Original Research Article

NIRS estimation of the nutritive value of sugarcane silage at different harvest seasons and with additives

ABSTRACT

This study aimed to evaluate the nutritive value of sugarcane silage at different harvest seasons and treated with additives, as well as its estimation by near-infrared reflectance spectroscopy (NIRS). The experimental design was completely randomized with four repetitions. The treatments were arranged in a 3×5 factorial scheme, being: three harvest seasons (March, May and July); and five additives: 10% corn flour; 10% disintegrated straw and cob corn (DSCC); 15% rice bran; 1,0% urea; no additive. Dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, indigestible neutral detergent fiber (iNDF) and estimated total digestible nutrients (TDN) contents were evaluated. The nutritive value of sugarcane silage improves with additives, when compared to sugarcane silage *in natura*. The moisture sequestering additives present better results when compared to urea, with the exception of CP content. The co-product rice bran provides reduced fiber content, and increased CP and TDN contents of the silage. The silage produced in July and with additives provides the highest contents of total digestible nutrients. The NIRS estimates are excellent ($R^2_{cv} > 0.95$) for CP, NDF, ADF, and ash, offering ranchers and researchers a fast and inexpensive service.

Keywords: near-infrared spectroscopy; rice bran; corn flour; harvesting age; Saccharum spp.

1. INTRODUCTION

The sugarcane is traditionally used in its raw form on small farms, which demands daily harvesting and short-term supply [1]. The ensiling of this crop, on the other hand, can promote greater efficiency in the management of ruminant feed, avoiding daily harvesting and enabling storage.

The sugarcane presents all the indispensable requirements in a plant to be ensiled, such as high soluble carbohydrate content, low buffer capacity and adequate dry-matter contents. However, the chemical nature of the carbohydrates (sucrose), in high quantities, favors alcoholic fermentation by the action of yeast, which converts sucrose into ethanol, CO_2 and water [2]. As a technical consequence, such a process drastically reduces the production of the food conservative agent, lactic acid, responsible for reducing the pH and inactivating the deteriorating microorganisms of the ensiled mass.

Concerning this, many additives are tested in order to improve the fermentation pattern and minimize losses in the silage production process [3]. Nevertheless, there is inconsistency in the results available in the literature on the effect of additives in silage, due to the use of different sugarcane varieties, the chemical composition of the plant at the time of silage, and the different types of additives used.

Urea is one of the most studied and used additives [3]. Inside the silo, urea is converted into ammonia (NH_3) due to the urease action. The ammonia produced reacts with the water present in the elemental matrix, forming ammonium hydroxide (NH_4OH), which increases the pH and acts on the metabolism of undesirable microorganisms, especially yeasts. In addition, it increases the crude protein (CP) content, improving the quality of the sugarcane silages produced [5].

Ingredients with high dry-matter (DM) content can act as a moisture absorbing or sequestering additive, which creates an unfavorable environment for yeast due to the reduced moisture content, and reduces DM losses.

According to Rech AF [6], the parameters most often evaluated for the nutritive value of forages and silages are DM, CP, acid detergent fiber (ADF), neutral detergent fiber (NDF), digestibility and energy contents. The reference methods or physico-chemical methods of analysis have as main limitations the long time needed for their performance, the use of chemical reagents with high toxicity and danger, and the generation of large volumes of chemical effluents.

As an alternative method, the near-infrared reflectance spectroscopy (NIRS) has been widely used as an accurate method to predict the nutritive value of forages and silages [7,8,8]. The NIR spectroscopy is based on the absorption of electromagnetic radiation at wavelengths in the range 780-2,500 nm. The NIR spectra always present absorption bands referring to overtones and combinations of vibrational modes associated with C—H, N—H, O—H and S—H bonds, called functional groups [10].

The great advantage of this technique is in the multiple analysis of constituents, greater speed, lower cost, not being polluting [10,11], in addition to dispensing with the use of surgically prepared animals. Therefore, the assessment of the nutritive value of a food matrix by NIRS can be done with a large number of samples, saving time and chemical reagents.

Many ranchers send samples of forage and silage obtained on their property for analysis, in order to reduce the production cost, by enabling the balancing of the diet and potentiate the feeding of their animals. Against this background, in order to provide ranchers and researchers with a reliable, fast, and low-cost answer, the NIRS technology emerges as an excellent alternative.

Thus, this study aimed to estimate the nutritive value of sugarcane silage at different maturation stages and treated with additives by NIRS.

2. MATERIAL AND METHODS

2.1 Location and experimental design

The experiment was developed in the Plant Production Sector of the Federal Institute of Education, Science and Technology of Rondônia, located in Colorado do Oeste, RO, Brazil, situated at 13°07’ South Latitude and 60°32’ West Longitude from Greenwich, with an altitude of 460 m. The region's climate, according to the Köppen-Geiger classification, is of type Am, tropical monsoon climate, with an average temperature of 24 °C and average annual rainfall of 2,200 mm, concentrated between the months of January and March.

The forage used was obtained from a sugarcane field planted in October 2014 with the medium-late cycle variety RB928064 (ratoon cane). In the field, the experimental unit was composed of ten rows of plants spaced at 1.3 m with 4.0 m in length (46.8 m^2), with the six central rows being the useful area, disregarding 1.0 m at each end.

The experimental design used was completely randomized with four repetitions. The treatments were arranged in a 3×5 factorial scheme, being: three sugarcane harvest seasons (March, May and July); and additives, three of which are moisture sequestrants (10% corn flour; 10% disintegrated straw and cob corn; 15% rice bran), 1% urea and the control treatment (no additive). The additives were applied based on the natural matter of the sugarcane forage. The chemical composition data of the additives are presented in Table 1.

Table 1. Chemical composition of additives used in silage of sugarcane harvested at different ages

Additive	Season	Composition			
		DM (%)	CP (% DM)	NDF (% DM)	ADF (% DM)
Corn flour	March	83.3	7.49	32.6	2.41
	May/July [1]	83.2	9.02	39.0	4.71
DSCC	March	70.1	8.40	39.5	9.56
	May/July [1]	80.3	8.24	46.3	8.97
Rice bran	March	83.9	16.7	28.6	4.48
	May/July [1]	85.1	17.7	25.0	2.26
Urea	March	96.5	281**	-	-
	May/July [1]	97.6	281**	-	-

*[1] The same additives were used in the harvesting seasons of May and July. * Protein equivalent. DSCC: disintegrated straw and cob corn. DM: dry matter. CP: crude protein. NDF: neutral detergent fiber. ADF: acid detergent fiber*

2.2 Filling and opening the experimental silos

At the predetermined harvest seasons, the sugarcane was harvested close to the ground and chopped into 1.0 to 2.0 cm particles, being manually homogenized with or without the application of additives.

The chopped forage was placed inside experimental glass pot silos [13] with a capacity of 1.3 L, and compacted manually until obtaining a density of 600 kg m^{-3} of forage. After filling, the silos were closed by applying a layer of silicone on the edge of the lids to seal. Water was placed in the "siphon" type valves, with the objective of making it impossible for external air to enter, but allowing the gases produced during the fermentation process to exit.

The silos were opened 35 days after ensiling. During sample collection, 5.0 cm of the upper and lower portions of the silos were discarded, and the silage located in the geometric center of the experimental silo was collected. Both during ensiling and opening, samples were placed in paper bags and dried in a forced air ventilation oven at a temperature of 55 °C for 72 hours. The pre-dried samples were weighed and milled, using a stationary Willey mill with a 10-mesh sieve, and stored in containers for later analysis of the final DM content, in an oven at 105 °C for 4 hours, according to AOAC [14].

2.3 Chemical analysis

Forage and silage samples were submitted to ash content analysis, described by Silva DJ and Queiroz AC [15]; CP, by the micro Kjeldahl method [13]; NDF and ADF according to Van Soest PJ, Robertson JB and Lewis BA [16]; iNDF according to Detmann E et al. [17]. The total digestible nutrient (TDN) content of silage and forage was estimated according to Cappelle ER et al. [18], using the following equations:

Eq. 1. Forage: $TDN = 83.79 – (NDF \times 0.4171)$

Eq. 2. Silage: $TDN = 74.49 – (FDA \times 0.5635)$

The data obtained were submitted to variance analysis and, when significant, to the Scott-Knott test at 5% error probability.

2.4 Calibration and validation curves

Approximately 15 g of milled forage and silage samples were transferred to a quartz bottom sample holder attached to an MPA FT-NIR device (BRUKER® OPTIK GmbH, Rudolf Plank Str. 27, D-76275 Ettlingen), and spectra were generated in triplicate with 64 different points scanned at 16 cm^{-1} resolution from 4,000 to 12,500 cm^{-1} wavenumber range.

The reference values of CP, NDF, ADF, ash and iNDF in % of DM were added to the spectra of the forage and silage samples. The construction of data pretreatment and chemometric models, i.e., development of calibration curves, was performed by Opus 7.5 software using the partial least-squares (PLS) model [19].

The calibration model was adopted based on the lowest root mean square error of cross-validation (RMSECV) and the highest value of the coefficient of determination (R^2). The ratio of performance to deviation (RPD) and the range error ratio (RER) above 3 and 10, respectively, values were also adopted [20].

Samples classified as discrepant in the graphs were detected and excluded from the models. A set of samples not included in the calibration step was used for external validation.

3. RESULTS

3.1 Forage chemical composition

Harvest seasons and the use of additives in sugarcane silage influenced (P = .05) the contents of DM, CP, ash, NDF, NDA, iNDF and TDN of the forage (Table 2). The forage obtained from the July harvest has higher DM content (P = .05). Among the additives, the highest DM contents (P = .05) were observed for rice bran in May and July, and corn flour in March and July.

Table 2. Dry matter (DM), crude protein (CP), ashes, neutral detergent fiber (NDF), acid detergent fiber (ADF) and total digestible nutrients (TDN) contents of sugarcane forage harvested at different seasons and treated with silage additives

Additive	Season	CV (%)

	March	May	July	
		DM (%)		
Corn flour (10%)	29.5 aB	30.4 bB	35.4 aA	
DSCC (10%)	28.1 bC	29.7 bB	34.2 bA	
Rice bran (15%)	28.7 bC	32.6 aB	36.0 aA	2.21
Urea (1%)	24.0 cB	24.0 cB	29.0 dA	
No additive	24.6 cC	22.1 dB	30.9 cA	
		CP (% DM)		
Corn flour (10%)	4.17 cA	4.20 cA	4.08 cA	
DSCC (10%)	4.45 cA	4.38 cA	3.66 cB	
Rice bran (15%)	9.02 bA	8.42 bB	7.10 bC	4.94
Urea (1%)	15.0 aB	21.5 aA	11.6 aC	
No additive	3.07 dA	3.43 dA	2.02 dB	
		Ash (% DM)		
Corn flour (10%)	3.86 cB	4.44 cA	3.20 bC	
DSCC (10%)	4.11 cB	5.01 bA	3.08 bC	
Rice bran (15%)	4.96 bA	5.20 bA	429 aB	8.91
Urea (1%)	5.73 aA	4.69 cB	3.48 bC	
No additive	4.99 bB	6.23 aA	3.12 bC	
		NDF (% DM)		
Corn flour (10%)	41.0 dC	46.3 dA	43.2 aB	
DSCC (10%)	47.7 cB	54.8 bA	43.9 aC	
Rice bran (15%)	41.2 dA	42.9 eA	38.4 bB	3.05
Urea (1%)	58.8 aA	51.3 cB	45.3 aC	
No additive	55.6 bB	59.2 aA	44.4 aC	
		ADF (% DM)		
Corn flour (10%)	20.6 cB	25.9 dA	20.8 bB	
DSCC (10%)	25.8 bB	29.0 cA	21.3 bC	
Rice bran (15%)	22.1 cA	21.9 eA	20.3 bB	4.30
Urea (1%)	34.6 aA	30.9 bB	25.8 aC	
No additive	34.3 aA	33.4 aA	24.4 aB	
		TDN (% DM)		
Corn flour (10%)	66.7 aA	64.5 bC	65.8 bB	
DSCC (10%)	63.9 bB	60.9 dC	65.5 bA	
Rice bran (15%)	66.6 aB	65.9 aB	67.8 aA	0.95
Urea (1%)	59.3 dC	62.4 cB	64.9 bA	
No additive	60.6 cB	59.1 eC	65.3 bA	

CV: Coefficient of variation. Means followed by lowercase letters in the column and uppercase letters in the row, differ by the Scott-Knott test (P = .05)

It was found that the highest CP contents (*P* = .05) were obtained with the urea and rice bran application in the three seasons evaluated. On the other hand, the advancement in the growth stage of sugarcane caused a decrease in the CP contents.

The ash content was higher (*P* = .05) when silage occurred in May and, lower with advancing sugarcane harvest age. The additives urea in March and rice bran in July gave the highest forage ash contents.

The additives corn flour in March and rice bran in March and July promoted a greater reduction in the forage NDF content (*P* = .05). With regard to the ADF, the lowest contents *(P* = .05) were obtained with the corn flour application in March and July, and rice bran application in July. In opposition, the highest contents were observed for forage without additive and with added urea.

The use of rice bran in July and corn flour in March and July promoted higher TDN contents of the sugarcane forage (*P* = .05), which may also be related to the lower NDF content verified.

The forage iNDF contents had an isolated effect (*P* = .05) for the additive factor in sugarcane silage (Table 3). The lowest iNDF contents were observed in the forage additived with the moisture sequestering additives (corn flour, rice bran, disintegrated straw and cob corn). At the same time, the iNDF content was not modified as a function of harvest season.

Table 3. Indigestible neutral detergent fiber (iNDF) content of forage harvested at different seasons and treated with silage additives

Additive	Season			Means	CV (%)
	March	May	July		
	iNDF (% DM)				
Corn flour (10%)	28.2	31.4	29.6	29.6 b	
DSCC (10%)	35.0	31.0	37.5	31.2 b	
Rice bran (15%)	25.6	31.5	25.6	27.6 b	11.6
Urea (1%)	33.2	35.6	35.8	34.9 a	
No additive	39.0	38.8	35.6	37.8 a	
Means	32.2 A	33.7 A	30.8 A		

CV: Coefficient of variation. Means followed by lowercase letters in the column and uppercase letters in the row, differ by the Scott-Knott test (P = .05)

3.2 Silage chemical composition

There was an interaction between additives and harvesting seasons (*P* = .05) for the variables DM, CP, ash, NDF, ADF, iNDF and TDN of the silage (Table 4). The highest DM contents (*P* = .05) were observed by using rice bran in March, May, and July; and corn flour in May. There was a reduction in the silage DM content when compared to the forage DM at ensiling.

Table 4. Dry matter (DM), crude protein (CP), ashes, neutral detergent fiber (NDF), acid detergent fiber (ADF), indigestible neutral detergent fiber (iNDF) and total digestible nutrients (TDN) contents of sugarcane silage harvested at different seasons and treated with silage additives

Additive	Season			CV (%)
	March	May	July	

		DM (%)		
Corn flour (10%)	24.7 cC	28.8 aB	29.7 bA	
DSCC (10%)	25.7 bB	25.6 bB	28.7 cA	
Rice bran (15%)	27.0 aC	29.5 aB	32.0 aA	2.11
Urea (1%)	21.1 dC	22.3 cB	23.9 dA	
No additive	20.4 dB	20.6 dB	22.4 eA	
		CP (% DM)		
Corn flour (10%)	5.07 cA	5.32 cA	5.14 cA	
DSCC (10%)	4.35 dB	5.09 cA	4.92 cA	
Rice bran (15%)	10.1 bA	9.33 bB	9.05 bB	3.68
Urea (1%)	17.5 aA	16.2 aB	16.3 aB	
No additive	3.62 eA	3.56 dA	3.56 dA	
		Ash (% DM)		
Corn flour (10%)	4.59 dA	4.84 cA	4.13 cB	
DSCC (10%)	5.34 cA	4.84 cB	3.87 cC	
Rice bran (15%)	5.65 bB	6.21 bA	5.18 aC	3.50
Urea (1%)	6.62 aA	6.24 bB	4.85 bC	
No additive	6.41 aA	6.57 aA	5.41 aB	
		NDF (% DM)		
Corn flour (10%)	54.2 dA	53.7 dA	54.2 dA	
DSCC (10%)	61.7 cA	56.3 cC	58.6 cB	
Rice bran (15%)	50.4 eB	52.6 dA	47.0 eC	1.65
Urea (1%)	66.2 bA	66.0 bA	60.2 bB	
No additive	70.9 aA	68.7 aB	70.2 aA	
		ADF (% DM)		
Corn flour (10%)	28.1 dA	29.0 dA	27.9 dA	
DSCC (10%)	34.1 cA	31.2 cB	29.7 cC	
Rice bran (15%)	27.0 eB	29.6 dA	25.5 eC	2.18
Urea (1%)	38.1 bB	39.7 bA	35.6 bC	
No additive	40.0 aB	41.2 aA	41.8 aA	
		iNDF (% DM)		
Corn flour (10%)	27.9 cB	33.3 bA	26.4 dA	
DSCC (10%)	33.0 cA	31.3 bA	28.6 bB	
Rice bran (15%)	25.8 eB	32.2 bA	28.6 bB	6.03
Urea (1%)	36.2 bB	37.0 aA	36.0 aA	
No additive	38.2 aB	38.4 aA	37.7 aA	
		TDN (% DM)		
Corn flour (10%)	58.6 bA	58.1 aA	58.7 bA	
DSCC (10%)	55.2 cC	56.9 bB	57.8 cA	
Rice bran (15%)	59.3 aB	57.8 aC	60.1 aA	0.73
Urea (1%)	53.0 dB	52.1 cC	54.4 dA	
No additive	52.0 eA	51.3 dB	51.0 eB	

CV: Coefficient of variation. Means followed by lowercase letters in the column and uppercase letters in the row, differ by the Scott-Knott test (P = .05)

The silage presented higher CP contents (*P* = .05) when the urea additive was used in every season evaluated. However, with regard to the ash content, high values were observed in the sugarcane silage without the use of additives, in all harvesting seasons, or when urea was used in the March harvest.

The sugarcane silage *in natura* showed higher NDF contents (*P* = .05), which is not desirable. The same pattern was seen in the ADF. Somewhat lower contents were observed with the use of urea, also at all harvesting seasons. On the other hand, the use of rice bran and corn flour seems to have positively influenced the silage fermentative process, promoting lower NDF and ADF contents in the different seasons.

The sugarcane harvesting in May contributes to higher iNDF contents in the silage (*P* = .05), which is not desired. Similarly, sugarcane silage *in natura* and added with urea, in May and July, also promote high iNDF contents.

The silage with the rice bran additive showed, in every season, the highest TDN contents (*P* = .05) when compared to the sugarcane silage *in natura* (no additive). The rice bran reduced the NDF and ADF contents in the silage, and thus all seasons that received this additive had silages with better nutritional value. The July harvest season was the one that promoted a higher TDN increment, compared to the fresh silage, due to the lower ADF content.

3.3 NIRS estimation

We used 120 and 18 samples for the calibration and external validation sets, respectively (Table 5). In the calibration, a maximum limit of 10% outliers was adopted (Table 6), and the ash content showed a lower percentage of outliers (5%).

At the calibration stage, it was observed that the coefficient of determination values of the cross-validation (R^2_{cv}), ratio of performance to deviation (RPD) and range error ratio (RER) were greater than 0.95, 3.0 and 10.0, respectively, demonstrating an excellent estimate of the CP, NDF, ADF and ash contents of sugarcane silage by NIRS (Table 6, Fig. 1). Another important parameter for calibration was the lowest value of the root mean square error of cross-validation (RMSECV). The most used preprocessing model in the calibration was *First derivative* + *Vector Normalization* (Table 6).

In the external validation stage, it was found that the values of the correlation coefficient (r) and the RPD were greater than 0.95 and 3.0, respectively, also demonstrating an excellent estimation of the CP, NDF, ADF and ash contents of sugarcane silage by NIRS (Table 7, Fig. 2). Similarly, in the external validation the values of the root mean square error of prediction (RMSEP) were low.

Table 5. Minimum and maximum contents of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and ash of sugarcane silage for the calibration and external validation sets

Variables	Calibration			External validation		
	Sample (n)	Min	Max	Sample (n)	Min	Max
CP (%)	120	1.43	22.1	18	4.43	17.6
NDF (%)	120	34.5	71.7	18	47.2	65.9

ADF (%)	120	17.8	42.9	18	23.0	38.8
Ash (%)	120	2.82	7.11	18	3.84	6.92

Table 6. Parameters and preprocessing models used for predicting crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and ash contents of sugarcane silage for the calibration sets

Variables	Spectra (n)*	Outliers (%)	RMSECV (% DM)	R^2_{cv}	RPD	RER	Preprocessing
CP (%)	326	9.44	0.49	0.98	8.42	42.1	FDNV
NDF (%)	325	9.72	1.72	0.96	5.19	21.7	SLS
ADF (%)	326	9.44	1.16	0.96	5.49	21.6	FDNV
Ash (%)	342	5.00	0.18	0.97	5.85	23.8	MSC

**Each silage sample gave rise to three spectra, RMSECV: root mean square error of cross-validation, R^2_{cv}: cross-validation coefficient of determination, RPD: ratio of performance to deviation, RER: range error ratio, FDVN: First Derivative + Vector Normalization, SLS: Straight Line Subtraction, MSC: Multiplicative Scattering Correction*

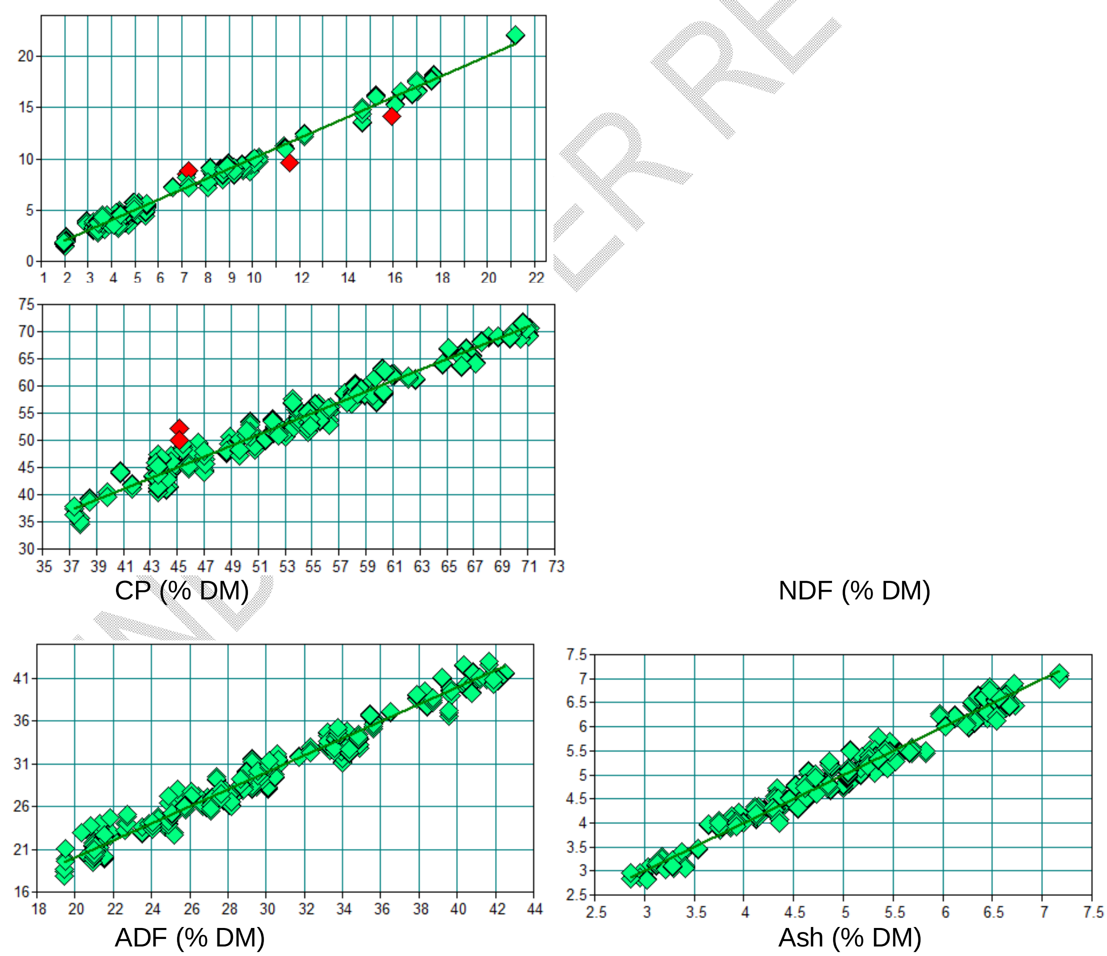

Fig. 1. Curve of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash contents of sugarcane silage for the calibration sets

Table 7. Adjusted parameters for estimation of crude protein (CP), neutral and acid detergent fiber (NDF) and ash contents of sugarcane silage for the external validation sets

Variables	Spectra (n)*	RMSEP (% DM)	r	RPD	Slope
CP (%)	54	0.59	0.99	8.26	0.90
NDF (%)	54	2.85	0.97	4.17	0.86
ADF (%)	54	2.83	0.96	3.65	0.91
Ash (%)	54	0.25	0.98	4.53	1.06

**Each silage sample gave rise to three spectra, RMSEP: root mean square error of prediction, r: correlation coefficient, RPD: ratio of performance to deviation*

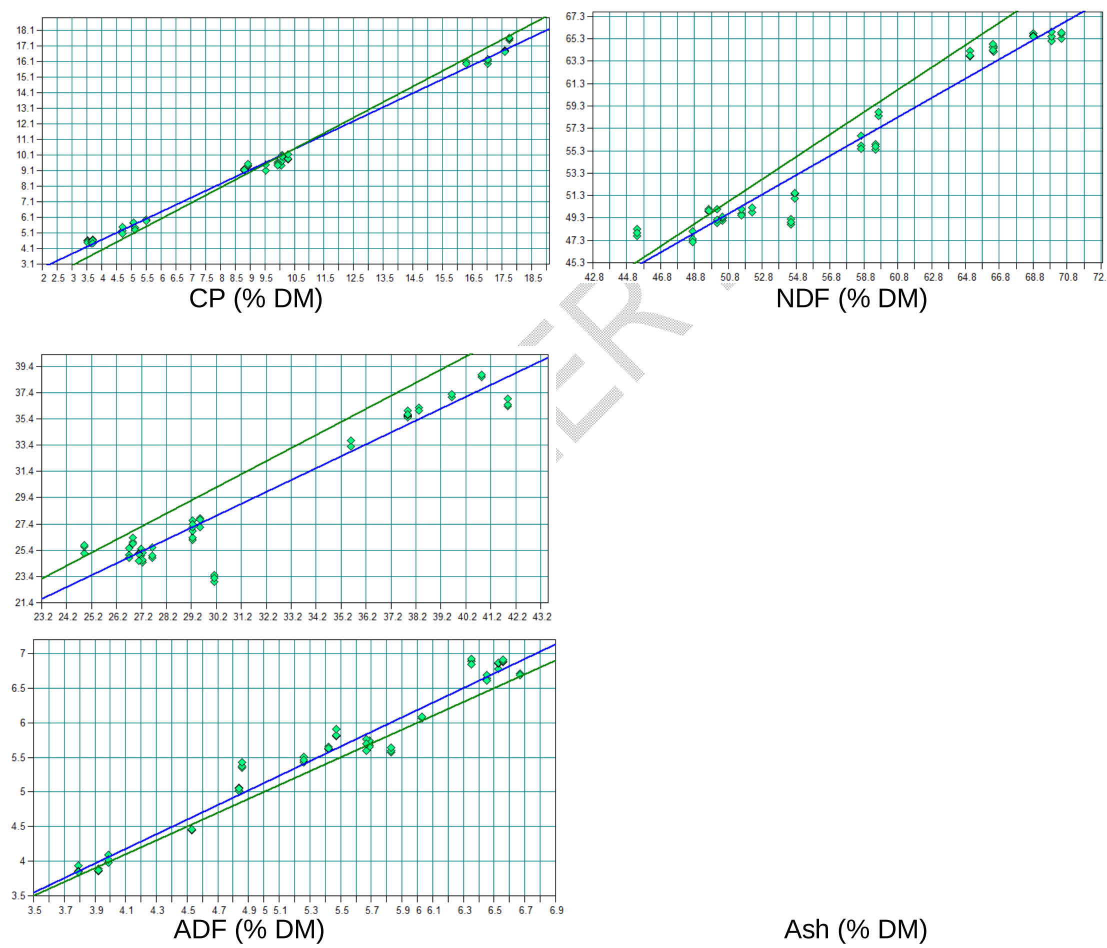

Fig. 2. Reference versus predicted values for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and ash contents of sugarcane silage for the external validation sample set

1. DISCUSSION

4.1 Forage chemical composition

The higher content of DM seen for the July harvest season is due to the degree of ripeness of the sugarcane at this time and, consequently, the presence of a greater participation of senescent material when compared to the other seasons. The contents obtained in July are close to the values recommended for silage (30% to 35%) for the process to occur satisfactorily [5].

The increase in DM content observed by the use of rice bran and corn flour is due to the high content of these additives, 84.5% and 83.2%, respectively, which, when added to the forage, increased DM specifically in July to the values of 36.0% and 35.4%, respectively.

The increase in CP content in the urea-additivated forage, as well as by rice bran, is due to the higher N content in these additives. Urea is commonly used in sugarcane silage to improve the low CP contents found in the raw material [3], besides helping in the silage preservation by the yeasts control.

Researchers reported higher CP contents at younger ages when studying the bromatological composition of sugarcane harvested at 18 and 12 months old, respectively [20,21], corroborating the result found in this research. For the most part, CP is concentrated in the leaves, which are abundant in the early stage of sugarcane development in March and May.

The ash content has an inverse relationship with the advancing maturity of the plant and its harvest. Similar results were obtained by Muraro GBP et al. [23], attributing the dilution effect on the content of other fractions, such as ash, to the advance in sugarcane development and the consequent sucrose accumulation promoted by the plant maturity.

The rice bran additive provided low NDF contents, which can be justified by the good participation of this additive in the ensiled mass (15%). This additive has a low NDF content and thus the fiber contribution to the forage was low, leading to its reduction. Likewise, the ADF content presented the same pattern, justified by the low contents of moisture sequestering additives.

The rice bran inclusion increased the TDN content, evidencing that the use of moisture sequestering additives, which have a higher nutritional value than forage *in natura*, improves the ensiled forage, ensuring greater maintenance of the quality and nutritional value of future silage.

The lower iNDF observed by using rice bran, DSCC and corn flour, reveal that these additives did not increase the iNDF content. As the concentrate levels in the diet of cattle fed sugarcane forage are increased, there is a reduction in the iNDF, a pattern that can be attributed to the increase in digestible energy [24].

4.2 Silage chemical composition

The additives rice bran and corn flour contributed as DM and soluble carbohydrates suppliers. Furthermore, they acted as moisture absorbers, which provided optimal conditions for the fermentation process. Consequently, this favored obtaining good silage, mainly by reducing DM losses.

The July season was the one that presented the highest DM contents, due to the advance in the vegetative stage of the plant. The RB928064 variety, a medium-late cycle variety, has a higher sucrose accumulation than the other varieties in the mid-crop and this condition explains the higher DM content at this harvest season.

The lowest DM contents were found when urea was used as a silage additive. Although the aim of urea is to reduce problems in the fermentation process by controlling the yeast population, this condition was not observed. The final DM content obtained by the other additives was higher than that of urea.

Urea, however, was responsible for the increase in the CP content of sugarcane silage, and this is due to the higher content of non-protein nitrogen present in this additive. According to Vilela HH et al. [5], the application of 1.5% urea in sugarcane silage promotes an increase in the CP content of silage (18.51%) compared to levels of 1% (9.69%) and 0.5% (6.12%).

The ash content observed in the fresh silage was lower than that found by Silva JG et al. [22] in a study of sugarcane silage without the use of additives (4.58%). The authors observed a 0.17% increase in ash content for each 1.0% increase of old man saltbush (*Atriplex nummularia* L.) in sugarcane silage.

The high NDF contents observed for sugarcane silage *in natura* are explained by the same condition pointed out by Lopes J and Evangelista AR [25]. For these authors, silage without additives has high fiber contents due to the non-inhibition of yeasts, which are mainly responsible for the reduction of cellular content in sugarcane silage. Moreover, an increase in the NDF content can be noticed due to the loss of cell content in the fermentation process [26].

The higher NDF content found in silage from the use of urea is possibly related to the non-effective yeasts control. This may be associated with the low input of carbohydrates or the low percentage of urea that was used, which may not have been enough to control the fermentation caused by the yeasts. This effect reduced cell content and increased NDF. On the other hand, the rice bran and corn flour inclusion, which had greater participation in the silage, promoted the dilution of the NDF contents, since they have low contents of 26.8% and 35.8%, respectively.

The lowest ADF content of silage was observed when rice bran and corn flour were used. On the other hand, the highest contents were observed when no additives were used, due to the uncontrolled undesirable fermentation inside the silo caused by yeast.

As it is a medium-late variety, the fibrous carbohydrates accumulation occurs at the end of the vegetative stage or at the beginning of maturation, a time corresponding to the second silage season. With advancing maturity or harvesting point, there is an increase in the ADF content, decreasing the fiber degradability, corroborating the results observed for the iNDF content.

It can be inferred that the higher TDN content in the silage obtained with the use of rice bran occurred due to the lower loss of soluble carbohydrates. As a consequence, there was a lower concentration of cell wall components, in addition to a higher nitrogen availability, due to the high CP content in silages with the inclusion of this additive.

The first two silage seasons (March and May) correspond to the end of vegetative growth. In July, the plant has low fiber content and high sucrose content, due to the advance in physiological maturity that occurs in the medium-late varieties.

4.3 NIRS estimation

The most commonly used parameters to evaluate the performance and validation of the models are RMSECV, RMSEP, coefficient of determination for calibration and validation set

(R^2), RPD and RER [27,28,28,30]. The RPD and RER values for CP, NDF, ADF, and ash are above 3.0 and 10.0, respectively, and are considered adequate for performing NIRS estimates of the nutritive value of sugarcane silage.

When evaluating the chemical composition of ryegrass (*Lolium multiflorum* Lam.), natural pasture and corn (*Zea mays* L.), Bezada SQ et al. [31], Yang Z et al. [32], Parrini S et al. [33], and Simeone MLF et al. [34] also found RPD and RER values for the CP, ether extract, crude fiber, ash and NDF contents above 3.0 and 10.0, respectively, and were considered adequate. However, the authors point out that the accuracy of a model depends on its application and its error of prediction (RMSEP).

The NIR region provided excellent estimates for CP, NDF, ADF and ash with $R^2_{cv} > 0.95$ and $r > 0.95$ values. Evaluating 232 samples of *Urochloa* forage crops (*U. brizantha* cvs. Marandu, Xaraés and Piatã; *U. ruziziensis* and *U. decumbens*), Gontijo Neto MM, Simeone MLF and Guimarães CC [35] also obtained similar results for the CP content ($R^2_{cv} = 0.98$; $R^2_v = 0.97$). The authors concluded that the analysis of the CP content by NIRS, allows the evaluation of a high number of samples and can be analyzed with lower cost, compared to the Dumas method, allowing better monitoring of the experimental area more frequently where the animals are located.

The results obtained for the CP contents were similar to those observed by Serafim CC [8] ($R^2_{cv} = 0.96$). The authors used forage and hay samples of Tifton-85 grass (*Cynodon* spp.), totaling 105 samples. Otherwise, the estimated contents for ash, NDF and ADF were lower than in the present work with low R^2_{cv} values of 0.84, 0.80, and 0.80, respectively.

The NIRS method can be used to predict the ADF and NDF contents of corn silage with high accuracy ($R^2_{cv} = 0.99$) [7]. Our study with sugarcane confirms this result by presenting estimates for NDF and ADF with $R^2_{cv} = 0.96$. Although Fontanelli RS et al. [7] did not obtain conclusive results about the ash fraction, the estimates for this variable in our research were excellent ($R^2_{cv} = 0.97$).

Molano LM et al. [35] used 1991 samples of tropical forage grasses and legumes for calibration by NIRS, and the models showed high coefficient of determination for the variables CP and NDF ($R^2_{cv} = 0.99$) and ADF ($R^2_{cv} = 0.95$), demonstrating excellent estimation by NIRS, in an indistinguishable way to this obtained in the present work.

In a study with 200 forage samples from different forage grasses and legumes, Massignani C et al. [8] observed high R^2_{cv} values of 0.94, 0.95 and 0.98 and r of 0.94, 0.95, and 0.97 for the parameters NDF, ADF and CP, respectively. Thus, the calibration curves were suitable for evaluating the quality of forages from multiple species and for their routine use in the laboratory.

5. CONCLUSION

The nutritive value of sugarcane silage improves with the use of additives, when compared to fresh sugarcane silage.

The moisture sequestering additives provide the best results when compared to urea, with the exception of crude protein content.

The co-product rice bran provides lower fiber content, high crude protein and total digestible nutrient contents of silage.

The silage produced in July and with the use of additives provides the highest total digestible nutrient contents.

The NIRS estimates are excellent ($R^2_{cv} > 0.95$) for crude protein, neutral detergent fiber, acid detergent fiber and ash, offering ranchers and researchers a fast and inexpensive service.

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