100 \quad (2)$$

$$EL\ (kg\ ton^{-1}) = [((WSSo - GMs) - (WSSc - GMf)) / GMf] \times 1000 \quad (3)$$

$$DMR\ (\%) = [(GMf \times \%DMf)/(GMs \times \%DMs)] \times 100 \quad (4)$$

where: *GMf* = green mass of the forage (kg); *%DMf* = dry matter content of the forage (%); *V*s = Silo volume (m³); *GL* = gas losses; *WSSc* = weight of silo sealed up on closing (kg); *WSSo* = weight of silo sealed up on opening (kg), *EL* = effluent losses (kg ton^{-1}); *GMs* = green mass of the silage (kg); *DMR(%)* = DM recovery index;*% DMs* = dry matter content of the silage (%).

Statistical analysis was run using analysis of variance, polynomial regression and Pearson correlation. The selection of the models was based on the significance of the linear or quadratic coefficients, using Student's t-test at 5% probability. As an aid to statistical analysis, we used the GLM procedure of the SAS package (STATISTICAL ANALYSIS SYSTEM, 2003).

RESULTS AND DISCUSSION

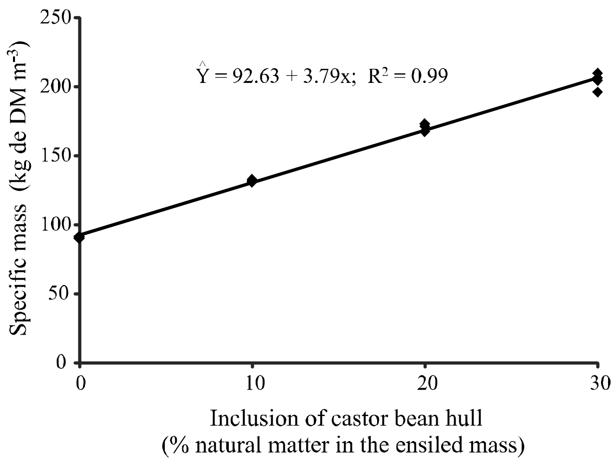
The inclusion of castor bean hull in elephant grass silage based on the natural matter increased linearly ($P<0.05$) the specific mass (SM) expressed in kg dry matter per cubic meter (kg DM m^{-3}), with observed values of 90.83; 131.73; 171.30 and 204.20 kg DM m^{-3}. Each percentage point of castor bean hull added increased SM in 3.79 kg DM m^{-3} (Figure 1). This result is due to variations in dry matter composition between castor bean hull (851.32 g kg^{-1}) and elephant grass (153.81 g kg^{-1}). According to Holmes and Muck (1999), a good fermentation of corn silages with few fermentative losses is obtained with SM value of 225 kg DM m^{-3}. However, this value is hardly reached in tropical grass silages (JOBIM *et al*., 2007), such as elephant grass, poor in DM

Table 1 - Chemical composition of elephant grass and castor bean hull

Variables	Elephant grass	Castor bean hull
Dry matter[1]	153.81	851.32
Organic matter[2]	933.52	945.68
Crude protein[2]	57.96	57.31
Ether extract[2]	17.41	5.76
Neutral detergent fiber[2]	633.21	686.32
Acid detergent fiber[2]	334.27	434.05
Hemicellulose[2]	298.94	252.27
Total carbohydrates[2]	858.15	882.61
Non-fibrous carbohydrates[2]	224.94	196.29
Cellulose[2]	334.30	434.10
Lignin[2]	24.00	28.50

[1]g kg^{-1} natural matter; [2]g kg^{-1} dry matter

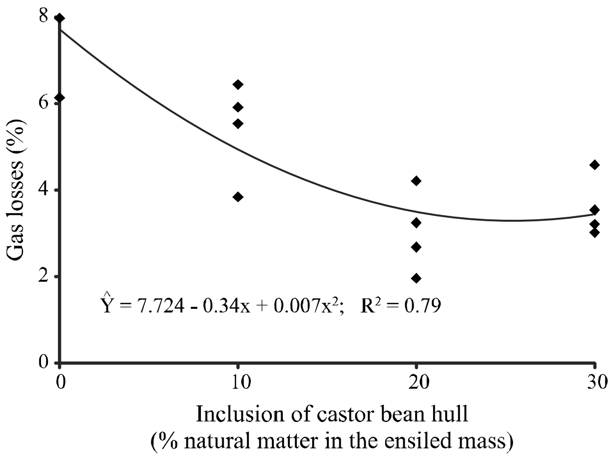
Figure 1 - Specific mass of elephant grass silages with castor bean hull



at the growth stage in which there is a balance between production and nutritive value.

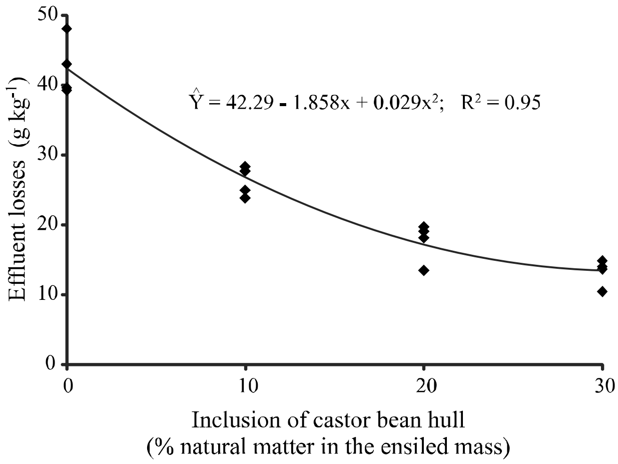
Gas losses (GL) were influenced (P<0.05) by the proportion of castor bean hull (Figure 2). It was observed a quadratic response with a minimum estimate of 3.29% losses with 25.63% of castor bean hull. This result is related to the environment and the type of fermentation occurred in silages with lower proportions of castor bean hull, characterized by the higher moisture content, providing more favorable conditions for clostridial bacteria and enterobacteria that are gas producers and generators of silages with inadequate fermentation (MCDONALD; HENDERSON; HERON, 1991; SILVA *et al*., 2011) and with low DM content. Amaral *et al.* (2007), working with different compactions to obtain different specific masses (100, 120, 140 and 160 kg DM m^{-3}) in ensiling marandu palisadegrass, verified higher intensities of gas production in silages produced with lower specific masses (100 and 120 kg DM m^{-3}), similar to that observed in this study.

Figure 2 - Gas losses of elephant grass silages with castor bean hull



Effluent losses (EL) (P<0.05) decreased in a quadratic manner with the inclusion of castor bean hull (Figure 3), with minimum values of EL with the inclusion of 31.19% castor bean hull. This result is related to the high DM content of castor bean hull (851.32 g kg^{-1}) and shows that castor bean hull is an additive with good moisture absorption capacity. This is reinforced by the high negative correlation between DM content and EL (r=-0.94, p<0.0001). Several studies in the literature confirm that the use of moisture-absorbing additives reduces the production of effluents in grass silages with low DM content (ANDRADE *et al*., 2012; FARIA *et al*., 2010; RIBEIRO *et al*., 2014). In a study by Andrade *et al.*

Figure 3 - Effluent losses of elephant grass silages with castor bean hull



(2010), the authors found a reduction in effluent production when coffee hull were included in different proportions (0, 10, 20 and 30%, based on the natural matter) during ensiling of elephant grass, with a lower value of EL in the proportion of 30% coffee hull. Ribeiro *et al.* (2014) observed a linear reduction in EL when castor bean cake was used up to 18% in elephant grass silage.

As the castor bean hull was included, there was a quadratic response (P<0.05) for the dry matter recovery index (DMR) with a maximum value of 99.38% with inclusion of 24.20% castor bean hull (Figure 4). The reduction in effluent and gas losses and consequent increase in DMR with the inclusion of castor bean hull shows the occurrence of reduced activity of heterofermentative and proteolytic bacteria that cause losses during the fermentation process. This evidences the efficiency of the castor bean hull in minimizing the losses of DM during the fermentation of the elephant grass in the silo throughout the fermentation, being attributed to the increase in DM and SM of the silages with the inclusion of castor bean hull. The efficiency of moisture absorbing additives in DMR has been demonstrated in several studies. Zanine *et al*. (2010) analyzed the inclusion of cassava scrape during elephant grass ensiling, and obtained higher DMR (94.34%) when adding 14.58% cassava scrape, showing that moisture-absorbing additives are efficient in improving DMR.

The pH increased linearly (P<0.05) with the inclusion of castor bean hull, presenting values of 3.53 and 4.54, at levels 0 and 30%, respectively (Figure 5). Each percentage point of castor bean hull included in the elephant grass silage increased the pH by 0.03. This result was similar to that obtained by Pires *et al.* (2004), who observed a higher pH value (4.6) when adding 15% coffee hull to elephant grass silage when compared to

Figure 4 - Dry matter recovery index of elephant grass silages with castor bean hull

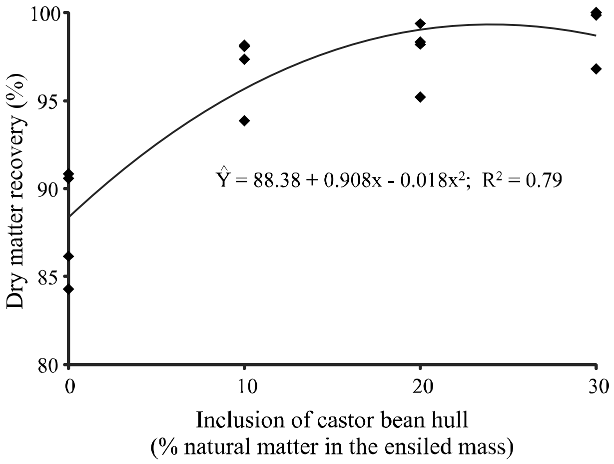
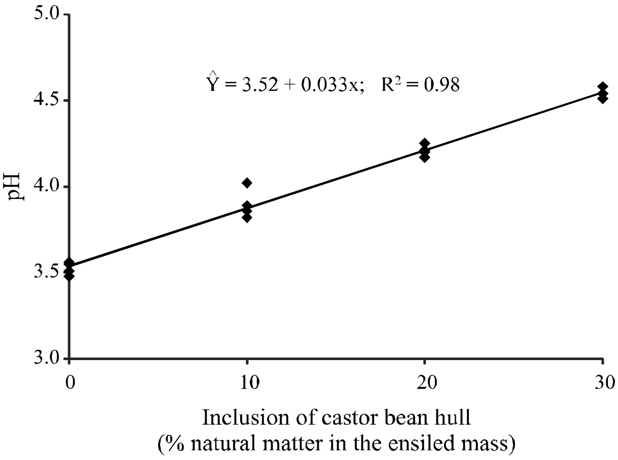


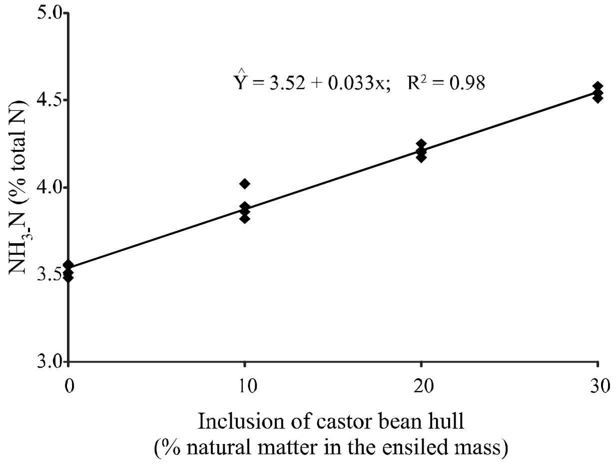
Figure 5 - Values of pH of elephant grass silages with castor bean hull



silage without the additive (4.2). In the present study, this difference is possibly due to the limited contribution of the castor bean hull with soluble carbohydrates for the fermentation process, making it difficult to reduce the final pH of the silage. In addition, the final pH value of the silage cannot be considered as the sole criterion for evaluation of the fermentation. Other variables need to be considered since the inhibition of secondary fermentations depends more on the pH decline rate, ionic concentration and moisture of the medium than on the final pH (WOOLFORD, 1984).

The inclusion of castor bean hull yielded a quadratic response ($P<0.05$) for NH_3-N contents of silages with a minimum estimated value of 6.51% total N with 28.07% castor bean hull (Figure 6). This result can be attributed

Figure 6 - Ammonia nitrogen (NH_3-N) of elephant grass silages with castor bean hull



to the elevation of DM of the silages that reduces the proliferation of *Clostridium* bacteria, which are the main responsible for the proteolysis of protein compounds (MOTA *et al*., 2011). The elephant grass silage with 0% inclusion of castor bean hull showed a NH_3-N value above 12%, a value reported by McDonald, Henderson and Heron (1991) as characteristic of low quality silage with high crude protein degradation, indicating this grass requires some additive to minimize losses of nitrogen compounds. Nevertheless, in silages with proportions of 10, 20 or 30% castor bean hull, this variable had values lower than 10% indicating adequate fermentation process and low hydrolysis of proteins and nitrogen compounds (VAN SOEST, 1994).

The regression analysis showed a linear effect ($P<0.05$) of castor bean hull proportions on the DM content of the silages, with values of 144.38 and 375.13 g kg^{-1} in the proportions of 0 and 30%, respectively (Table 2). Each percentage point of added castor bean hull increased the DM content by 7.69 g kg^{-1}, proving that the castor bean hull is efficient in raising the DM content of the silages as a consequence of the disparity between DM contents of the elephant grass (153.81 g kg^{-1}) and the castor bean hull (851.32 g kg^{-1}). According to McDonald, Henderson and Heron (1991), good quality silages have a DM content between 30 and 35%. In the present study, these contents were obtained by including 20.24% and 26.74% of castor bean hull in elephant grass silage.

Despite the similarity in crude protein (CP) content between elephant grass (57.96 g kg^{-1} DM) and castor bean hull (57.31 g kg^{-1} DM), the CP content of the silages increased ($P<0.05$) with the inclusion of castor bean hull, estimating increases of 0.47 percentage points for each 1% castor bean hull added. Positive correlation between

Table 2 - Chemical composition of elephant grass silages added with castor bean hull

Variables	Inclusion of castor bean hull (% NM)				Equation	R^2
	0	10	20	30		
Dry matter[1]	143.03	225.15	294.58	376.28	$\hat{Y} = 144.38 + 7.69x$	0.99
Crude protein[2]	56.28	63.88	64.49	71.72	$\hat{Y} = 57.03 + 0.47x$	0.83
Ether extract[2]	24.68	16.51	12.08	8.60	$\hat{Y} = 23.38 - 0.53x$	0.94
NDF[2]	661.55	669.88	685.28	674.40	$\hat{Y} = 659.88 + 1.98x - 0.05x^2$	0.62
ADF[2]	399.05	427.45	450.72	465.24	$\hat{Y} = 402.36 + 2.22x$	0.94
Hemicellulose[2]	262.49	242.43	234.52	209.17	$\hat{Y} = 262.33 - 1.68x$	0.88
Cellulose[2]	349.55	372.51	391.19	407.78	$\hat{Y} = 351.25 + 1.93x$	0.93
Lignin[2]	21.87	25.03	24.63	25.62	$\hat{Y} = 24.29 \pm 2.97$	-
Total carbohydrates[2]	825.35	835.64	840.47	854.07	$\hat{Y} = 825.23 + 0.9x$	0.84
NFC[2]	163.80	165.76	155.23	179.68	$\hat{Y} = 166.20 - 1.32x + 0.06x^2$	0.47

[1]g kg^{-1} natural matter; [2]g kg^{-1} dry matter; NM - natural matter; NDF - neutral detergent fiber; ADF - acid detergent fiber; NFC - non-fibrous carbohydrates; R^2 - coefficient of determination

DM and CP (r = 0.92, p = 0.0001) and negative between DM and NH_3-N (r = -0.85, p = 0.0001) and NH_3-N and CP (r=-0.88, p=0.0001), strengthen the idea that, with the elevation of DM content of the silages, there were losses of nitrogen compounds in the form of NH_3-N, thus increasing the protein content of the silage. These reductions were mainly due to the inhibition of effluent production with increasing DM. This shows that the DM content plays a key role in obtaining an adequate fermentative process when ensiling tropical grasses, because the castor bean hull contributed little as a source of soluble carbohydrates.

The inclusion of castor bean hull resulted in a linear decreasing effect (P<0.05) on the ether extract (EE) content of silages with a reduction of 0.53 g kg^{-1} DM for each percentage point of castor bean hull added. This result is justified by the EE content of the castor bean hull (5.76 g kg^{-1}DM), lower than that of elephant grass (17.4 g kg^{-1} DM). In general, the use of byproducts in elephant grass silage raises the EE content when the byproduct contains higher EE content than elephant grass (ANDRADE *et al*., 2010; RÊGO *et al*., 2010) and reduces the content of EE when the byproduct presents lower content of EE than elephant grass, as occurred herein and in other studies. This points out that the EE content in the silage is due to the chemical composition and proportion of the grass and the additive used, being little influenced by the fermentative process.

The neutral detergent fiber (NDF) content was influenced by the inclusion of castor bean hull, with a quadratic response (P<0.05) with estimated maximum content of 680.29 g kg^{-1}DM with 20.62% castor bean hull. Up to this level, the NDF was strongly influenced by acid detergent fiber (ADF), which were increased with the inclusion of castor bean hull, with an increase of 2.22 g kg^{-1} DM for each 1% castor bean hull added to silage. Nevertheless, this effect of ADF on NDF was reversed by the reduction of hemicellulose contents from the point of maximum NDF. This result suggests a possible nutritional limitation with higher proportions of castor bean hull, due to the high levels of ADF, which correlates negatively with DM digestibility (VAN SOEST, 1994).

Hemicellulose reduced linearly (P<0.05) with inclusion of castor bean hull with estimated values of 262.33 and 201.89 g kg^{-1} DM when castor bean hull was included in 0 and 30%, respectively. Among the three possible sources of hemicellulose degradation reported by McDonald, Henderson and Heron (1991), bacterial hemicellulose or those present in the forage are the possible causes of reduction in the hemicellulose content of the silages in the present study. Dewar, McDonald and Whittenbury (1963) verified pH influence on the action of hemicellulose present in the plant, with increased enzymatic activity when there is a rise in pH from 4.0 to 6.0. According to McDonald, Henderson and Heron (1991), although only small changes in soluble carbohydrate content occur as a result of plant enzyme activity, this response is maximized when pH approaches neutrality. This justifies our results, where the pH increased linearly and the hemicellulose contents were reduced with the inclusion of castor bean hull, a fact corroborated by the negative correlation between pH and hemicellulose (r=-0.94, p<0.0001). Thus, with the increase in pH of the silages, there was a higher enzymatic hydrolysis of the hemicellulose, impacting on the reduction of NDF from the inclusion of 20.62% castor bean hull in the silages.

The content of cellulose increased ($P<0.05$) with castor bean hull levels with an estimated value of 351.25 and 409.26 g kg^{-1} DM at levels 0 and 30%, respectively. During the fermentation process, the cellulose fraction remains stable with little variation of its content in the silage (VAN SOEST, 1994). Thus, this result is attributed to the higher cellulose content in castor bean hull (434.10 and 334.30 g kg^{-1} of DM) compared to elephant grass. Similarly, lignin also remains stable during fermentation in the silo (AMARAL *et al*., 2007), corroborating the results obtained in the present research, where the levels of castor bean did not influence the lignin contents of the silages with mean values of 24.29 g kg^{-1}DM.

The total carbohydrate (TC) content of the silages presented an increasing linear response ($P<0.05$) to the inclusion of castor bean hull. At each 1% addition of castor bean hull, there was an increase in TC content of 0.91 g kg^{-1} DM, ranging from 825.24 to 857.99 g kg^{-1} DM at levels 0 and 30%, respectively. This result may be explained by the increasing proportion of fiber constituents in the silages, especially cellulose that showed a high correlation with the TC ($r=0.87$, $p<0.0001$).

The non-fibrous carbohydrates (NFC) content of the silages was influenced ($P<0.05$) by inclusion of the castor bean hull, and a quadratic effect was observed with a minimum estimated content of 158.50 g kg^{-1} DM with inclusion of 11.70% castor bean hull. Although part of the soluble carbohydrates that integrate the NFC was used in the fermentation process as energy source, initially reducing the NFC content, there was degradation of hemicellulose releasing monosaccharides to the medium and contributing to the elevation in the NFC content, a process intensified by the inclusion of the castor bean hull to the silage.

The use of castor bean hull in elephant grass silage was efficient as a moisture-absorbing additive, since it increased the pH, but also increased SM, DM content and reduced the NH_3-N content and losses of DM by gases and effluents. Although it increased the fiber fractions, the addition of up to 25% castor bean hull favored the fermentation process without compromising the chemical composition of the silage. This opens up new perspectives for the use of castor bean hull in animal nutrition. Nevertheless, the lack of information regarding possible toxic effects due to the contamination from seed fragments present in the castor bean hull when added to the elephant grass silage, shows the need for further research evaluating the elephant grass silage with castor bean hull in ruminant feed.

CONCLUSIONS

1. The castor bean hull was efficient as a moisture-absorbing additive when ensiled with elephant grass;
2. The inclusion of up to 25% castor bean hull, based on the natural matter, during the ensiling of elephant grass, minimizes losses of the fermentation process and does not compromise the chemical composition of the silage.

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