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Use of NIRS technology for predicting the nutritional value of silage made from tropical grasses enriched with corn ethanol co-products and intercropped with corn

Uso da tecnologia NIRS para predição o valor nutritivo da silagem de capins tropicais enriquecida com coprodutos do etanol de milho e em consórcio com o milho

Joadil Gonçalves de Abreu[1]; Daniele Cristina da Silva Kazama[2]; Wender Mateus Peixoto[3]*; Edegar Matter[4]; Luciano da Silva Cabral[1]; Ernando Balbinot[5]; Patrícia Orlando Royer[6]; Eduardo André Ferreira[7]

Highlights

Silage enriched with DDG enhances grass nutritive value.
Intercropping with corn enhances ruziziensis grass silage quality.
NIRS provides precise silage analysis and routine nutrient assessment.

Abstract

This study aimed to evaluate the chemical composition of tropical grass silage with added co-products from the production of corn ethanol, dried distillers' grains (DDG) and wet distillers' grains (WDG), and

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[1] Profs. Drs., Department of Animal Science and Rural Extension, Faculty of Agronomy and Animal Science, Universidade Federal do Mato Grosso, UFMT, Cuiabá, MT, Brazil. E-mail: joadil.abreu@ufmt.br; lucianoufmt@gmail.com

[2] Profª Drª, Department of Animal Science and Rural Development, Agrarian Sciences Center, Universidade Federal de Santa Catarina, UFSC, Florianópolis, SC, Brazil. E-mail: daniele.kazama@ufsc.br

[3] PhD Candidate, Postgraduate Program in Tropical Agriculture, Faculty of Agronomy and Animal Science, UFMT, Cuiabá, MT, Brazil. E-mail: wender.mpeixoto@gmail.com

[4] Master's in Animal Science, Postgraduate Program in Animal Science, Faculty of Agronomy and Animal Science, UFMT, Cuiabá, MT, Brazil. E-mail: edegarmatter@gmail.com

[5] Prof. Dr., Department of Agrarian Sciences, Instituto Federal de Educação, Ciência e Tecnologia de Rondônia, IFRO, Colorado do Oeste, RO, Brazil. E-mail: ernando.balbinot@ifro.edu.br

[6] Master's Student, Postgraduate Program in Animal Science, Faculty of Agronomy and Animal Science, UFMT, Cuiabá, MT, Brazil. E-mail: patricia.royer.ta@hotmail.com

[7] Researcher, Empresa Mato-Grossense de Pesquisa, Assistência Técnica e Extensão Rural, EMPAER-MT, Cuiabá, MT, Brazil. E-mail: eduardoferreira@empaer.mt.gov.br

* Author for correspondence

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grass intercropped with corn. The estimation of the chemical composition was performed with near-infrared reflectance spectroscopy (NIRS). In Experiment I (elephant grass), the experimental design was completely randomized with four replicates, and treatments were arranged in a 2×6 factorial scheme with two factors (additives: DDG and WDG, and application levels: 0, 5, 10, 15, 20, and 30%). In Experiment II (tanzania grass), the experimental design was completely randomized with four replicates, and the treatments included five DDG levels (0, 5, 10, 15, and 20%). In Experiment III, the experiment was conducted in randomized blocks with five replicates, and the treatments were organized in a 2×4 arrangement. Factor 1 included two cultivation methods, monocropped corn and corn intercropped with ruziziensis grass, and factor 2 involved four parts of the corn plant: whole plant, half plant, cobless plant, and cob with husk. The dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid (ADF), ash, and estimated total digestible nutrient (TDN) contents were evaluated. Reference values were added to the spectra of forage samples. Data preprocessing and chemometric model building, that is, calibration curve development, were performed using the Opus 7.5 software employing partial least squares (PLS) regression. The calibration model was selected based on the lowest root mean square error of the cross-validation (RMSECV) and the highest coefficient of determination (R^2_{cv}). The nutritive value of elephant grass and Tanzania grass silage improved with the use of DDG when compared with that of in natura silage. The NDF and ADF contents were lower, and DM was higher in ruziziensis grass silage intercropped with corn, highlighting the importance of adopting integrated production systems. Estimates by NIRS presented high R^2_{cv} values (>0.95), demonstrating the potential of this technology for routine analysis of tropical grass silages for CP, NDF, ADF, and ash.

Key words: Elephant grass. Near-infrared spectroscopy. Ruziziensis grass. Tanzania grass. *Zea mays.*

Resumo

Este estudo teve como objetivo avaliar a composição química da silagem de capins tropicais aditivada com coprodutos da produção de etanol de milho, grãos secos de destilaria (DDG) e grãos úmidos de destilaria (WDG), e consorciada com milho. A estimativa da composição química foi realizada por meio de espectroscopia de reflectância no infravermelho próximo (NIRS). No Experimento I (capim-elefante), o delineamento experimental foi inteiramente casualizado com quatro repetições, e os tratamentos foram dispostos em um esquema fatorial 2×6, sendo dois fatores (aditivos: DDG e WDG, e níveis de aplicação: 0, 5, 10, 15, 20 e 30%). No Experimento II (capim-tanzânia), o delineamento experimental foi inteiramente casualizado com quatro repetições, e os tratamentos foram cinco níveis de DDG (0, 5, 10, 15 e 20%). No Experimento III, o delineamento experimental foi conduzido em blocos casualizados com cinco repetições, e os tratamentos foram organizados em um arranjo 2×4. O fator 1 incluiu dois métodos de cultivo: milho cultivado em monocultura e milho consorciado com capim-ruziziensis, e o fator 2 envolveu quatro partes da planta de milho: planta inteira, meia-planta, planta sem espiga, espiga com palha. Os teores de matéria seca (MS), proteína bruta (PB), fibra em detergente neutro (FDN) e ácido (FDA), cinzas e a estimativa de nutrientes digestíveis totais (NDT) foram avaliados. Os valores de referência foram adicionados aos espectros das amostras de forragem. A construção de pré-tratamento de dados e modelos quimiométricos, ou seja, desenvolvimento de curvas de calibração, foi realizada pelo software Opus 7.5 utilizando o modelo de mínimos quadrados parciais. O modelo de calibração foi adotado com base na menor raiz quadrada do erro médio de validação cruzada (RMSECV) e maior valor do coeficiente de determinação (R^2_{cv}). O valor nutritivo da silagem de capim-elefante e capim-tanzânia melhorou com o uso de DDG quando comparado à silagem *in natura.* Os teores de FDN e FDA foram menores e a MS foi maior na silagem de capim-ruziziensis consorciado com milho, destacando a importância da adoção de sistemas integrados de produção. As estimativas por NIRS apresentaram altos valores de R^2_{cv} (>0,95), demonstrando o potencial dessa tecnologia para a análise rotineira de silagens de capins tropicais para PB, FDN, FDA e cinzas.

Palavras-chave: Capim-elefante. Capim-ruziziensis. Capim-tanzânia. Espectroscopia no infravermelho próximo. *Zea mays.*

Introduction

Ruminant feeding is influenced by the seasonality of pasture production, compelling ranchers to explore strategies for feeding animals during the dry season. Among these options, silage production from pasture areas with surplus growth during the rainy season has garnered significant research attention. Another alternative is the concurrent cultivation of corn (*Zea mays*) and tropical grasses, with a focus on increasing silage production in intercropping systems.

Elephant grass (*Pennisetum purpureum*) and tanzania grass (*Megathyrsus maximus*) are distinguished by their high dry matter productivity and adaptability to diverse climatic and soil conditions (Pereira et al., 2016; Jesus et al., 2021), rendering them suitable choices for preservation in ensiled states. Within the ambit of intercropping with corn through the Barreirão System, the foremost forage species employed is marandu grass (*Urochloa brizantha*) (Gomes et al., 2021; M. H. Oliveira et al., 2021).

Silage is a product obtained through the controlled fermentation of forage material with the aim of preserving the nutritional quality following silo opening (McDonald et al., 1991). However, for this to transpire, assessment of dry matter (DM) content, soluble carbohydrate levels, and buffering capacity during ensiling is important, contingent upon the chosen forage type (Weissbach et al., 1974).

Elephant grass and tanzania grass, when they have better nutritional value, present low contents of DM and soluble carbohydrates and a high buffering capacity, which allows the occurrence of undesirable fermentation, effluent production, and consequent reduction in the nutritional quality of the silage. To overcome this limitation, additives must be added to the forage at the ensiling stage to improve the fermentation pattern and the chemical composition of future silage (A. C. Oliveira et al., 2017).

The additives commonly employed in the ensilage of elephant grass are moisture-absorbing agents that increase the DM content and concomitantly increase the prospects of achieving optimal fermentation dynamics. In addition to rectifying the DM aspect, certain additives provide soluble carbohydrates and serve as fermentation stimulants (Rêgo et al., 2013; Penso et al., 2016). Illustrative examples of such additives include citrus pulp, by-products of the cassava (*Manihot esculenta*) and passion fruit (*Passiflora edulis*) industries, remnants of soybean (*Glycine max*), and cotton (*Gossypium* spp.) harvesting, as well as various cake and meal formulations.

The utilization of co-products, characterized by a superior cost-to-benefit ratio, has emerged as an additional strategy that exerts substantial influence on the economic sustainability of livestock enterprises (A. M. Silva et al., 2014). Currently, co-products derived from the corn ethanol manufacturing process, namely dried distillers' grains (DDG) and wet distillers' grains (WDG), have garnered significant attention in the market. In addition to their potential role as moisture-absorbing additives, these co-products provide a pathway to increase nutritional value, which is attributable to their high crude protein content and correspondingly low proportions of neutral and acid detergent fibers (Dian et al., 2021). According to data from the Companhia Nacional de Abastecimento [CONAB] (2020), corn ethanol production reached 791,431,000 liters during the 2018/2019 harvest season, culminating in the generation of 751,850 tons of co-products.

Within crop-livestock integration systems, an elevated harvest height of corn for silage engenders a heightened grain representation within the resultant mass, thereby enhancing the nutritive profile of the silage derived from the summer/autumn intercropping season. Moreover, this practice augments the regrowth potential of marandu grass, thereby facilitating lamb grazing and culminating in semi-feedlot finishing within the same field during the winter-spring interval (Pariz et al., 2017).

Near-infrared reflectance spectroscopy (NIRS) has been used in food analysis to assess samples and obtain quantitative insights into the interaction of near-infrared electromagnetic waves with their inherent components. Among the salient merits of NIRS-based technologies are their rapidity, nondestructive nature, and limited requirements for sample preparation, thereby supplanting conventional laboratory analysis techniques across a spectrum of domains encompassing food analysis and agricultural products (Pasquini, 2018).

According to Souza et al. (2018), to integrate NIRS analytical methodology into a laboratory setting, the establishment of multivariate calibration models is imperative. This task requires initial steps involving sample preparation strategies, wherein meticulous attention is directed toward the drying and grinding phases. These procedures ensure adherence to the prescribed drying temperatures adapted to the respective matrix types as well as obtaining the required particle size distribution profile.

When selecting samples for the calibration set, it is important to encompass samples exhibiting maximum variability in their physicochemical composition while simultaneously accounting for homogeneity within a specific species or botanical family (Souza et al., 2018). NIRS is highly accurate in predicting the nutritive attributes of forage and silage (Fontaneli et al., 2002; Massignani et al., 2021; Serafim et al., 2021; Abreu et al., 2023).

The aim of this study was to assess the chemical composition of tropical grass silage enriched with co-products from corn ethanol production, intercropping with corn, and its potential for estimation using NIRS.

Material and Methods

Site and experimental design

Experiments I (elephant grass) and II (tanzania grass) were conducted at the Experimental Farm of the Federal University of Mato Grosso (Santo Antônio do Leverger, MT, Brazil), situated at coordinates 15º47′ South Latitude and 56º04′ West Longitude, with an elevation of 140.0 meters. The climate, categorized under the Köppen classification as type Aw (tropical megathermal climate), is characterized by two distinct seasons: the dry season (May–September) and the rainy season (October–April). Annual precipitation amounts to 1,500 mm, peaking in December, January, and February.

The prevailing soil type is Plinthosol (Plinthosol Tb albic moderate), which is characterized by a medium texture and flat relief. This texture promoted effective water infiltration, soil aeration, root penetration, and robust root system development.

In Experiment I, the experimental design was completely randomized with four replicates. The treatments were distributed in a 2×6 factorial scheme with two factors (additives: DDG and WDG; and application levels: 0, 5, 10, 15, 20, and 30%). After the standardized harvest, maintenance fertilization was applied using 200 kg N ha^{-1} and 200 kg K_2O ha^{-1} (Ribeiro et al., 1999). The harvest date was when the elephant grass cv. Canará was 1.50 m tall, 70 days old, and was harvested 10 cm from the ground.

In Experiment II, the experimental design was completely randomized with four replicates. Treatments consisted of five different DDG concentrations (0, 5, 10, 15, and 20%). After the standardization harvest, covering maintenance fertilization was applied using 100 kg N ha^{-1} and 100 kg K_2O ha^{-1} (Ribeiro et al., 1999). The harvest date was when tanzania grass cv. tanzania had a pre-grazing height of 70 cm at 30 days of age, and was harvested at a residue height of 35 cm from the ground.

Experiment III (corn+grass) was conducted at Fazenda Nossa Senhora Aparecida (Colorado do Oeste, RO, Brazil), located at 13º07′ South Latitude and 60º31′ West Longitude, with an elevation of 460.0 meters. The climate, according to the Köppen classification, is categorized as Awa type, signifying a hot and humid tropical climate. This climate is characterized by two well-defined seasonal variations: summer, spanning from May to September, during which the lowest rainfall levels are observed, ranging from approximately 750 to 810 mm; and winter, extending from October to April, coinciding with the peak rainfall, which ranges between 1,470 and 1,500 mm. The experiment was conducted in randomized blocks with five replicates and the treatments were organized in a 2×4 arrangement. Factor 1 included two cultivation methods: monocropped corn and corn intercropped with ruziziensis grass, and factor 2 involved four parts of the corn plant: whole plant, half-plant (plant harvested 50 cm above the soil), cobless plant, and cob with husk.

The corn used in the experiment encompassed the hybrid Galo, endowed with the biotechnological trait Agrisure Viptera 3, thereby conferring tolerance to the herbicide glyphosate and ensuring the effective control of the paramount caterpillar species. The intercropping component, *U. ruziziensis* cv. Kennedy grass, commonly referred to as ruziziensis grass, was used at a sowing density of 3.25 kg ha^{-1} of pure live seeds. Grass was sown manually prior to corn sowing. The initial fertilization incorporated 25 kg N ha^{-1} and 45 kg P_2O_5 ha^{-1}, employing ammonium monophosphate and single superphosphate, respectively. Subsequent topdressing comprised 197 kg N ha^{-1}, administered in two installments (at 15- and 30-days post plant emergence), in conjunction with 58 kg K_2O ha^{-1}, utilizing urea and potassium chloride as sources, respectively (Ribeiro et al., 1999).

In Experiments I and II, the forage underwent fragmentation using a stationary crusher, resulting in 2-cm particulates, which were subsequently homogenized alongside the two additives, according to their respective treatment regimens. The chemical compositions of DDG and WDG were 92.39%, 35.64%, 36.94%, and 30.15%; 42.45% and 40.65%; 16.71% and 16.58%; and 1.73% and 1.75% of DM, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash, respectively.

In Experiment III, plant harvesting was carried out manually and timed to coincide with the attainment of the corn floury grain development stage. Aligned with the specific treatments, the harvesting protocol encompassed two distinct heights: 20 cm above the soil surface (representing the entire plant) and 100 cm above the soil surface (reflecting the upper portion of the plant). The latter mimicked the elevation of the harvesting platform of a mechanized silage apparatus. In cases in which the "cob with husk" treatment was applied, the cob was manually detached from the plant.

Filling and opening the experimental silos

Before ensiling was performed, approximately 500 g of forage from the mixtures was sampled for each treatment (DDG, WDG, and intercropped with corn). The samples were placed in paper bags and sent for pre-drying at a temperature of 55 ºC for 72 hours.

The pre-dried samples were weighed and subsequently milled using a stationary mill equipped with a 1.0 mm sieve. After milling, these samples were transferred and preserved within polyethylene receptacles intended for the subsequent determination of DM content via the Association of Official Analytical Chemists [AOAC] (1995) protocols prior to ensiling.

In Experiments I and II, each distinct experimental silo (plot) was constructed using polyvinyl chloride (PVC) pipes with a diameter of 10 cm and a height of 50 cm. The silos were manually compacted to attain a target density of 600 kg m^{-3} of forage. To ensure hermetic containment, the sealing process was executed using PVC lids designed to accommodate a Bunsen valve, thereby facilitating the venting of fermentation gases while simultaneously thwarting the ingress of atmospheric air. Subsequently, the lids were securely affixed using an adhesive tape. The opening of the silos transpired 40 days after the completion of the ensiling process.

In Experiment III, the prepared chopped forage was deposited in specialized experimental silos crafted from glass jars with a volumetric capacity of 1.3 L each. The silos were manually compressed with precision to achieve an intended density of

600 kg m^{-3} of forage. Upon completion of the loading process, the silos were meticulously sealed with the edges of the lids affixed through the judicious application of sealing silicone. Water was judiciously introduced into the siphon valves, serving the dual purpose of impeding the influx of extraneous air while simultaneously facilitating the release of fermentation-generated gases. At a designated interval of 135 days after ensiling, the silos were unsealed.

During the sample collection process, segments measuring 5.0 cm from both the upper and lower extremities of the silos were discarded, and the silage positioned at the precise geometric center of the experimental silo was gathered. After this, the collected samples were delicately placed within paper bags, after which they underwent desiccation within a convection drying oven set at 55 ºC for a duration of 72 hours.

Following the preliminary desiccation phase, the pre-dried samples were quantified by weight and subsequently comminuted using a stationary Willey mill equipped with a 1.0 mm sieve. Thereafter, the comminuted samples were securely stored within the designated receptacles, awaiting subsequent analysis of their definitive DM content. This analysis was conducted within an oven maintained at a temperature of 105 ºC for a duration of 4 hours, meticulously adhering to the stipulations outlined by the AOAC (1995).

Chemical analysis

Forage and silage samples were subjected to analysis aimed at determine their ash content using a procedure meticulously outlined by D. J. Silva and Queiroz (2002). CP was quantified using the micro-Kjeldahl method as described by Detmann et al. (2012). NDF and ADF contents were evaluated in strict accordance with the methodologies established by Van Soest et al. (1991).

The profound intricacies of total digestible nutrient (TDN) content within the forage and silage matrices were estimated using the guidance of Cappelle et al. (2001). This estimation was effectuated through the judicious utilization of the prescribed equations, which were diligently tailored for this purpose:

$$\text{Forage: TDN} = 83.79 - (\text{NDF} \times 0.4171) \qquad (1)$$

$$\text{Silage: TDN} = 74.49 - (\text{ADF} \times 0.5635) \qquad (2)$$

The acquired data were subjected to a rigorous analysis of variance using SISVAR, version 5.6 (Ferreira, 2019), where statistical significance was manifested, followed by scrutiny via Tukey's Honest Significant Difference Test, maintaining a stringent threshold of 5% probability of error.

Calibration and external validation curves

A total of 209 samples were used for the calibration set and 18 samples were used for the external validation set. For calibration, 94 samples from Experiment I, 38 from Experiment II, and 77 from Experiment III were used.

Approximately 15 g of milled forage and silage sample were transferred onto a quartz-bottom sample holder affixed to an MPA FT-NIR apparatus (BRUKER® OPTIK GmbH, Rudolf Plank Str. 27, D-76275 Ettlingen). The ensuing spectra were generated in triplicate, encompassing 64 discrete scan points with a spectral resolution of 16 cm^{-1} spanning the wavenumber range of 4,000 to 12,500 cm^{-1}.

The reference values of CP, NDF, ADF, and ash as a percentage of DM were integrated into the spectra of both the forage and silage samples. The pre-processing of data and establishment of a chemometric model, namely the development of calibration curves, were executed using the Opus 7.5 software, employing the partial least squares (PLS) modeling approach (Bjorsvik & Martens, 2001).

The calibration model was based on the lowest root mean square error of cross-validation (RMSECV) and the highest coefficient of determination (R^2_{cv}). Furthermore, the ratios of deviation performance (RPD) and error range (RER) were adopted, each surpassing the thresholds of 3 and 10, respectively, as described in the study by Williams and Sobering (1993).

In the calibration, a maximum limit of 10% outliers was adopted. Samples identified as outliers in the graphical representations were detected and subsequently excluded from the modeling process. A distinct set of samples not encompassed within the calibration phase, obtained from the UFMT farm and rural producers, was used for external validation.

The validation model was based on the lowest root mean square error of prediction (RMSEP), highest coefficient of determination (R^2_v), ratio of deviation performance (RPD) greater than 3, and ratio of error range (RER) above 10.

Results and Discussion

Chemical composition of elephant grass and tanzania grass silages

As shown in Table 1, the interplay between the additives and their respective inclusion levels exerted a discernible influence ($P<0.05$) on the DM, CP, NDF, ADF, ash, and TDN constituents within the silage. Notably, among the various additives investigated, irrespective of the inclusion level employed, the highest concentrations of DM, CP, and TDN ($P<0.05$), accompanied by the lowest quantities of NDF and ADF, were conspicuously manifested by DDG.

In all treatment scenarios in which DDG and WDG additives were integrated into both grass varieties, the resulting silages displayed a distinctive greenish-yellow tint complemented by a pleasant olfactory profile devoid of any fungal presence. Intentional incorporation of DDG (92.36% DM) and WDG (35.64% DM) additives was designed to enhance the DM composition of forage earmarked for ensilage. This deliberate enhancement, in turn, cultivates the potential to establish an optimal fermentative environment, thereby imparting superior silage quality.

An increasing linear trend pertaining to the levels of DDG and WDG was observed for the constituents of DM, CP, and TDN within the silage. These increments amounted to 0.53% and 0.11% for every 1.0% increase in inclusion, 0.55% and 0.28%, and 0.31% and 0.22%, respectively.

The utilization of DDG exhibited more pronounced efficacy in augmenting the DM content of elephant grass and Tanzania grass silages, inducing incremental enhancements of 48.41% and 64.37%, respectively, when juxtaposed with the non-addition (zero dose) and 20% inclusion scenarios. Silveira (2017) reported an analogous linear effect pertaining to the inclusion of macaw palm (*Acrocomia aculeata*) cake within elephant grass forage, manifesting a progressive increase of 0.66% in DM for every 1.0% inclusion.

Meanwhile, in an evaluation conducted by Penso et al. (2016), wherein elephant grass silage was enriched with rice (*Oryza sativa*) bran as an additive, a discernible increase of 0.56% in DM content was observed for each incremental 1.0% incorporation of rice bran into the mass.

Table 1
Means and regression equations of the dry matter, crude protein, neutral detergent fiber, acid detergent fiber, ash, and total digestible nutrients of Elephant grass silage with different inclusion levels of DDG and WDG

Additive	Levels (%)						Regression equation	R^2	CV (%)
	0	5	10	15	20	30			
	DM (%)								
DDG	20.56	26.35a	28.64a	30.52a	32.63a	38.13a	$\hat{y}= 22.2817 + 0.5393x^{**}$	0.96	3.20
WDG	20.56	20.84b	22.64b	22.00b	23.07b	24.05b	$\hat{y}= 20.6405 + 0.1166x^{**}$	0.89	
	CP (%)								
DDG	5.19	10.63a	14.63a	16.24a	18.10a	23.06a	$\hat{y}= 7.2032 + 0.5581x^{**}$	0.95	3.32
WDG	5.19	7.57b	9.46b	11.19b	12.32b	13.78b	$\hat{y}= 6.1112 + 0.2855x^{**}$	0.94	
	NDF (%)								
DDG	70.07	64.31b	63.86b	63.57	63.28	61.31b	$\hat{y}= 67.4500 - 0.2287x^{**}$	0.69	1.88
WDG	70.07	70.50a	66.15a	65.30	64.27	63.38a	$\hat{y}= 70.0093 - 0.2547x^{**}$	0.84	
	ADF (%)								
DDG	46.50	37.34b	35.25b	33.00b	31.16b	27.39b	$\bar{y}= 42.6641 - 0.5654x^{**}$	0.87	3.23
WDG	46.50	45.75a	40.73a	39.00a	37.90a	35.21a	$\hat{y}= 46.1626 - 0.3986x^{**}$	0.93	
	Ash (%)								
DDG	10.06	8.53b	6.46b	5.88b	5.26b	4.97b	$\hat{y}= 9.1240 - 0.1698x^{**}$	0.83	2.62
WDG	10.06	9.07a	8.56a	8.14a	7.56a	6.94a	$\hat{y}= 9.7278 - 0.1004x^{**}$	0.96	
	TDN (%)								
DDG	48.28	53.44a	54.56a	55.89a	56.93a	59.05a	$\hat{y}= 50.4485 + 0.3186x^{**}$	0.87	1.30
WDG	48.28	48.71b	51.53b	52.51b	53.13b	54.64b	$\hat{y}= 48.4772 + 0.2245x^{**}$	0.93	

Means followed by lowercase letters in the column differ according to Tukey's Honest Significant Difference Test ($P<0.05$); **Significant at the 1.0% probability level using the F test. DDG, dried distiller grain; WDG, wet distiller grain; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; TDN, total digestible nutrients; CV: Coefficient of variation; R^2: Coefficient of determination.

In instances of the elevated DM content, compaction becomes arduous, which simultaneously fosters fungal growth. Conversely, excessive moisture levels lead to a preponderance of butyric acid fermentation and effluent loss. Thus, the prescribed DM content for optimal silage performance is inherently variable, although it is generally within 30%–35% (Nussio et al., 2002; Rezende et al., 2008). Notably, the incorporation of DDG aligned with this recommended threshold for both grass cultivars.

Concomitant with the progressive augmentation in inclusion levels, an evident linear decrease was observed in the NDF, ADF, and ash contents, with reductions of 0.22% and 0.25%, 0.56% and 0.39%, and 0.16% and 0.10% for the DDG and WDG, respectively.

For every incremental increase of 1.0% in DDG inclusion, corresponding increases of 0.77%, 0.92%, and 0.18% were noted in the DM, CP, and NDT contents, respectively (Table 2).

An observable linear decrease was evident in the NDF, ADF, and ash content. With each successive 1.0% augmentation in DDG inclusion, corresponding reductions of 0.56%, 0.32%, and 0.23% were observed.

Moreover, the integration of DDG as an additive holds promise, in contrast to the use of pre-dried forage. This is since pre-drying results in an escalation of undesirable microorganisms within the silage matrix (Neres et al., 2013), subsequently elevating the risk of compromising the aerobic stability of the silage product.

A prerequisite for a minimum DM content of 25% is to curtail effluent losses within the silo, thereby safeguarding the retention of nutrients within the forage silage (McDonald et al., 1991). It is salient to underscore that the WDG additive exhibits a comparatively lower DM content (35.64%). Consequently, the utilization of up to 30% WDG within elephant grass resulted in a marginal 4.13% increase in DM content. Correa and Pott (2002) corroborated this observation by noting that silages derived from 55-day-old Tanzania grass exhibited DM contents of 20% (control) and 24% (8% citrus pulp).

The incorporation of 5.0% to 30% DDG and WDG into elephant grass silage resulted in an augmentation of CP content spanning from 9.99% to 23.95% and 7.54% to 14.68%, respectively. Similarly, with the application of 5.0% to 20% DDG to tanzania grass, the CP content in the silage exhibited a corresponding ranged from 11.17% to 24.99%. The superior performance of the DDG can be attributed to its inherent chemical composition, boasting a CP proportion of 36.94%, as opposed to 30.15% for the WDG.

Table 2
Means and regression equations of the dry matter, crude protein, neutral detergent fiber, acid detergent fiber, ash, and total digestible nutrients of Tanzania grass silage with different levels of DDG inclusion

Variables	DDG levels (%)					Regression equation	R^2	CV (%)
	0	5	10	15	20			
DM (%)	23.98	27.64	31.73	35.47	39.32	$\hat{y}= 23.9275 + 0.7702x^{**}$	0.99	3.82
CP (%)	6.53	11.31	14.80	22.04	24.21	$\hat{y}= 6.5625 + 0.9215x^{**}$	0.98	3.83
NDF (%)	70.49	66.47	62.13	59.23	60.07	$\hat{y}= 69.2935 – 0.5612x^{**}$	0.88	3.15
ADF (%)	32.41	30.94	29.36	29.08	25.17	$\hat{y}= 32.6585 - 0.3265x^{**}$	0.91	4.47
Ash (%)	10.90	9.32	8.04	6.66	6.30	$\hat{y}= 10.6175 – 0.2371x^{**}$	0.96	3.19
TDN (%)	56.22	57.05	57.94	58.10	60.30	$\hat{y}= 56.0255 + 0.1841x^{**}$	0.90	1.28

DDG, dried distiller grains; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; TDN, total digestible nutrients; **Significant at the 1.0% probability level using the F test. CV: Coefficient of variation; R^2: Coefficient of determination.

Within the context of Central Brazilian conditions, where pastures exhibit diminished nutritional potency during the dry season, the persistent deficiency of CP content remains consistently below the critical threshold required to satisfy the exigencies of rumen microbiota (7.0% CP). This deficiency in CP inevitably translates into diminished DM intake by forage (Medeiros et al., 2015). Evidently, the incorporation of 5.0% DDG or WDG into the silage of both grass varieties has emerged as a viable alternative for animal sustenance during the dry season, as it effectively addresses the minimal protein requirements essential for ruminant well-being.

To attain optimal performance from lactating cows, the forage must have a CP content of 15%, whereas for the maturation of growing animals, the range falls within 11% to 12% (Rodrigues et al., 2011). Adhering to these stipulations, the incorporation of 13.97% and 9.16% DDG into elephant grass and Tanzania grass silages, respectively, would effectively fulfill the essential minimal protein requirements pertinent to lactating cows. Conversely, despite its potential, the highest inclusion rate of WDG (30%) in elephant grass silage would fall short of meeting the CP demands of lactating cows, yet sufficiently satisfy the needs of developing animals.

Rêgo et al. (2013), in their study involving three different harvesting ages of elephant grass (70, 90, and 110 days) and incremental inclusion levels of mesquite (*Prosopis juliflora*) bran, identified a linear upward trend in CP content, amounting to 0.17% for every 1.0% addition. However, the outcomes of this study, particularly in relation to the DDG, substantially exceeded those achieved for both grass varieties. The DDG additive, notable for its high protein content and moisture-absorbing properties, exhibited commendable outcomes concerning the fermentative and nutritional qualities of the silages. As aforementioned, the maintenance of appropriate dietary CP levels is pivotal about fostering microbial proliferation, whereas inadequacies in CP content can potentially lead to reduced feed intake and diminished animal performance.

The inclusion of DDG and WDG in both grass varieties induced a notable reduction in NDF and ADF contents. This response can be attributed to the substantially lower NDF content (42.45% and 40.65%) and ADF content (16.71% and 16.58%) observed in DDG and WDG, respectively, when compared to the contents detected in fresh elephant grass silages (NDF: 70.07%, ADF: 46.50%) and tanzania grass silages (NDF: 70.49%, ADF: 32.41%). In a similar vein, E. M. Santos et al. (2006), in their investigation involving the supplementation of elephant grass silage with 15% wheat (*Triticum aestivum*) bran, reported analogous declines in NDF and ADF contents.

Rêgo et al. (2013), while examining harvesting ages of 70, 90, and 110 days within elephant grass, corroborated similar findings. For each incremental 1.0% incorporation of mesquite bran, reductions of 0.54%, 0.44%, and 0.42% in ADF content within the silage were observed.

It is imperative to emphasize that an NDF content surpassing 60% is negatively correlated with animal intake. In regard to the ADF content, values around 30% are deemed ideal for optimal animal intake, whereas contents exceeding 40% tend to correspond to lower intake levels (Van Soest, 1994). Consequently, for fostering adequate intake (ADF≤ 40%), DDG and WDG inclusion rates exceeding 5.0% and 15%, respectively, would be requisite for elephant grass silage. For tanzania grass silage, an inclusion rate of DDG exceeding 8.0% would be necessary to ensure optimal animal intake (ADF≤ 30%).

The incorporation of both DDG and WDG into the two grass cultivars resulted in a discernible reduction in the ash content, which was attributable to the relatively diminished levels of DDG (1.73%) and WDG (1.75%). Conversely, the inclusion of these additives concurrently increased the TDN content in both grasses. Dian et al. (2021) analyzed the chemical composition of DDG and elucidated a TDN spectrum ranging from 73% to 79% across their samples. This observation underscores the noteworthy TDN richness inherent to DDG, thereby undoubtedly influencing the augmentation of this variable within elephant grass and tanzania grass silages.

Chemical composition of corn silage intercropped with ruziziensis grass

No discernible interaction between the cultivation method and distinct segments of the corn plant was observed (P<0.05) with respect to the DM, CP, NDF, ADF, ash, and TDN constituents within the silage matrix (Table 3).

The corn silage intercropped with ruziziensis grass had a higher DM content (37.21%) than that of the monocropped system (34.88%). Conversely, Gomes et al. (2021) investigated corn intercropped with marandu grass and converted grass (interspecific hybridization: *U. ruziziensis x U. decumbens x U. brizantha*), both with and without pigeon pea (*Cajanus cajan*) in an integrated crop-livestock system for silage production, with no statistically significant variations in DM content. In instances without pigeon peas, the recorded DM contents were 36.71% and 38.45% for Marandu grass and converted grass, respectively.

Table 3
Means of the dry matter, crude protein, neutral detergent fiber, acid detergent fiber, ash and total digestible nutrients contents in the silage of parts of the corn plant (*Zea mays* L.) in monocropping and intercropped with Ruziziensis grass (*Urochloa ruziziensis*)

Method	Parts of the corn plant				Mean
	Whole plant	Half plant	Cobless plant	Cob with husk	
			DM (%)		
Monocropped	30.95	34.09	28.20	46.29	34.88 b
Intercropped	31.72	37.20	32.89	47.06	37.21 a
Mean	31.33 C	35.64 B	30.54 C	46.67 A	CV (%) = 6.99
			CP (%)		
Monocropped	7.50	7.58	7.88	7.31	7.57 a
Intercropped	7.09	7.20	7.22	6.77	7.07 b
Mean	7.30 AB	7.39 A	7.55 A	7.04 B	CV (%) = 4.09
			NDF (%)		
Monocropped	32.55	27.82	50.49	15.03	31.47 a
Intercropped	31.59	25.56	48.47	13.25	29.72 b
Mean	32.07 B	26.69 C	49.48 A	14.14 D	CV (%) = 6.13
			ADF (%)		
Monocropped	15.29	11.79	24.43	5.14	14.16 a
Intercropped	14.63	10.89	24.31	4.53	13.59 b
Mean	14.96 B	11.34 C	24.37 A	4.84 D	CV (%) = 7.28
			Ash (%)		
Monocropped	4.18	3.92	7.01	1.46	4.14
Intercropped	4.38	3.47	6.84	1.48	4.04
Mean	4.28 B	3.70 B	6.93 A	1.47 C	CV (%) = 11.5
			TDN (%)		
Monocropped	65.87	67.85	60.73	71.59	66.51
Intercropped	66.25	68.36	60.79	71.94	66.83
Mean	66.06 C	68.10 B	60.76 D	71.77 A	CV (%) = 0.85

Means followed by lowercase letters in the column and uppercase letters in the row differ according to Tukey's Honest Significant Difference Test ($P< 0.05$). CV, coefficient of variation; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; TDN, total digestible nutrients.

Intercropping practices and the use of husked cobs resulted in elevated silage dry matter content, suggesting an advantage in the chemical composition of the cultivated material. Increased CP concentrations were attained through monocropping and among plants devoid of cobs.

Cultivating corn for silage has implications for greater nutrient depletion from the soil than grain-only harvests. The extent of this depletion is influenced by variables, such as climatic conditions, genetics, crop attributes, and production levels. Conversely, integrated systems featuring corn intercropped with forage plants enhance soil quality through the contribution of plant residues from both aerial parts and roots, subsequently improving physical and biological soil attributes, increasing organic matter content, and reducing pest and disease incidence.

The chosen cultivation approach had a negligible influence on the ADF, ash, and NDT contents. Lower ADF quantities and higher TDN values were observed in silage derived from husked cob. Conversely, augmented ash content was notable in cobless plants.

Monocropped corn silage (7.57%), half-plant (7.55%), and cobless plant (7.39%) silages had higher CP contents. Variations in the CP content among corn silages have been documented in several studies (Santos et al., 2020; Almeida et al., 2020). A reduction in CP content in intercropped silage may be linked to resource competition. Alves et al. (2013) and J. F. Silva et al. (2013) observed that intercropping corn with ruziziensis grass led to reduced corn growth, particularly during the off-season, which is characterized by diminished light, lower temperatures, and reduced water availability.

The adopted cultivation method aimed to optimize agricultural productivity while also ensuring production system sustainability, thus highlighting the role of structural components in silage and intercropping within the context of the local environment. Consequently, evaluating the economic viability of corn intercropped with tropical forage plants and its resultant yield vis-à-vis the applied production system becomes a crucial consideration. Gomes et al. (2021) in their study achieved greater dry matter yields in the corn intercropped with marandu grass (13.65 t DM ha^{-1}) as opposed to convert grass (10.99 t DM ha^{-1}), both without pigeon pea presence.

It was further ascertained that the least NDF contents were achieved through intercropping, alongside the utilization of husked cobs. The NDF and ADF contents were lower in corn silage intercropped with ruziziensis grass (29.42% and 13.59%, respectively) and cob with husk silage (14.4% and 4.84%, respectively). Vasconcelos et al. (2005) attributed this phenomenon to the basal portion of the corn plant, which is characterized by higher fiber concentrations (NDF and ADF), primarily within the column. Strategically adjusting harvesting height can yield more digestible silage for more demanding animal categories, such as high-producing dairy cows facing limitations in DM intake stemming from rumen-filling constraints (F. C. L. Oliveira et al., 2011).

Notably, previous research underscores myriad avenues for ensiling corn plants, with diverse results contingent on the plant segments subjected to ensiling and varying outcomes. The adaptability of forage harvesters to different harvesting heights allows ranchers to leverage silage production, while leaving the residual material as a protective soil cover.

Corn cropping methods did not significantly affect ash and TDN content. However, the highest ash content was observed in cobless plant silage (6.93%), whereas the cob with husk silage exhibited the highest TDN content (71.77%). The use of cobs with husk silage in lactating cow diets alters milk composition and reduces dry matter intake. However, it does not impede milk production and leads to enhanced feed efficiency driven by increased ruminal starch digestibility (Akins & Shaver, 2014). Justino (2022) highlighted those cobs with husk silage streamlined feedlot logistics by substituting three feeds with cobs with husk silage. However, given its lower dry matter production per hectare, it requires a greater planting area to meet production requirements when compared to whole-plant corn silage.

Prediction models performance

Table 4 lists the maximum and minimum values obtained during the calibration and external validation stages. The assessment of model performance and validation primarily relied on key parameters, including RMSECV, RMSEP, and R^2 for the calibration and validation sets, RPD, and RER (Fonseca et al., 2020).

Table 4
Reference values for the crude protein, neutral detergent fiber, acid detergent fiber, and ash of tropical grass silages for calibration and validation sets

Variables	Calibration				Validation			
	Minimum	Maximum	Mean	SD	Minimum	Maximum	Mean	SD
CP (%)	5.05	24.56	10.62	4.94	5.24	23.02	9.95	5.13
NDF (%)	11.34	75.88	55.32	17.71	15.37	75.43	51.03	19.61
ADF (%)	3.81	49.92	28.38	12.85	5,64	48.40	28.32	14.14
Ash (%)	1.17	11.21	6.35	2.54	1,51	9.56	6.57	2.37

CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; SD: Standard deviation.

During the calibration phase, the cross-validation coefficients of determination (R^2_{cv}), the RPD, and RER exceeded thresholds of 0.95, 3, and 10, respectively. This unequivocally underscores the exemplary precision exhibited in the estimation of CP, NDF, ADF, and ash content in tropical grass silages using NIRS (Table 5). Another pivotal parameter influencing the calibration was the lowest RMSECV value along with the highest R^2_{cv}.

Table 5

Parameters and preprocessing models used for the prediction of the crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash contents of tropical grass silages for the calibration sets

Variables	RMSECV (% DM)	R^2_{cv}	RPD	RER	Preprocessing	nLV
CP (%)	0.685	0.981	7.26	28.31	FDVN	10
NDF (%)	1.950	0.987	9,08	33.10	FDMSC	9
ADF (%)	1.690	0.982	7.60	27.28	SLS	10
Ash (%)	0.393	0.976	6.51	25.74	FDVN	10

RMSECV, root mean square error of cross-validation; DM, dry matter; R^2_{cv}, cross-validation coefficient of determination; RPD, ratio of deviation performance; RER, ratio of error range; nLV, number of latent variables; FDVN: First Derivative + Vector Normalization; FDMSC: First Derivative + Multiplicative Scattering Correction; SLS: Straight Line Subtraction.

The RPD and RER values for CP, NDF, ADF, and ash exceeded 3 and 10, respectively, signifying their adequacy as NIRS estimates of the nutritive value of corn silage. Similar RPD and RER values were observed in a study evaluating ryegrass (*Lolium perenne* L.) chemical composition by Bezada et al. (2017) for CP, ethereal extract, ash, and NDF content, reinforcing their appropriateness.

Within the NIR spectral range, the estimates for CP, NDF, ADF, and ash exhibited impressive R^2_{cv} values exceeding 0.95. This is consistent with Fontaneli et al. (2002), who analyzed 246 corn silage samples from diverse regions in Rio Grande do Sul, Brazil and obtained remarkable accuracy (R^2_{cv} = 0.99) for CP, NDF, ADF, and DM parameters. While mineral determination's R^2 ranged from 0.92 to 0.94, a more comprehensive study of spectrum behavior remains warranted, in order to decipher mineral complex formation and its interaction with physicochemical methods. The mean CP, NDF, ADF, and DM values were recorded as 7.86%, 60.66%, 29.87%, and 94.17%, respectively.

In Chile, the study by Ibáñez and Alomar (2008) assessed 920 grass silage samples and reported the efficacy of NIRS in predicting silage chemical composition. High predictive accuracy was demonstrated, with R^2_{cv} surpassing 0.89, and RMSECV values of 6.69, 16.01, and 9.15 for CP, NDF, and ADF, respectively. The CP content results closely aligned with those of Serafim et al. (2021) (R^2_{cv} = 0.96) who evaluated 105 Tifton-85 (*Cynodon* spp.) grass and hay samples. In contrast, the ash, NDF, and ADF content estimates were lower, with R^2_{cv} values of 0.84, 0.80, and 0.80, respectively.

In the course of external validation, it was ascertained that the determination coefficient (R^2_v) and the RPD values exceeded the threshold values of 0.95 and 3, respectively, further substantiating the precision manifested in the prediction of CP, NDF, ADF, and ash contents within tropical grass silages through the application of NIRS (Table 6). Correspondingly, within the realm of external validation, the root mean square error of the prediction (RMSEP) values was observed to be diminutive.

Table 6
Adjusted parameters for the estimation of the crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF), ash contents of tropical grass silages for the external validation sets

Variables	RMSECV (% DM)	R^2_{cv}	RPD	RER
CP (%)	1.77	0.910	2.90	10.05
NDF (%)	2.60	0.985	7.56	23.10
ADF (%)	2.22	0.980	6.37	19.26
Ash (%)	0.55	0.971	4.31	14.64

RMSEP = root mean square error of prediction; DM = dry matter; R^2_v = validation coefficient of determination; RPD = ratio of deviation performance; RER = ratio of error range.

A parallel study conducted by Cozzolino et al. (2003) in Chile, which assessed 200 corn silage samples, showed the potential of NIRS for routine analysis, yielding calibration coefficients (R^2_{cv}) and RMSECV values of 0.94 (RMSECV = 0.54%), 0.91 (RMSECV = 1.8%), and 0.90 (RMSECV = 3.8%) for CP, ADF, and NDF, respectively) on a dry mass basis. The authors stated that NIRS predictability diminishes with parameter complexity, as evidenced by NDF and ash.

In the infrared region, it is difficult to determine complex entities, such as NDF, because NIRS spectral data more accurately represent the sample's chemical structure than elements established via wet chemistry, such as fractions, acid, or neutral fibers, or their constituents that make up the plant cell wall. Notably, the inorganic and mineral substances did not exhibit absorption in the infrared range. Nonetheless, minerals and inorganic elements may be associated with the organic structures of oxides, chelates, or other complexes, allowing indirect NIRS estimation.

Massignani et al. (2021) analyzed 200 forage samples encompassing diverse grasses and legumes and reported robust R^2_{cv} values of 0.94, 0.95, and 0.98, with R^2_v values of 0.94, 0.95, and 0.97 for NDF, ADF, and CP parameters. This underscores the suitability of calibration curves for assessing forage quality across various species, thereby warranting routine laboratory use.

Restaino et al. (2009) evaluated 120 grass silage samples and ascertained the utility of NIRS for predicting DM, ADF, and CP contents with moderate accuracy. Their analysis yielded R^2_{cv} and RMSECV values of 0.73 (SECV = 1.2%), 0.81 (SECV = 2.0%), 0.75 (SECV = 6.6%), 0.80 (SECV = 4.0%), 0.60 (SECV = 3.6%), and 0.70 (SECV = 0.34) for ash, CP, NDF, ADF, *in vitro* dry matter digestibility, and pH, respectively.

Conclusions

DDG utilization resulted in an enhancement of the nutritive value of both elephant grass and tanzania grass silage relative to their fresh counterparts.

The contents of neutral and acid detergent fibers were found to be diminished, and the dry matter content was elevated in the corn silage cultivated under an intercropping system with ruziziensis grass, thus underscoring the significance of implementing integrated production systems.

Estimates by NIRS had high R^2_{cv} values (>0.95), thus highlighting the potential of this technology for the routine analysis of tropical grass silages with respect to parameters such as crude protein, neutral detergent fiber, acid detergent fiber, and ash.

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