



## Elephant grass silage with the addition of crambe bran conjugated to different specific mass

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**ABSTRACT.** This study aimed to evaluate the effects of inclusion of crambe bran concentrations (*Crambe abyssinica* Hochst.) with different specific masses in elephant grass silage (*Pennisetum purpureum* Schum.). For that, the bromatological and microbiological compositions of the experimental silages were determined. We used 48 mini silos distributed in a completely randomized design, arranged in a factorial  $4 \times 3$ , four levels of inclusion of crambe bran (0; 10; 20 and 30%) and three specific masses (400; 500 and 600 kg MN  $m^{-3}$ ), with four replications. After 240 days of fermentation the silos were opened. It was observed linear effect on DM, CP, NDFap, ADFap, HEM, LIG, NFC, TC and population of bacteria that produce lactic acid with the addition of crambe meal. There was interaction between the crambe bran factors and specific masses on the values of pH and N-NH<sub>3</sub>. For MM variables and yeast count there was a negative linear effect due to the evaluated specific mass. The inclusion of crambe bran helps to increase the fermentative profile and the bromatological composition of elephant grass silages studied, and the best results were obtained with the addition of 30% of this coproduct, based on the natural matter.

**Keywords:** additive, coproduct, *Pennisetum purpureum*, bromatological composition, fermentation.

## Silagem de capim Elefante com adição do farelo de crambe conjugado a diferentes massas específicas

**RESUMO.** Objetivou-se avaliar os efeitos da inclusão de concentrações do farelo de crambe (*Crambe abyssinica* Hochst.), com diferentes massas específicas, na ensilagem de capim-elefante (*Pennisetum purpureum* Schum.). Para tanto foram determinadas as composições bromatológica e microbiológica das silagens experimentais. Foram utilizados 48 minissilos distribuídos em delineamento inteiramente casualizado, arranjado em esquema fatorial  $4 \times 3$ , sendo quatro concentrações de inclusão do farelo de crambe (0; 10; 20 e 30%) e três massas específicas (400; 500 e 600 kg de MN  $m^{-3}$ ), com quatro repetições. Após 240 dias de fermentação os silos foram abertos. Foi observado efeito linear para os teores de MS, PB, FDNcp, FDAcp, HEM, LIG, CNF, CT e para a população de bactérias produtoras de ácido láctico com a inclusão do farelo de crambe. Houve interação entre os fatores farelo de crambe e massas específicas sobre os valores de pH e N-NH<sub>3</sub>. Para as variáveis MM e contagem de leveduras houve efeito linear negativo em função da massa específica avaliada. A inclusão do farelo de crambe contribuiu para incrementar o perfil fermentativo e a composição bromatológica das silagens de capim-elefante estudadas, sendo os melhores resultados obtidos com a adição de 30% deste coproduto, com base na matéria natural.

**Palavras-chave:** aditivo, coproduto, *Pennisetum purpureum*, composição bromatológica, fermentação.

### Introduction

The silage production of tropical grasses either in an area designed for direct production or as a way of using produced surplus in pasture or forage areas, during the favorable period of time to the forage growth, made up an interesting strategy for the livestock roughage supplementation (Faria et al., 2010). In Brazil one of the main forage used for this goal is the elephant grass (*Pennisetum purpureum*

Schum.), characterized by its high capacity of biomass production and proper nutritional value.

However, tropical grass silage requires some understanding about these forages specific fermentation profile, in order to get an adequate quality final product. In this case, when the cutting of these forages with good nutritional value is desired, the dry matter content (DM) is reduced (18 to 22%), which negatively influences both in the fermentation process, and also promotes some

increase in the nutrients loss due to effluents production. In addition, the reduced carbohydrate ratio (2.5% on average) also constitutes an important obstacle for the exclusive tropical grasses silage (Muck, 2010; Santos et al., 2010).

Thus, the high moisture content together with the low carbohydrate ratio of these forages at the moment of cutting can result in secondary fermentations, mainly the ones promoted by *Clostridium* bacteria. Such microorganisms act on the breakdown of sugars, lactic acid, proteins and amino acids, promoting excessive productions of butyric acid, acetic acid, ammonia and carbon dioxide, which results in effective losses in silage quality (Cândido, Neiva, & Rodriguez, 2007).

In this stream, the use of products aiming to enhance the silage efficiency of these forages, either through the improvement of fermentation characteristics or by the reduction of inherent to the process losses, constitute fundamental practice for obtaining good quality grasses silage (Ferrari Junior, Paulino, Possenti, & Lucenas, 2009). The use of DM rich additives (generally higher than 80%) and with adequate supply of nutrients can enable the moisture absorption, increasing the DM content of the ensiled material, and even promote improvements in the bromatological composition of the produced silage.

The number of studies regarding the inclusion of moisture absorbents additives in grass silage has grown over the last years (Pires et al., 2009; Rêgo et al., 2010; Santos et al., 2014). In tropical regions the crambe bran (*Crambe abyssinica* Hochst.) a co-product of the biofuel agribusiness, presents certain particularities in its bromatological composition, such as: high DM content and crude protein (Carrera et al., 2012; Souza, Goes, Silva, Yoshihara, & Prado, 2015). Thus, the use of crambe bran as an additive for tropical grasses ensilage will enable the increase of dry matter content and even enhance the crude protein content of the silage, making it possible to reduce the costs with the use of protein concentrates in diets, besides reducing possible impacts with the discharge of this co-product directly in the environment.

In order to obtain the proper results for the action of lactic acid producing bacteria, besides the DM and carbohydrate ratio content, the efficient removal of oxygen from the ensiled mass is a necessary condition to enable the growth of anaerobic lactobacilli. The regulation of efficiency of the oxygen removal from the mass to be ensiled is directly modulated by the specific mass adopted in the silage, which is influenced by the type of silo, applied pressure load, total compression time,

thickness of added layers and DM content and average size of forage particle (Jobim, Nussio, Reis, & Schmidt, 2007).

We aimed at evaluating the effect of increasing the inclusion of crambe bran as an additive in elephant grass ensilage with different specific masses, and for this the bromatological and microbiological compositions of silage were determined.

## Material and methods

The study was carried out on Fazenda Experimental do Moura which belongs to the Federal University of Vales do Jequitinhonha and Mucuri (UFVJM), located in Curvelo, Minas Gerais, Brazil.

For producing silage elephant grass (*Pennisetum purpureum* Schum) was used, Napier cultivar, derived from a 1.2 ha forage area, implanted in the second half of October 2012. The crambe bran (*Crambe abyssinica* Hochst.) was provided by the company Caramuru Alimentos S.A., to be used as an additive in the ensilage. The use of crambe bran was performed with no previous treatment, being physically similar to soybean meal for this co-product.

The elephant grass cutting happened in the middle of January 2014, respecting the average height of it – 1.7 m –, which corresponded to 60 days of growth after the standardization cut. The grass was manually cut, with the help of cutting tools (machete and cleaver), and it was performed close to the ground. Immediately after the necessary amount cutting, grass was chopped in a stationary machine set to obtain  $\pm 10$  mm length particles. The chopped material was homogenized and reserved for filling the mini silos.

The mini silos were made in polyvinyl chloride (PVC) tubes, whose dimensions were 60 cm height and 15 cm diameter. The mini silos were made up of a top cover with inner rubber seal on the side and valve type “Bunsen” for gases free escape. After closing the mini silos, they were properly sealed with silica gel to avoid oxygen inlet.

We used a completely randomized design, arranged in  $4 \times 3$  factorial scheme, with four concentrations of crambe bran inclusion (0; 10; 20 and 30%), regarding natural matter (NM) and three specific masses (400; 500 and 600 kg of NM  $m^{-3}$ ), with four repetitions.

The laboratory analysis concerning the fermentation evaluation and chemical compositions of silages were performed in the Laboratory of Animal Nutrition of the Zootechnology

Department (DZO - UFVJM). The microbiological evaluations were carried out in the Integrated Laboratory of Multiuser Research of Vales do Jequitinhonha and Mucuri (LIPEMVALE/UFVJM, Diamantina-MG). The bromatological composition of elephant grass and crambe bran, both collected right before the ensilage, is presented in Table 1.

After 240 days of fermentation the silos were opened. The silages were individually removed from the mini silos, homogenized and, afterwards, we took some samples for laboratory analysis.

**Table 1.** Bromatological composition of elephant grass and crambe bran (*Crambe abyssinica* Hochst.) used for producing the experimental silages.

Item	Elephant grass	Crambe bran
Dry matter (%)	18.40	92.50
Crude protein (%DM)	6.85	36.40
Ether extract (%DM)	2.26	1.22
Mineral matter (%DM)	6.79	7.83
Neutral detergent fiber <sup>1</sup> (%DM)	68.14	32.60
Acid detergent fiber <sup>1</sup> (%DM)	45.50	18.50
N-NH <sub>3</sub> (%)	14.90	10.70
Total carbohydrate (%DM)	83.10	54.60
Non fibrous carbohydrate (%DM)	14.96	21.50

<sup>1</sup>corrected for ash and protein.

For evaluating the fermentation parameters, determinations of hydrogenionic potential (pH) and ammonia nitrogen (N-NH<sub>3</sub>) were carried out. For determining the pH, the juice of each experimental silage was extracted, with the help of a hydraulic press, and afterwards the direct reading with the digital pHmeter was performed. To quantify the content of N-NH<sub>3</sub>, a new silage juice was extracted, properly identified, kept in sulfuric acid to 0.036 N and frozen for being determined through the distillation with magnesium oxide later (AOAC, 2005).

The sample preparation for microbiological analysis was constituted through previous dilution, weighing 25 g of silage, adding to 225 mL of peptone bacteriological water 0.1% sterile (121°C for 15 minutes) and stirred for 20 minutes at 150 rpm. From the diluted extracts (10<sup>-1</sup> to 10<sup>-6</sup>) the inoculations for evaluating the lactic acid bacteria (LAB), yeast and filamentous fungi (mold) were performed. For the total score of microorganisms 0.1 mL of each dilution as distributed, in triplicate and spread with Drigalsky handle in *Lactobacilli* MRS agar (De Man Rogosa Sharpe, Difco) added nystatin (0.4%), for counting lactic bacteria, and the Petri dishes were incubated at 30°C for 96 hours (Ávila, Pinto, Sugawara, Silva, & Schwan, 2008).

For scoring the filamentous fungi the Potato Dextrose Agar (PDA) was used, adding chloramphenicol (100 mg L<sup>-1</sup>), and the samples were

incubated at 28°C for 120 hours and the pH of the middle of culture was adjusted for 3.5. In the yeast scoring the YEPG means was used (0.3% yeast extract; 0.3% malt extract; 0.5% peptone; 1.0% glucose; 2.0% agar per liter, containing 100 mg L<sup>-1</sup> of chloramphenicol), and the samples were incubated at 28°C for 72 hours, adjusting the pH to 3.5 (Ávila et al., 2008). Plaques presenting between 30 and 300 colony forming units (CFU) per Petri dish were considered able to be scored. The present microorganisms numbers were counted as CFU and expressed as logarithm to the base 10.

In order to evaluate the bromatological composition the samples were pre-dried in a forced ventilation oven at 55°C for 72 hours and, afterwards, scrunched in a knife mill *Willey* type with a 1 mm sieve. The dry matter content (DM) of the experimental silages was determined in final drying oven at 105°C for 16 uninterrupted hours. The crude protein analysis (CP), mineral matter (MM) and ether extract (EE) were carried out secondly (AOAC, 2005).

The neutral detergent fiber analysis corrected for ash and protein (NDFap) and acid detergent fiber corrected for ash and protein (ADFap), using thermostable amylase, were determined according to the methodology described by Mertens (2002). The total carbohydrates (TC), the non-fibrous carbohydrates (NFC) were obtained according to Sniffen, O'Connor, Van Soest, Fox, and Russell (1992), through the application of the equations:

$$TC = 100 - (\%CP + \%EE + \%MM), e$$

$$NFC = 100 - (\%CP + \%EE + \%NDFap + MM).$$

Data were analyzed by multiple regressions through the software R (R. development team, 2006). We considered level 5% of probability ( $p < 0.05$ ) through the method of least squares.

## Results and discussion

The effect ( $p < 0.05$ ) of crambe bran addition for the bromatological composition was observed: DM, CP, NDFap, ADFap, TC and NFC contents; and for the microbiological composition: lactic acid producing bacteria (LAB) and filamentous fungi (FF) (Table 2).

Concerning specific mass there was effect ( $p < 0.05$ ) for MM contents and yeast population. As for EE contents, differences were not observed ( $p > 0.05$ ) for any factors. Interaction effect was observed (crambe bran x specific mass) for the variables pH and N-NH<sub>3</sub> (Table 2).

**Table 2:** Effects of increasing addition of crambe bran (*Crambe abyssinica* Hochst.) and specific masses on the evaluation of fermentation parameters and on the bromatological composition of elephant grass silages.

Item	Crambe bran(%)				SM (kg NM m <sup>-3</sup> )			Effects <sup>1</sup>			Regression equations	R <sup>2</sup>
	0	10	20	30	400	500	600	CB	SM	CB*SM		
DM	20.0	28.6	33.8	39.9	28.8	30.9	32.7	*	M	M	$\hat{Y} = 21.36 + 0.6042x$	0.91
pH	4.7	4.5	4.4	4.4	4.6	4.53	4.49	*	*	*		
N-NH <sub>3</sub>	7.6	6.8	4.8	3.9	7.1	6.27	6.07	*	*	*		
LAB	6.3	6.9	7.6	7.6	7.0	7.19	7.33	*	M	M	$\hat{Y} = 4.98 + 0.13x$	0.55
FF	3.9	3.2	3.2	2.9	3.43	3.28	3.16	*	M	M	$\hat{Y} = 3.75 - 0.03x$	0.25
YE	4.0	4.0	3.6	4.1	3.94	3.89	3.84	M	*	M	$\hat{Y} = 6.50 - 0.0051x$	0.41
MM	9.1	8.4	8.4	8.6	8.72	8.58	8.43	M	*	M	$\hat{Y} = 10.41 - 0.0048x$	0.30
CP	5.8	14.1	21.2	24.7	14.8	16.7	18.6	*	M	M	$\hat{Y} = 4.50 + 0.68x$	0.95
NDFap	71.0	63.7	52.4	47.4	60.9	58.4	56.0	*	M	M	$\hat{Y} = 76.35 - 0.8268x$	0.95
ADFap	45.0	40.3	34.5	32.5	38.9	37.7	36.5	*	M	M	$\hat{Y} = 44.62 - 0.4399x$	0.88
TC	82.0	74.3	67.2	62.9	73.2	71.4	69.5	*	M	M	$\hat{Y} = 81.41 - 0.65x$	0.96
NFC	11.0	10.5	14.9	15.6	12.3	13	13.5	*	M	M	$\hat{Y} = 10.35 + 0.18x$	0.39
EE	3.0	2.9	2.8	2.7	3.22	3.26	3.44	M	M	M	$\hat{Y} = 2.35 - 0.08x$	0.45

Dry matter contents (DM) are in %. Crude protein contents (CP), neutral detergent fiber corrected for ash and protein (NDFap), acid detergent fiber corrected for ash and protein (ADFap), lignin (LIG), total carbohydrates (TC) and non-fibrous carbohydrates (NFC), are expressed in % of DM and the ammoniac nitrogen (N-NH<sub>3</sub>) is expressed in % of TOTAL-N. Lactic acid bacteria (LAB), filamentous fungi (FF) yeast (YE) are expressed in LOG UFC g<sup>-1</sup> of forage <sup>1</sup>Probabilities of P < F, CB: Concentration of crambe bran, SM: specific masses and CB\*SM interaction between the crambe bran and specific masses concentrations. \* p < 0.05. M = meaningless.

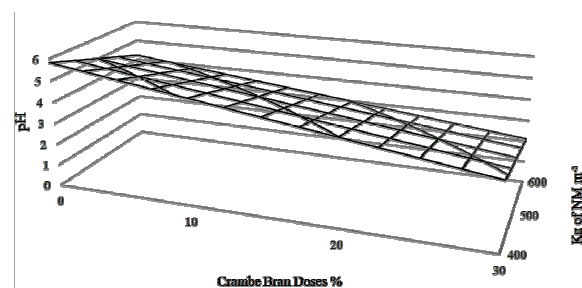
The increasing addition of crambe bran concentrations (CB) in the ensiled mass of elephant grass promoted linear growth ( $p < 0.05$ ) upon the dry matter DM content of the experimental silages. This fact was influenced by the high DM content present in the crambe bran composition (Table 1). The CB addition resulted in an increase of 8.0; 13.8 and 19.9 percentage points for the concentrations of 10; 20 and 30% of CB, respectively. In corroboration Zanine, Santos, Ferreira, Oliveira, and Pereira (2006) reported linear increase of DM due to the increasing addition of citrus pulp in the elephant grass ensilage, and this response is also explained by the high DM content present in the bromatological composition of this additive.

Data from this study pointed that the CB addition in elephant grass ensilage is an efficient technique to enhance the DM contents, turning this co-product an interesting additive absorbent for the ensilage process. Although this study has not quantified it, the increase in DM content through the CB inclusion might have enabled lower losses per effluents in the ensilages with higher addition of this co-product.

Regarding the CB addition, the concentrations of 10 and 20% provided an increase of DM to the proper values (28.6 and 33.8%, respectively) for good quality ensilage production. These results are within the interval reported by Zopollatto, Daniel, and Nussio (2009) for tropical grass with additive silages (28 to 35% of DM). The treatment without adding CB resulted in low DM content (20%), which may have affected this silage quality. The high amount of water in the ensiled mass turns the environment more likely for the development of undesirable microorganisms (*Clostridium* bacteria and yeast), thus enabling the secondary fermentations, besides significantly increasing the

losses by effluents (Santos & Zanine, 2007). The addition of 30% of CB resulted in a higher increase of interval than the recommended one for adequate quality silage (39.9%). The high DM content of the ensiled material can bring problems in the ensiled mass compression, which will directly reflect on the fermentation process due to the great quantity of oxygen that will be held in this material (Santos et al., 2010).

The combination of the treatments with 30% of CB and SM of 600 kg NM m<sup>-3</sup> resulted in silage with smaller pH value, on the other hand, the combination of the treatment without CB addition and 400 kg MV m<sup>-3</sup> of SM presented higher pH among the evaluated silages (Figure 1). These results highlighted the great importance of the increase of DM contents associated with the high intensity of specific matter for the production of proper quality tropical grass silages. Thus, to obtain a proper fermentation process, it is suggested that the inclusion of absorbent additives is connected with high intensity of specific matter, which may be determining to provide greater quality to tropical grass silages (Jobim et al., 2007).



**Figure 1.** Effects of interaction between the crambe bran (CB) concentrations and specific masses (SM) in the pH of elephant grass silages. Equation  $\hat{Y} = 9.01 - 0.13 \cdot CB - 0.008 \cdot SM + 0.0001 \cdot CB \cdot SM$ ;  $R^2 = 0.76$ .

It is noteworthy to emphasize that besides the beneficial effects due to the DM and specific mass increases, the bromatological composition of CB can also have specifically contributed towards the reduction of pH. Mainly the non-fibrous carbohydrates content (NFC) take fundamental role to module the fermentation profile of the newly ensiled masses, once they constitute the main source of energy for the development of lactic acid producing bacteria (LAB).

Cândido et al. (2007) reported that the NFC acts as a substrate for the development of LAB, influencing on the fermentation process and improving the nutritive value of silage. Thus, as the CB presented higher content of NFC compared to the elephant grass (Table 1), the addition of this co-product directly influenced a greater reduction of the pH over the higher levels of inclusion.

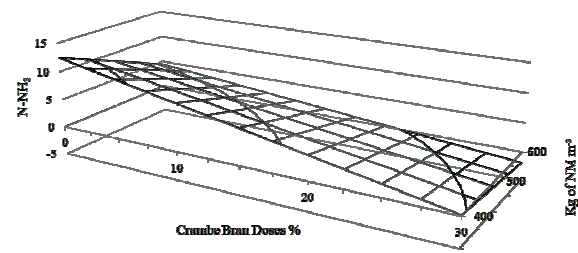
Although the pH of the silage is not isolated considered a good criterion for the evaluation of fermentation, we can suggest that the experimental silages with 10; 20 and 30% of CB inclusion present and adequate standard of conservation and final pH value close to 4.2. In a study reviewing the use of different additive in tropical grass ensilage, Zopollatto et al. (2009) reported great variation in the interval of the evaluated pH value (3.5 to 5.3), which emphasizes the discrepancy of this parameters due to the adopted additive. However, all the results from this study presented values close to the interval described by the mentioned authors (mean pH 4.4), which suggests an adequate pH value for the evaluated silages.

The combination of treatments with 30% do CB e SM of 600 kg NM m<sup>-3</sup> resulted in silage with lower N-NH<sub>3</sub> content (Figure 2). This answer is based on the increase of the DM content associated with greater removal of oxygen from the ensiled mass. The DM elevation through CB addition was efficient to decrease the N-NH<sub>3</sub> production, once the proteolysis extends during the fermentation when there are not enough acid conditions so that the undesirable microorganisms are avoided. It is important to highlight that, in this study; the acid conditions were adequately established, according to the presentation of pH values.

On the other hand the decrease of N-NH<sub>3</sub> content, influenced by the increase of specific mass, can be explained by better conditions of fermentation due to a lower presence of oxygen in the newly ensiled mass (Santos et al., 2010).

The silage N-NH<sub>3</sub> content is a real indicator of the extension of the proteolytic activity of the *Clostridium* bacteria, once it is produced in small amounts by other silage microorganisms and a small

enzymatic action of the plant itself. Well-preserved silage must present N-NH<sub>3</sub> contents lower than 10% (Ferreira et al., 2005). Zopollatto et al. (2009) reported that the N-NH<sub>3</sub> contents frequently found for tropical grass silage are higher than 15%. In this study all the treatments presented N-NH<sub>3</sub> contents lower than the recommended value.



**Figure 2.** Effect of the interaction between the levels of crambe bran (CB) inclusion and specific masses (SM) in the N-NH<sub>3</sub> of elephant grass silages. Equation:  $\hat{Y} = 28.44 - 0.81 \cdot CB - 0.039 \cdot SM + 0.0013 \cdot CB \cdot SM$ ;  $R^2 = 0.62$ .

The populations of lactic acid bacteria (LAB) in silages had a linear increase ( $p < 0.05$ ) with the crambe bran addition. This probably happened due to the elevation of silages DM, what resulted in the reduction of bacterial activity in the experimental silages. In these conditions, the *Clostridium* type activity is substantially reduced or does not exist, allowing the lactic acid bacteria to produce their products to rapidly stabilize the pH of the silage.

The elevation of the LAB population consists in a great indicator to determine the good fermentation quality during the ensilage, once the intense activity of these microorganisms inhibits the possible development of *Clostridium* type bacteria (Bernardes, Reis, & Moreira, 2005).

A linear decrease ( $p < 0.05$ ) in the fungi population of the silage with an increasing addition of CB was observed. According to Ávila et al. (2008) the filamentous fungi grow better under aerobic conditions and in higher pH (5.0 to 6.0), i.e., with the increase of specific mass there was a consequent reduction of oxygen in the ensiled mass, narrowing the fungi growth, and also a greater concentration of LAB indicates bigger production of acids, which decreases these fungi's growth.

The increasing addition of CB promoted an increase in the DM content, which together with a greater specific mass, resulted in a proper pH value in the experimental silages. In these conditions there is greater development of LAB due to the microorganisms that perform secondary fermentation. In addition, the inclusion of CB can probably have enriched the energy and protein contents of the ensiled mass, too. And this may have

enhanced the LAB development in relation to the other microorganisms. This explanation can be grounded when the composition of the co-product analyzed in this study is observed (Table 1).

A linear decrease ( $p < 0.05$ ) was observed in the yeast population of the silage with SM increase. In general, it has been reported that the yeast are associated with the presence of oxygen in the newly ensiled mass (Muck, 2010). Thus, the SM increase caused greater oxygen reduction and, consequently, decreased the development of yeast population, providing better conditions of anaerobiosis for the ensilage process.

The compared results between the fermentation process (pH and N-NH<sub>3</sub>) and the microbiological composition prove the potential of using the CB as an additive for the elephant grass ensilage. In this chain, when the effects of the CB inclusion with the greater SM are evaluated the final product quality is enhanced. Based on this answer, the CB inclusion in concentrations of 20 and 30% in relation with the natural matter, under more intense SM (600 kg de NM m<sup>-3</sup>), would be the most indicated treatments for obtaining a better quality product. However, although we did not observe any problems related with the profile of silages fermentation, the high DM content obtained with the greater concentration of CB can result in possible problems at the moment of compression, mainly when the ensilage is performed in large scale (on the field). In this case, adopting a more conservative view, the addition of 20% of CB is the best way to use this co-product.

The increasing addition of CB in the elephant grass ensilage linearly increased ( $p < 0.05$ ) the crude protein contents (CP) of the experimental silages. This answer is due to the high crude protein content (36.4%) present in the bromatological composition of CB (Table 1). The increasing addition of CB resulted in expressive increases of 8.30; 15.40 and 18.90% of CP for the treatments with addition of 10; 20 and 30% of CB in relation with the control treatment (Table 2). The increase in the CP contents, due to the use of additives in the ensilage, has been reported only for products which present higher CP contents than the mass to be ensiled, and this effect is more intensified when the CP content is higher or even, due to the added concentration (Santos et al., 2010; Zopollatto et al., 2009).

It is noteworthy to mention that even presenting an expressive increase in the CP contents due to the increasing inclusion of CB; the results did not affect the pH variables, N-NH<sub>3</sub> and the microbiological composition. It would be expected that the sharp increase in the nitrogen content could enhance the

elephant grass buffering capacity, promote expressive increase in the N-NH<sub>3</sub> content that, consequently, would damage the fermentation profile of the ensiled mass. Probably, this response was not observed due to the adequate protein:energy ratio for the evaluated treatments. In this case the hypothesis of ensilage enrichment with the CB addition can be consolidated, once this co-product presents high CP contents and a satisfactory NFC content in its constitution (Table 1).

In general the elevation in the crude protein content obtained in this study can represent an important protein increase in ruminant diets, in such way it reduces the use of protein foods, which constitute the most expensive part in the costs with animal nutrition. Additionally, the inclusion of CB in animal nutrition, independently of the way it is used, constitutes a rational and efficient way to provide a proper destiny to the high volume of this co-product, easing possible impacts with the direct dumping of this "waste" in the environment.

A linear decrease ( $p < 0.05$ ) was observed in the silage MM contents with the increase of SM. In this case, the compression pressure might have brought bigger losses by effluents, which can explain the reduction in the MM contents under more intense SM. Tavares et al. (2009) working with Tanzania grass silage, observed that as the density was higher, there was an increase in the nutrients losses (carbohydrates, proteins and minerals) through the effluent. Although the effluents are mainly composed by water, big amounts of other compound soluble are present, such as proteins, organic acids, sugars, carbohydrates and the minerals.

The silages NDFap and ADFap were influenced ( $p < 0.05$ ) by the CB concentrations and presented a decreasing linear response, once this co-product has lower contents of these constituents in its bromatological composition (Table 1).

The reductions in the NDFap and ADFap contents under the bigger inclusion level (30% of CB) decreased from 23.6 and 12.5 percentage points, respectively, in relation with the treatment without CB. According to Macedo Júnior, Zanine, Borges, & Pérez (2007), voluminous with cell wall higher than 60% have their consumption limited by the effect of ruminal repletion. In this study the treatments from 20% of crambe bran, presented NDFap concentration lower than 60%, which suggests a bigger consumption and, possibly, greater digestibility due to the reduction in the lignin contents of these silages compared to the control silage.

The TC content of the silages presented decreasing linear response ( $p < 0.05$ ) towards the crambe bran concentrations. This co-product has low total carbohydrates content, which is influenced by the high concentration of crude protein in its constitution. This response is easily explained when we analyze the way how the TC content is calculated (Sniffen et al., 1992). In this case, the higher the CP and EE contents are, the lower the carbohydrate proportion in the food will be. Thus, when adding an increasing amount of crambe bran in the mass to be ensiled, there will be a lower concentration of total carbohydrates. However, we must highlight that the decrease in the TC contents happens more sharply due to the effective reduction of fibrous carbohydrates, as previously observed in the variables NDFap and ADFap. It is important to note that the fibrous carbohydrates do not constitute instant energy source for the fast pH reduction by the LAB action. Thus, the soluble carbohydrates contents are more important to provide a better quality fermentation profile. This way, as the pH contents for the experimental silages were similar to the evaluated treatments, the TC reduction through the increasing inclusion of CB did not bring effective losses for the ensilage process.

The increasing inclusion of CB promoted a linear increase ( $p < 0.05$ ) in the NFC content of the experimental silages. The CB can be considered an important source of NFC because it presents a similar value (21.5%) to the foods which are traditionally used as ensilage additives, such as: cottonseed meal (19.2%), corn gluten meal (20.0%) and soybean hulls (17.6%), according to what was reported in literature Rocha Júnior et al. (2003). The NFC constitution of the additives for ensilage constitutes a major factor for enhancing the fermentation profile of the mass to be ensiled. The simple sugars constitute the main energy source rapidly available for the LAB populations.

The ether extract contents (EE) were not different for any of the studied treatments. Probably the reduced EE contents in the crambe bran and in the elephant grass ensiled mass can have been decisive for this response. Although crambe is defined as an oleaginous plant, the crambe bran consists in the co-product obtained after the extraction of vegetal oil through specific solvents (Carrera et al., 2012). In this case, the high efficiency of the extraction through solvents provides an effective reduction in the EE contents of this co-product (Table 1).

The presented results for the bromatological composition of the experimental silages demonstrated the potential of using the CB as an

additive to enrich the nutritional value of tropical grasses silages. In this study, the CP and NFC contents increased and the NDFap and ADFap contents linearly reduced as there was linear effect for the increasing CB addition, the use of this co-product aiming to nutritionally enrich the elephant grass silage must suggest the 30% addition of CB based on the natural matter.

Through the results evaluation we can suggest the 30% addition of crambe bran (based on the natural matter) in the elephant grass ensilage. Although there was a DM content higher than the recommended one (39.9%) for a proper quality silage, this treatment presented the best results for the parameters: fermentation profile and bromatological composition. However, we presume that the CB doses which are higher than the studied ones in this experiment can promote an excessive increase of DM in the ensiled mass, which can affect the compression of the ensiled mass in the silo, directly damaging the fermentation profile and, consequently, the final product quality. It is noteworthy to mention that the knowledge regarding the moisture content of tropical grasses at the moment of cutting is fundamental for determining the level of inclusion of this additive in the ensiled mass. Thus, the higher the grass moisture content, the greater the necessity of additive inclusion will be to increase the dry matter content of the ensiled mass.

## Conclusion

The crambe bran presents great potential to be used as an additive in the elephant grass ensilage, and the indicated dose, based on the natural matter, is 30% of this co-product.

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