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Chemical composition of sugarcane silages with crambe bran

Composição química de silagens de cana-de-açúcar adicionadas de farelo de crambe

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ABSTRACT

We evaluated the contents of dry matter (DM), crude protein (CP), mineral matter (MM), soluble carbohydrates (CHOS), ammonia nitrogen (N-NH_3), neutral detergent fiber (NDF), and acid detergent fiber (ADF) in sugarcane silages to which different levels of inclusion (LI) of crambe bran (CB) were added in proportions of 0, 3, 10, 17 and 20% at different storage times (ST). Laboratory silos were used, predetermined ST being 0, 3, 7, 12, 24, 36, 41, and 60 days after silage. For statistical analysis, the response surface methodology was used, in which LI and ST served as independent factors and response variables as dependent factors. The levels of DM and CP increased linearly with NI; however, it did not show any significant response regarding ST. The CHOS contents were reduced linearly with NI and displayed a quadratic response with ST. The interaction between NI and ST for the levels of N-NH_3 was significant, as the levels reduced with the NI and ST. The levels of NDF and ADF reduced linearly with NI and displayed a quadratic response with ST. The CB showed high efficacy to improve fermentation of ensiled cane, with the addition and conservation of nutrients in all NI evaluated and over the storage time. In addition to the residues generated with the production of biodiesel in a sustainable way, CB is a viable alternative to the sugarcane silage.

Index terms: Biodiesel; co-product; response surface; sustainability.

RESUMO

Avaliou-se os teores de matéria seca (MS), proteína bruta (PB), matéria mineral (MM), carboidratos solúveis (CHOS), nitrogênio amoniacal (N-NH_3), fibra em detergente neutro (FDN) e fibra em detergente ácido (FDA), de silagens de cana-de-açúcar adicionadas de diferentes níveis de inclusão (NI) de farelo de crambe (FC), nas proporções de 0; 3; 10; 17 e 20%, e avaliados em diferentes tempos de armazenamento (TA), em dias. Utilizou-se silos laboratoriais, abertos nos tempos 0; 3; 7; 12; 24; 36; 41 e 60 dias após a ensilagem. Para a análise estatística utilizou-se a metodologia de superfície de resposta, em que os fatores independentes foram os NI e os TA, e os fatores dependentes foram as variáveis respostas. Os teores de MS e de PB aumentaram linearmente com os NI, mas não apresentaram respostas significativas quanto aos TA. Os teores de CHOS reduziram linearmente com os NI e apresentaram resposta quadrática com os TA. Houve efeito significativo da interação entre NI e TA para os teores de N-NH_3, pois os teores reduziram com os NI e TA, de forma conjunta. Os teores de FDN e FDA reduziram linearmente com os NI, e apresentaram resposta quadrática com os TA. O FC apresentou eficácia para melhorar o processo fermentativo da cana ensilada, com adição e conservação de nutrientes, em todos os NI avaliados e ao longo do tempo de armazenamento. O FC é uma alternativa viável para a ensilagem da cana-de-açúcar, além de destinar os resíduos gerados com a produção de biodiesel de forma sustentável.

Termos para indexação: Biodiesel; coproduto; superfície de resposta; sustentabilidade.

INTRODUCTION

The use of renewable sources to generate energy, particularly biofuels, has increased substantially in Brazil and the rest of the world (Santos et al., 2017). In 2019, Brazil produced approximately 6 billion liters of biodiesel (Agência Nacional de Petróleo - ANP, 2020). Moreover, the growing production of biodiesel generates large volumes of co-products that add nutritional value for both humans and animals (Bonfá et al., 2018; Guimarães et al., 2018).

Among the crops studied in Brazil as possible substitutes for conventional sources of oil for producing biodiesel, crambe (*Crambe abyssinica*) has been reported as a potential candidate. Moreover, its co-products, such as pies and bran, can be used in animal diets. According to Mendonça et al. (2014), the productivity of crambe can vary from 1,000 to 1,500 kg/ha, and the oil extracted from grains corresponds to 36 to 38%. The protein content of crambe bran is approximately 40%, making it a potential ingredient for use in animal feed.

Sugarcane (*Saccharum* spp.) is an important agricultural crop in the Brazilian economy. With Brazil being the world's largest producer of sugarcane, this crop has great agribusiness significance for the country. It is estimated that sugarcane production in Brazil for the 2019/20 harvest could reach 642.7 million tons, an increase of 3.6% over the previous harvest (Companhia Nacional de Abastecimento - CONAB, 2019).

Sugarcane silage is an alternative to forage during dry periods (Queiroz et al., 2015). Furthermore, Queiroz et al. (2015) reported that ensiling sugarcane reduces the daily work with cultural treatments necessary to obtain high productivity and longevity in the cane fields.

However, the exclusive silage of sugarcane presents certain obstacles, such as low levels of dry matter (DM), crude protein (CP), and mineral matter (MM) (Cantoia Júnior et al., 2020). These factors can negatively affect the fermentation of the ensiled material due to the high humidity of the cane and nutritional quality of silages, which cannot be improved without the use of additives when ensiled.

To curtail the losses associated with both silage fermentation and after the opening of silos, additives are used for sugarcane ensilage (Siqueira et al., 2010). Additives have humidity-absorbing properties and create a favorable environment for beneficial microorganisms to produce organic acids and conserve ensiled material during fermentation. Furthermore, additives add nutrients to the material, thus enhancing the quality and nutritional value of the silages produced (McDonald; Henderson; Heron, 1991).

Crambe bran can be used as a humidity-absorbing additive in ensiled cane, as it contains high levels of DM (Oliveira et al., 2016; Barbosa et al., 2017), thus reducing nutritional losses during the process. In addition, crambe bran has high levels of proteins and minerals, which add nutritional value to the co-product of the sugarcane ensilage.

The present study evaluated the potential use of crambe bran in sugarcane ensilage at different LIs and monitored the ST of the ensiled material. In addition, the study verified the effectiveness of the co-product in enhancing the nutritional value and reducing the losses during the process.

MATERIAL AND METHODS

The experiment was performed at the Federal University of Vales do Jequitinhonha and Mucuri (UFVJM), Campus JK, located in Diamantina, Minas Gerais, Brazil. The municipality of Diamantina is located in the Alto Jequitinhonha region, northeast of Minas Gerais and southeast of Brazil, in "Serra do Espinhaço."

The analyses were conducted at the Biofuels Laboratories (Bioprocesses and Microbiology), Animal Nutrition Laboratory, and the Integrated Multiuser Research Laboratory of the Jequitinhonha and Mucuri Valleys (LIPEMVALE), all parts of UFVJM.

Different LIs of the co-product from biodiesel production, the crambe bran (CB), were studied in the sugarcane silage, in the following proportions: 0, 3, 10, 17, and 20%, in relation to the DM of sugarcane. Further, different LIs were evaluated at different storage times (ST): 0, 3, 7, 12, 24, 36, 41, and 60 days after ensiling. The ST = 0 refers to the ensiled material and opened immediately after closing.

The sugarcane (RB867515) was obtained from the Experimental Farm of Moura (FEM) at UFVJM, located in the municipality of Curvelo, Minas Gerais, Brazil. The sugarcane culture was harvested following 12 months of development and represented the third cut. The cane was cut at 10 cm from the ground level. At the JK Campus, the entire plant material was minced in a stationary ensilator (Nogueira brand, Model EN-6600) regulated to obtain particles of approximately 2 cm. The crambe bran was supplied by Caramuru Alimentos SA, Itumbiara, Goiás, Brazil.

The DM contents of sugarcane and crambe bran at the time of ensiling were calculated to be 25.1 and 84.57%, respectively, using the AOAC methodology (Association Official Analitical Chemists – AOAC, 1995). After adding the co-product to the chopped sugarcane, in the aforementioned proportions, the material was homogenized and ensiled in laboratory silos made of PVC tubes 100 mm in diameter and 450 mm in length. The material was ensiled with a density of 600 kg/m^3.

The silos were sealed during ensiling with PVC caps. The PVC caps were provided with a Bunsen type valve, allowing the gases generated during the fermentation inside the silos to escape. The caps were later sealed with adhesive tape. The silos were opened at the aforementioned ST. During the opening of the silos in all ST, the material was homogenized, and the content was separated into two equal parts: one part was used in its wet form, and the other was pre-dried in a forced ventilation oven at 55 ºC for 72 h according to the AOAC (1995). Next, the samples of the dried part were weighed again and grounded in a stationary mill type Willey using a 1-mm sieve (AOAC, 1995) and packed in plastic bags for further analysis.

The contents of DM and MM were analyzed according to the AOAC (1995); neutral detergent fiber (NDF) and acid detergent fiber (ADF) based on the sequential method proposed by Van Soest, Robertson, and Lewis (1991). The contents of alcohol-soluble carbohydrates (CHOS) were analyzed by the method proposed by Bailey (1967).

For crude protein (CP) analysis, the nitrogen percentage of each sample was determined using the elementary analyzer LECO CHNS/O, model Truspec Micro, with further conversion of the nitrogen content (N) to crude protein (PB) content.

From the wet part of the material, separated after the opening of the silos, a sample of known weight was taken that was pressed with a hydraulic press (Reinalab brand, Model TE-097) to extract the silage broth, following which its volume was measured. In this extract, the levels of ammoniacal nitrogen were determined by distillation with magnesium oxide (AOAC, 1995). This process was repeated in all ST evaluated, including ST = 0. After closing the silos, they were opened at ST = 0 for better visualization of the results.

On the day of silage, the mixtures of sugarcane, with LIs of crambe bran, showed chemical–bromatological characteristics as described in Table 1.

For statistical analysis, data were evaluated using the response surface methodology and the software *Statistica 7.0* at a contingency level of 5% probability. According to Marone et al. (2015), this statistical methodology is used for modeling and analyzing problems where the response variable is influenced by several factors. The methodology is used to optimize this response. Therefore, the response variables can be influenced by independent factors, that is, they can be influenced both by different LIs of the CB and by different ST of the silos in days.

The Central Composite Design (CCD) is one of the most suitable designs for adjusting response surface models (Box; Wilson, 1951). The obtained equations were interpreted and adjusted, according to the level of significance, to obtain the actual behavior of the response variables using the model below. Thus, the response surface methodology helped to get a broader view of the variables under study with all the factors involved.

Table 1: Chemical composition of the mixture of sugarcane with different levels of inclusion of crambe bran to ensilage:

Variables (%)	Proportions of CB in sugarcane ensilage (%)				
	0.0	3.0	10.0	17.0	20.0
DM	25.1	26.89	31.05	35.21	37.01
CP	2.43	3.85	7.17	10.49	11.91
MM	2.54	2.73	3.18	3.62	3.81
CHOS	5.5	5.34	5.0	4.64	4.50
NDF	47.83	47.92	48.15	48.37	48.48
ADF	28.59	28.58	28.58	28.58	28.58

CB–crambe bran; DM–dry matter; CP–crude protein; MM–mineral matter; CHOS–soluble carbohydrates; NDF–neutral detergent fiber; ADF–acid detergent fiber.

$$Y = b_0 + (b_1 \cdot LI) + (b_1 \cdot LI^2) + (b_2 \cdot ST) + (b_2 \cdot ST^2) + (b_{1;2} \cdot LI \cdot ST) + e,$$

where Y = answer considered in its real values; b_0 = regression coefficients of the second order statistical model; b_1 and b_2 = model coefficients to be determined; LI = study variable “level of inclusion” (independent factor); ST = study variable “storage time” (independent factor); LI ST = interaction between two independent factors; e = experimental error.

To project the data on a response surface, that is, on the surface graphs, the “white dots” represent the evaluated treatments and the response surface is the projection (optimization) of the data obtained, using statistical planning initial as the reference.

RESULTS AND DISCUSSION

Different LIs of the CB in the sugarcane silage and different ST in days were tested as independent factors and their possible interactions. Further, the content of the DM, MM, CP, CHOS, N-NH_3, NDF, and ADF was evaluated.

The DM contents were evaluated with a 5% significance level ($R^2 = 0.82$), in which the LI affected the CB in the sugarcane ensilage (linear behavior: $P = 0.0001$; coefficient of variation = 8.27) and general equation:%DM = 24.47 + 0.66·LI. The exclusive sugarcane silages had 24.47% DM during the 60 days evaluated, contents close to those of sugarcane during ensiling. With the inclusion of 3, 10, 17, and 20% CB, the DM levels increased to 26.45, 31.08, 35.72, and 37.70%, respectively. It was observed that CB favored the increase in the DM content of the ensiled sugarcane, as the cane presented 25.10% DM in the ensilage and 84.57% in the CB.

The CB was used for ensiling a material with reduced humidity so that fermentation was not compromised. For sugarcane to be ensiled with higher DM levels to reduce losses, and without the use of humidity-absorbing additives, it would have to be harvested later. However, this would compromise the nutritional quality of the silages produced due to plant's age (Ávila; Bravo Martins; Schwan, 2010; Schmidt et al., 2014). Thus, the higher the LI of the CB, the higher the DM contents of the ensiled material, as shown in Figure 1.

Because there were no effect on the ST, the DM contents did not change over the evaluated time. However, these changed with the LI of the CB, showing the additive effect of DM on the ensiled cane. Consequently, the humidity-absorbing effect favored fermentation and the conservation of silages produced. With the highest levels of DM, the humidity of the material reduced, hindering the proliferation of undesirable microorganisms and using the nutrients in the medium as substrates for their growth and development. Thus, they compete with desirable microorganisms and compromise the conservation of ensiled material, reducing the quality of the final product (Bonfá et al., 2018; Guimarães et al., 2018).

The CP contents were evaluated with a 5% significance level ($R^2 = 0.95$), in which LI of the CB affected in the sugarcane ensilage (linear behavior: $P = 0.0001$; coefficient of variation = 3.48); and general equation: $\%CP = 2.42 + 0.51 \cdot LI$. The exclusive sugarcane silages showed low levels of CP, approximately 2.42%, which is practically the same content found in sugarcane *in natura*. With the inclusion of CB to ensilage, which presented 49.85% CP during ensilage, the CP levels increased. For LI of 3, 10, 17, and 20%, the CP levels increased to 3.95, 7.51, 11.08, and 12.61%, respectively.

When compared to the data presented in the current study, literature reports lower levels of CP for crambe bran. Cardoso et al. (2016) worked on CB containing 36.4% of CP, Mendonça et al. (2014) used a CB with 35% CP, and Mizubuti et al. (2011) worked on CB with 37.07% CP. Different levels of CP can be related to the efficiency of oil extracted from crambe seeds, as this step determined the concentration of nutrients in the obtained co-products. However, in these studies, the high protein content found in CB and its contribution in increasing the CP levels of the produced silages were evidenced.

In the present study, all the LIs could efficiently increase the CP levels of sugarcane silages. However, only LI above 10% could reach minimum CP levels of 6 to 8% in silages, as per the recommendation of Van Soest (1994) and Mertens (1994), so that this nutrient is not limiting for fermenting structural carbohydrates by the rumen microbial flora. This can negatively affect voluntary intake and the digestibility coefficient of forage. Therefore, all LIs evaluated responded satisfactorily to the increased CP levels of sugarcane silages (Figure 1), thus improving the nutritional quality of sugarcane, in addition to being an alternative to reduce costs with protein supplementation of animals.

The MM contents were evaluated with a 5% significance level ($R^2 = 0.82$), which included the effect of the LI of the CB on the sugarcane ensilage (linear behavior: $P < 0.0001$; coefficient of variation = 0.53) and effect of ST (linear behavior: $P < 0.0001$; coefficient of variation = 0.29); general equation: $\%MM = 2.48 + 0.08 \cdot LI – (19 \cdot 10^{-4}) \cdot ST$. The sugarcane *in natura* presented 2.54% MM and 8.9% CB in the ensilage. The exclusive sugarcane silages showed,

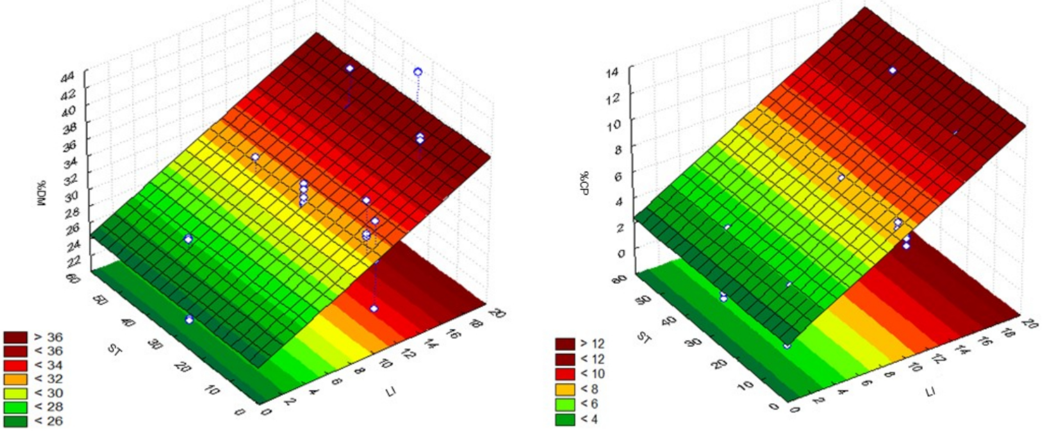

Figure 1: Response surfaces of the variable dry matter (%DM) and crude protein (%CP), depending on the levels of inclusion of the crambe meal, evaluated for 60 days. ST: Storage Times; LI: Levels of Inclusion.

at ST = 0, approximately 2.47% of MM and, on the last day of storage, the silages contained 2.36% MM. With the inclusion of 3% of the CB in the ensiling, the percentage of MM increased to 2.74% in the first evaluated ST (ST = 0). During fermentation, the percentage of MM decreased to 2.62% of MM (ST = 60) in the silages produced. The same behavior was observed with the inclusion of 10, 17, and 20% of CB, in which the percentages of MM were approximately 3.36, 3.98, and 4.25% at ST = 0. The response was similar to fermentation of exclusive cane and to the inclusion of 3% CB, in which the percentages of MM reduced to 3.24, 3.86, and 4.13% at ST = 60, respectively.

In addition to the low levels of CP, the exclusive sugarcane silages have limitations related to the levels of MM. In this case, the CB contributed to increase the supply of minerals in the sugarcane silages, which can be seen in Figure 2.

The CHOS contents were evaluated with a 5% significance level ($R^2 = 0.92$), which included the effect of LI of the CB on the sugarcane ensilage (linear behavior: $P = 0.0037$; coefficient of variation = 0.35) and the effect of ST (linear behavior: $P < 0.0001$; variation coefficient = 1.63; quadratic behavior: $P < 0.0001$, variation coefficient = 0.96); general equation: $\%CHOS = 4.25 – 0.05 \cdot LI – 0.13 \cdot ST + (10 \cdot 10^{-4}) \cdot ST^2$.

The sugarcane exclusive silages showed CHOS contents of 4.24% on the first day of opening the silos (ST = 0). Over the 60 days evaluated, a reduction in CHOS contents was observed, in which the silages, after fermentation, started to contain 0.55% of residual CHOS. The difference in CHOS in fresh forage (5.5) and silage in ST = 0 (4.24%) can be explained by the intense cellular activity that continues to occur in plants even after cutting. This is because the cells using oxygen from the air are still available, resulting in the metabolic burning of sugars (CHOS) forming CO_2, water, and heat, and has the effect of reducing the CHOS contents between cutting the material, silage, and opening the silo. The longer the time between these steps, the greater the CHOS consumption of the material (McDonald; Henderson; Heron, 1991).

The same behavior was observed with the LI of 3 and 10% of CB, in which the silages contained, in the first evaluated ST, 4.10 and 3.76% of CHOS. Further, at ST = 60, the concentration CHOS reduced to 0.40 and 0.05%, respectively, of residual CHOS. With LI of 17 and 20%, CHOS concentrations of 3.41 and 3.27% were observed at ST = 0, respectively. However, as the CHOS supply was lower in these two LIs, the exhaustion of CHOS was verified during fermentation inside the silos. For the LI of 17%, CHOS were consumed up to ST = 42; from this ST, CHOS were not detected in the silages. With the LI of 20%, the CHOS were depleted before this ST, as from ST = 39, the presence of CHOS in silages was not detected (Figure 2).

Sugarcane has high concentrations of soluble carbohydrates (sucrose) and a large population of yeasts, which can convert sugars to ethanol, CO_2, and water, which reduce the levels of soluble carbohydrates and increase the components of the cell wall and DM losses. These affect the quality and nutritional value of silage (Silva et al., 2008). The CB was used to increase the DM content of ensiled sugarcane so that nutrient losses are reduced due to the lesser stimulus to secondary fermentations.

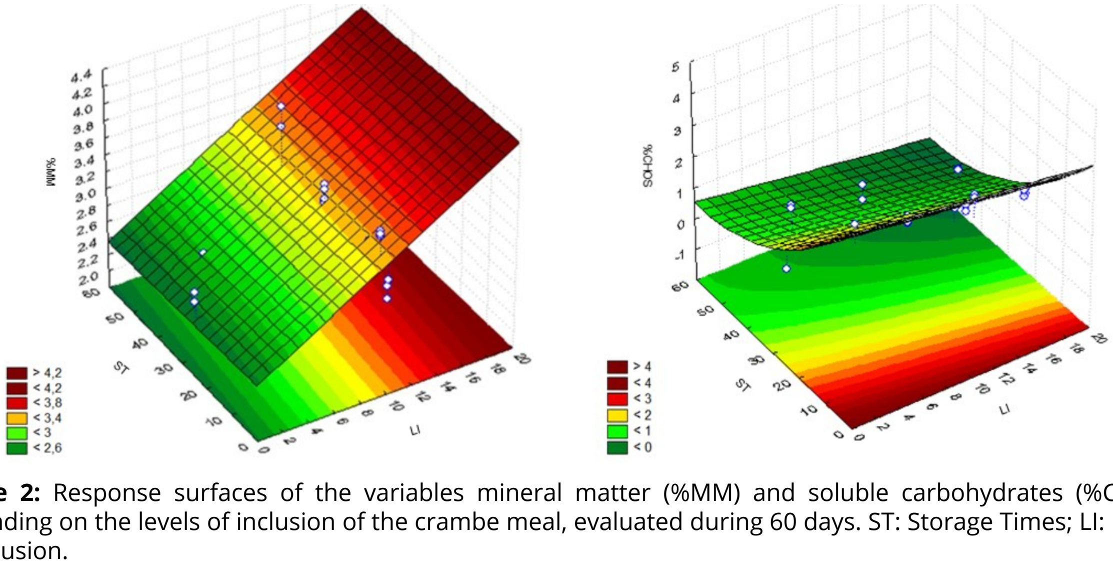

Figure 2: Response surfaces of the variables mineral matter (%MM) and soluble carbohydrates (%CHOS), depending on the levels of inclusion of the crambe meal, evaluated during 60 days. ST: Storage Times; LI: Levels of Inclusion.

In the present study, we found that the CB reduced the input of CHOS from ensiled cane because the CB presented 0.51% CHOS and the cane 5.55%, during ensiling. However, CB-induced reduction in CHOS was insufficient to compromise the activity of microorganisms beneficial to the process because the silages had residual CHOS at the end of ST (ST = 60). According to this parameter, the material was conserved well, and nutrients were preserved at the end of fermentation. It is necessary to consider the amount of easily fermentable CHOS that will be available to microorganisms beneficial to fermentation. In the absence of CHOS, desirable microorganisms can reduce their activity due to a lack of substrates and, thus, compromise the conservation of the material (McDonald; Henderson; Heron, 1991).

Microorganisms metabolize the substrates available for fermentation into lactic acid, the main product of fermentation by lactic bacteria, or other products, such as ethanol and CO_2, which are undesirable, both due to the loss of energy in the silage fermentation and because it is a neutral compound for the nutrition of ruminant animals. An adequate population of lactic acid bacteria is necessary to ensure proper lactic acid production, which rapidly reduces the pH and suppresses the activity of microorganisms harmful to silage, such as enterobacteria, clostridia, and others, increasing the conservation of ensiled nutrients (Bonfá et al., 2018; Guimarães et al., 2018; Van Soest, 1994).

Therefore, in the context of CHOS variable, the use of LI of up to 10% of CB to be ensiled for sugarcane is indicated to ensure no exhaustion of CHOS from silages until fermentation. This provides sufficient substrates for microorganisms to produce organic acids, which are responsible for conserving the ensiled material—the final products of their metabolism. However, as per the parameters evaluated and the highest levels of inclusion of crambe bran, the exhaustion of CHOS, which occurred during the stabilization phase of the silages, was insufficient to impair the conservation and quality of nutrients in the produced silages.

The $N\text{-}NH_3$ contents, presented as a percentage of total nitrogen, were evaluated with a 5% significance level ($R^2 = 0.93$), which included the effect of the LI of the CB on the sugarcane ensilage (linear behavior: $P < 0.0001$; coefficient of variation = 2.06) and effect of ST (linear behavior: $P < 0.0001$; variation coefficient = 3.71; quadratic behavior: $P = 0.0048$; variation coefficient = 1.31) and the interaction between the two factors ($P = 0.0032$; coefficient of variation = 2.41); general equation: $\%N\text{-}NH_3 = -3.29 + 0.63 \cdot LI + 0.32 \cdot ST - (14 \cdot 10^{-4}) \cdot ST^2 - 0.01 \cdot LI \cdot ST$. The exclusive sugarcane silages and those with the lowest LI of CB (3% of LI) did not result in $N\text{-}NH_3$ at ST = 0. However, the silages added with 10, 17, and 20% of the CB produced $N\text{-}NH_3$ on the first day of storage of silos (ST = 0), suggesting that the epiphytic microflora of the ensiled material decomposed the high PC (due to the inclusion of CB [49.85% of CP] in the sugarcane [2.43% of CP]) in the medium.

The production of $N\text{-}NH_3$ started after 11 days of storage of sugarcane (0.12%), reaching 11.01% at ST = 60. The same behavior was observed with the inclusion of 3% CB to ensilage, in which the presence of $N\text{-}NH_3$ remained undetected until the fifth day of the storage of the material. From the sixth day onward, 0.03% of $N\text{-}NH_3$ was detected, reaching 10.85% $N\text{-}NH_3$ at ST = 60. With the LI of 10%, 3.03% $N\text{-}NH_3$ was detected at ST = 0. During fermentation, the percentage of $N\text{-}NH_3$ increased to 10.46% at ST = 60. With the LI of 17 and 20% of the CB, the PC of the ensiled material increased further, causing the levels of $N\text{-}NH_3$ to be higher on the first day of opening the silos (ST = 0). However, despite the high concentration of $N\text{-}NH_3$ observed at ST = 0, the production of $N\text{-}NH_3$ was lower at the end of fermentation.

When 17% of the CB was used, 7.46% $N\text{-}NH_3$ was observed at ST = 0. After 60 days, the percentage of $N\text{-}NH_3$ increased to 8.02%, this concentration being lower than that found in exclusive sugarcane silages. A similar finding was observed when 20% CB was used. At ST = 0, silages contained 9.36% $N\text{-}NH_3$, which increased to 9.91% after 60 days, also lower than that found in exclusive sugarcane silages.

We could detect $N\text{-}NH_3$ on the first day of opening the silos due to the presence of crambe bran in PC, which did not occur with the exclusive sugarcane silages. After cutting the forage, the plant cells continued cellular respiration, thus maintaining the epiphytic microflora present in the material for a certain duration (McDonald; Henderson; Heron, 1991). With the high concentration of CP due to the inclusion of crambe bran, these microorganisms continued decomposing the proteins (abundant in the medium) and generating ammonia as the final product, consequently increasing the levels of $N\text{-}NH_3$ at ST = 0.

Studies on a higher concentration of $N\text{-}NH_3$ in exclusive sugarcane silages are scarce in the literature. According to Van Soest (1994), levels above 10% indicate the excessive breakdown of proteins to ammonia during fermentation to neutralize the organic acids formed and prevent a decrease in pH. In addition, high levels of $N\text{-}NH_3$ could be the reason for the low consumption of silages by animals. In well-preserved silages, amino acids make up the bulk of the fraction of non-protein nitrogen, and ammonia is present in low concentrations. Therefore, the quality of the silage produced decreases with the increase in the N-NH_3 content. We observed that CB reduced the levels of N-NH_3 in sugarcane silages, as shown in Figure 3.

The NDF contents were evaluated with a 5% significance level ($R^2 = 0.63$), which included the effect of the LI of the CB on the sugarcane ensilage (linear behavior: $P = 0.0006$; coefficient of variation = 3.54) and effect of ST (linear behavior: $P < 0.0001$; variation coefficient = 4.81; quadratic behavior: $P = 0.0289$; variation coefficient = 3.53); general equation:$\%FDN = 50.42–0.71 \cdot LI + 0.01 \cdot ST + (39 \cdot 10^{-4}) \cdot ST^2$.

We observed that the exclusive sugarcane silages had the highest NDF contents, with 57.31% at ST = 0. During fermentation, the NDF contents increased to 65.24% (ST = 60). The inclusion of 3% CB allowed the silages to have NDF content of 48.27% at ST = 0. After 60 days of storage of the ensiled material, the NDF content increased, reaching a concentration of 63.10% (ST = 60). The same behavior was observed for silages to which 10, 17, and 20% of CB were added at ST = 0, with NDF contents of 43.26, 38.25, and 36.11% and, at ST = 60, the NDF contents increased to 58.09, 53.08, and 50.93%, respectively.

The NDF content, as well as the concentration of the cell wall in the plant (cellulose, hemicellulose, and lignin-insoluble or remaining in neutral detergent), decreased in the silages when the crambe co-product was added (Figure 4). However, these levels increased throughout fermentation because the CB resulted in a higher NDF content than sugarcane in silage (51.10 and 47.83%, respectively).

The NDF content is an important parameter that can define the quality of the forage. It is considered as the main chemical constituent and related to the voluntary intake of the roughage by animals because it is directly associated with the increase in the rumen filling effect and reduction of the energy density of the food (Castro et al., 2010). High levels of NDF correlate negatively with voluntary intake by animals, and very low levels can impair the optimal conditions for ruminal fermentation (Van Soest, 1994). As reported by McDonald, Henderson, and Heron (1991), the increased NDF content throughout fermentation is relative and occurs due to the loss of cellular content during fermentation. Sugarcane silages produced without additives often result in materials with high fiber contents due to the absence of yeast inhibition, which are primarily responsible for reducing the cellular content in these silages. Therefore, the inclusion of CB in the sugarcane ensilage reduced the NDF contents of silages produced and improved the quality. According to this parameter, it is inversely correlated to the intake of food by the animals and contributes to the use of sugarcane in the form of silage.

The ADF contents were evaluated with a 5% significance level ($R^2 = 0.68$), which included the effect of the LI of the CB on the sugarcane ensilage (linear behavior: $P = 0.0009$; coefficient of variation = 1.94) and effect of ST (quadratic behavior: $P = 0.0051$; coefficient of variation = 2.65); general equation:$\%ADF = 34.41–0.78 \cdot LI + (29 \cdot 10^{-4}) \cdot ST^2$. Sugarcane exclusive silages showed the highest percentage of ADF, with 34.41% at ST = 0. After fermentation, the percentage increased to 35.59% (ST = 60). At 3, 10, 17, and 20% of the CB, the silages presented ADF contents of 32.05, 26.56, 21.07, and 18.72% at ST = 0, respectively. These levels increased during fermentation of the ensiled material, reaching concentrations of 33.23, 27.74, 22.25, and 19.89%, respectively, at ST = 60 (Figure 4).

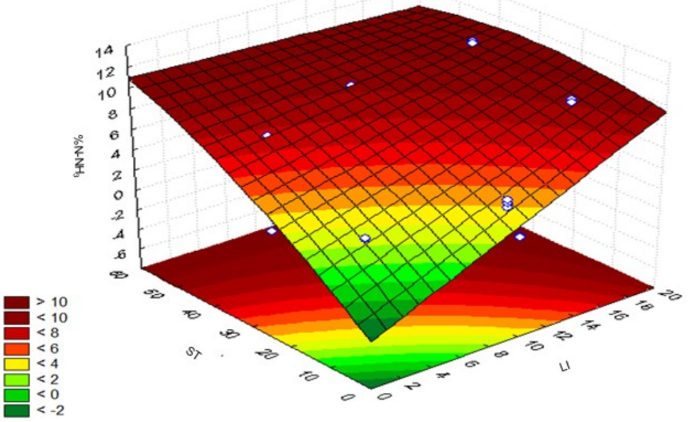

Figure 3: Response surfaces of the variable ammonia nitrogen (%N-NH_3), depending on the levels of inclusion of the crambe meal, evaluated for 60 days. ST: Storage Times; LI: Levels of Inclusion.

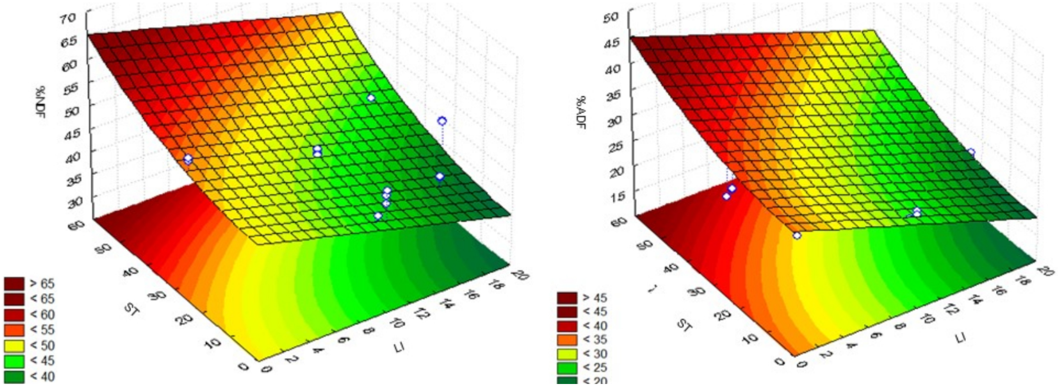

Figure 4: Response surfaces of the variables neutral detergent fiber (%NDF) and acid detergent fiber (%ADF), depending on the levels of inclusion of the crambe meal, evaluated during 60 days. ST: Storage Times; LI: Levels of Inclusion.

The levels of ADF showed the same behavior as the variable NDF, i.e, the levels of ADF decreased with the increased addition of CB to sugarcane ensilage. However, it increased during fermentation due to loss of cellular content. The ADF content, which determines the quality of the cell wall and reflects the insoluble and less digestible fraction of it (cellulose and lignin-insoluble or remaining in acid detergent), is directly associated with food digestibility (Van Soest, 1994). Thus, the higher the ADF content of a given food, the greater the indigestible fraction and, as a consequence, the lower the digestibility.

Therefore, the addition of CB to sugarcane ensilage reduced the constituents of the cell wall of the evaluated silages, as verified by NDF and ADF analyses, and shown in Figure 4. Figure 4 illustrates the behavior of these variables during the 60 days of fermentation and evaluation of the material.

CONCLUSIONS

The use of crambe bran in sugarcane ensilage increased the quality of silages produced at all levels. Moreover, it preserving the nutrients during 60 days of fermentation of the material. The co-product of sugarcane could be a valuable source of silage, as it improves the quality of this bulk and destines co-products from biodiesel production, in a correct and sustainable way, for feeding ruminant animals.

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