

Fermentative profile and nutritive value of maize, legume and mixed silage

Perfil fermentativo e valor nutritivo da silagens de milho, leguminosas e mistas

Luciana Maria da Silva^{1*}; Kátia Aparecida de Pinho Costa²; João Antônio Gonçalves e Silva¹; João Victor Campos Pinho Costa³; Adriano Carvalho Costa²; Eduardo da Costa Severiano²; Patrick Bezerra Fernandes²; Katryne Jordana Oliveira³; Kamilly Tiffany Magalhães Mendonça³; Gercileny Oliveira Rodrigues³

Highlights

Legume exclusive silage shows losses in the fermentative process.

Mixed silage with legumes is a alternative to increase the CP content of silage maize.

Abstract

The partial substitution of maize by tropical legumes for the production of silage has aroused interest, for bringing benefits of increasing the crude protein content of corn-only silage, constituting an important alternative for the production of food. In this context, the objective of this study was to evaluate the effect of addition 30% tropical legumes on the fermentative characteristics and nutritive value of maize silage. The experimental design was entirely randomized with three replications. The treatments consisted of silages: Maize; *Stylosanthes* cv. Campo Grande (80% *S. capitata* and 20% *S. macrocephala*), *Stylosanthes* cv. Bela; Pigeon pea (*Cajanus cajan* cv. BRS Mandarim); maize + 30% Campo Grande; maize + 30% Bela and maize + 30% Pigeon pea. The results show that exclusive legume silage without preservatives present fermentative losses that compromise the silage quality. Addition of 30% legumes to maize silage improves the nutritional quality of the silage without compromising its fermentation profile. *Stylosanthes* cv. Campo Grande and Bela are the most recommended locations for maize silage. Thus, a mixed silage of maize and legumes is an alternative to improve the crude protein content of exclusive maize exclusive silage and reduce fermentative losses of legume silage.

Key words: *Cajanus cajan* cv. BRS Mandarim. Forage conservation. *Stylosanthes* cv. Campo Grande. *Stylosanthes guianensis* cv. Bela. *Zea mays* L.

¹ Students of the Graduate Program in Agricultural Sciences, Agronomy, Instituto Goiano Federal, IF Goiano, Rio Verde, GO, Brazil. E-mail: luy.mari@hotmail.com; joao.antonioigs@hotmail.com

² Profs. Drs., Researcher Graduate Program in Agricultural Sciences, Agronomy and Animal Science, IF Goiano, Rio Verde, GO, Brazil, E-mail: katia.costa@ifgoiano.edu.br; adriano.costa@ifgoiano.edu.br; eduardo.severiano@ifgoiano.edu.br; zoo.patrick@hotmail.com

³ Undergraduate Students in Animal Science, IF Goiano, Rio Verde, GO, Brazil. E-mail: joao.campos@estudante.ifgoiano.edu.br; katrynejordana25@gmail.com; kami_tiffany@hotmail.com; cilenyrv@gmail.com

* Author for correspondence

Resumo

A substituição parcial do milho por leguminosas tropicais para a produção de silagem tem despertado interesse, por trazer benefício de aumentar o teor de proteína bruta da silagem exclusiva de milho, constituindo importante alternativa para a produção de alimento. Neste contexto, objetivou-se avaliar o efeito da inclusão de 30% de leguminosas tropicais sobre as características fermentativas e o valor nutritivo da silagem de milho. O delineamento experimental foi inteiramente casualizado com três repetições. Os tratamentos consistiram das silagens: Milho; *Stylosanthes* cv. Campo Grande (80% *S. capitata* e 20% *S. macrocephala*), *Stylosanthes* cv. Bela; Feijão Guandu (*Cajanus cajan* cv. BRS Mandarin); milho + 30% Campo Grande; milho + 30% Bela e milho + 30% Feijão Guandu. Os resultados mostram que a silagem exclusiva de leguminosas sem conservantes apresenta perdas fermentativas que comprometem a qualidade da silagem. A adição de 30% de leguminosas à silagem de milho melhora a qualidade nutricional da silagem sem comprometer o seu perfil fermentativo. O *Stylosanthes* cv. Campo Grande e Bela são os mais recomendados para a ensilagem com milho. Desta forma, silagem mista de milho e leguminosas é uma alternativa para melhorar o teor de proteína bruta da silagem exclusiva de milho e reduzir as perdas fermentativas da silagem de leguminosas.

Palavras-chave: *Cajanus cajan* cv. BRS Mandarin. Conservação de forragem. *Stylosanthes guianensis* cv. Campo Grande. *Stylosanthes guianensis* cv. Bela. *Zea mays* L.

Introduction

The seasonality of forage production in the tropics, throughout the year, has compromised the animal production systems based on the exclusive exploitation of pasture, resulting in high supply of animal products in summer and decreased production in dry season (Rufino et al., 2022), mainly due to the reduction in nutritional quality of forage that negatively affects animal feed and consequently their performance (Barreto et al., 2020).

Alternatives must be sought to supplement the forage deficit, as the requirements of animals remain constant throughout the year. In this regard, the production of high-quality silage is a viable alternative to the maintenance of forage systems by restricting the feed shortage period and contributing to the improvement

of the zootechnical indices of the herd (Souza et al., 2019).

Maize (*Zea mays* L.) is generally used for silage production because it exhibits ease of cultivation, good green matter yield, good fermentative patterns, maintenance of the nutritive value of the ensiled mass, and good palatability (Guan et al., 2020). However, maize silage has a protein content between 70 and 90 g kg⁻¹ and cannot meet the protein requirements of ruminants (Paludo et al., 2020). In addition, the high cost of maize silage production (Edson et al., 2018) has made its production difficult on a large scale, which may compromise the quantity offered to animals.

In this context, the partial replacement of maize silage with tropical forage crop silages has sparked interest in recent years and has shown positive results for cattle feed

(Paludo et al., 2020; Rufino et al., 2022). In addition, the presence of legumes improves the soil-plant-animal system, mainly in terms of forage quality and atmospheric nitrogen fixation (Boddey et al., 2020), maintaining greater sustainability for food production (Epifanio et al., 2019b).

The introduction of legumes in silage of annual crops, can add benefits such as, balancing the nutritive value, presenting better qualitative characteristics in the dry matter (Pereira et al., 2019; Ligoski et al., 2020), thereby providing higher nutrient production per unit area (S. S. Oliveira et al., 2020), besides its flexibility of use, constituting an important alternative in the period of low forage production (N. C. Oliveira et al., 2021).

Among tropical legumes, *Stylosanthes* cv. Campo Grande stands out as a promising crop with a great potential for silage production, as it is a suitable source of ruminant nutrition (Bao et al., 2022; Silva et al., 2022). *Stylosanthes* cv. Bela, on the other hand, was recently released and has shown positive results due to its high crude protein content (Assis et al., 2018; Braga et al., 2020; Prado et al., 2023), making it a viable alternative for ruminant feed. Another legume that has been highlighted for silage production is the Pigeon pea (*Cajanus cajan*), which is an excellent source of protein in animal feed (Ludkiewickz et al., 2022).

Considering the scarcity of information on maize silage with the addition of cultivars of *Stylosanthes* and Pigeon pea, there is a need for more information, especially regarding the best legume to be added to silage, to improve its nutritional value. Thus, we hypothesized that the addition of 30% tropical legumes to maize silage would positively influence

the bromatological characteristics of the silage without compromising its fermentation process. Thus, the objective of this study was to evaluate the effect of addition 30% tropical legumes on the fermentative characteristics and nutritive value of maize silage.

Materials and Methods

This experiment was conducted in a field (17°48' S, 50°55' W at 748 m altitude) in the municipality of Rio Verde, Goiás in central Brazil during the second crop season of 2021 in a Latossolo Vermelho Acriférico (Santos et al., 2018).

The experimental design was entirely randomized with three repetitions. The treatments consisted of silages: maize (hybrid B 2800 PWU); *Stylosanthes* cv. Campo Grande (80% *S. capitata* and 20% *S. macrocephala*), *Stylosanthes* cv. Bela: Pigeon pea (*Cajanus cajan* cv. BRS Mandarin), maize + 30% Campo Grande; maize + 30% Bela and maize + 30% Pigeon pea.

The crops were then sown separately. The plots consisted of eight rows, three meters long, each row spaced at 0.50 m. Maize and Pigeon pea were sown at a depth of 3 cm, and *Stylosanthes* at a depth of 2 cm. At seeding, 150 kg ha⁻¹ of P₂O₅ was applied as a simple superphosphate, and 30 kg ha⁻¹ of FTE BR 12. For maize, nitrogen and potassium fertilization was performed when the plants had three to six fully developed leaves, and 180 and 120 kg ha⁻¹ of N and K₂O in urea and potassium chloride, respectively. In legumes, 30 and 60 days after sowing (DAS), covering fertilization was performed with 60 kg ha⁻¹ of K₂O (potassium chloride).

Two manual weeding campaigns were performed to control weeds in both the crops. Maize crop was provided with phytosanitary control throughout the development, including two applications of the insecticide Lannate (methomyl active ingredient) in the proportion of 0.4 L ha⁻¹ of commercial product.

The maize was harvested for silage when it presented 340.2 g kg⁻¹ DM (dry matter), the legumes in the development cycle of 100 days with 260.3 g kg⁻¹ DM for *Stylosanthes* cv. Campo Grande, 266.1 g kg⁻¹ DM for *Stylosanthes* cv. Bela, and 278.5 g kg⁻¹ DM for Pigeon pea. The crops were harvested 20 cm from the soil. Subsequently, the components were separately chopped in a stationary forage grinder into particle size of approximately 10 mm.

Soon after, the maize was homogenized with legumes for the treatments with the addition of 30% legumes, calculated based on natural matter. No chemical additive (preservative) was applied to improve silages fermentation. The material was stored in PVC experimental silos measuring 10 cm in diameter and 40 cm in length. Subsequently, the silos were compacted with a pendulum, closed with PVC lids, and sealed with adhesive tape to prevent air from entering. The silos were stored in the laboratory at room temperature and were protected from rain and sunlight.

Before ensiling, analyses were performed on the raw materials (Maize, *Stylosanthes* cv. Campo Grande, Bela, and Pigeon pea) to determine the dry matter (DM), crude protein (CP), lignin, and ether extract (EE) according to the method described by the Association Official Analytical Chemists [AOAC] (1990), neutral detergent fiber (NDF),

and acid detergent fiber (ADF) using the method described by Mertens, (2002). The total digestible nutrients (TDN) were obtained using the equation proposed by Chandler (1990) and the *in vitro* dry matter digestibility (IVDMD) was determined by the technique of Tilley and Terry, (1963), adapted to the artificial rumen, developed by ANKON®, using the "Daisy incubator" instrument of Ankom Technology (*in vitro* true digestibility [IVTD]).

The silos were opened after 50 days of fermentation, and the upper and lower portions were discarded. The central portion was homogenized and placed in a plastic tray. The analysis of the fermentative parameters, including buffering capacity, pH, and ammoniacal nitrogen per total nitrogen (N-NH₃/NT) was performed following the methodology of Bolsen et al. (1992).

The pH and buffering capacity were determined when the silos were opened, so as to avoid changes in the expected values due to heat and humidity. To determine ammoniacal nitrogen, the silage was frozen to inactivate the activity of anaerobic bacteria, thus avoiding nitrogen volatilization, and the samples were later thawed for juice extraction (Bolsen et al., 1992).

The total dry matter (DM) loss and effluent production were determined using the methodology of Jobim et al. (2007). Organic acids (lactic, acetic, butyric, and propionic acids) were determined using a Shimadzu SPD-10A VP liquid chromatograph (HPLC) coupled with an ultraviolet (UV) detector at 210 nm wavelength (Kung & Shaver, 2001).

The remaining portion of the material (approximately 0.5 kg) was weighed and dried in a forced ventilation oven at 55°C until a constant weight was obtained. The samples

were then ground in a knife mill, cleared using a 1 mm sieve, and stored in plastic containers. The chemical and bromatological

characteristics of the silage were analyzed using the methodology described for fresh material.

Table 1

Chemical-bromatological composition of Maize, *Stylosanthes* cv. Bela, *Stylosanthes* cv. Campo Grande and Pigeon pea, before silage

Chemical composition	Maize	Campo Grande	Bela	Pigeon pea
DM (g kg ⁻¹)	340.1	260.2	266.1	278.5
CP (g kg ⁻¹ DM)	78.0	162.1	150.5	161.5
NDF (g kg ⁻¹ DM)	554.0	566.4	574.7	615.2
ADF (g kg ⁻¹ DM)	281.8	338.6	340.7	372.1
Lignin (g kg ⁻¹ DM)	26.6	30.8	295.4	43.9
EE (g kg ⁻¹ DM)	46.8	22.2	223.6	21.3
IVDMD (g kg ⁻¹ DM)	668.8	657.1	653.5	556.2
TDN (g kg ⁻¹ DM)	623.5	527.5	523.6	550.3

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; IVDMD, in vitro dry matter digestibility; TDN, total digestible nutrients.

The data were subjected to analysis of variance using the R program version R-3,1,1 (2014) and the ExpDes package. Means were compared using Tukey's test, with a significance level of 5% probability.

Multivariate factor analysis was performed considering the means of the variables for each treatment using the MVar.pt computational package of the R computer program. Subsequently, a regression analysis of the scores of the first factor was performed as a function of dosage.

Results and Discussion

Fermentative characteristics (pH, buffering capacity, DM, N-NH₃, total losses DM, effluent production, lactic acid, and acetic

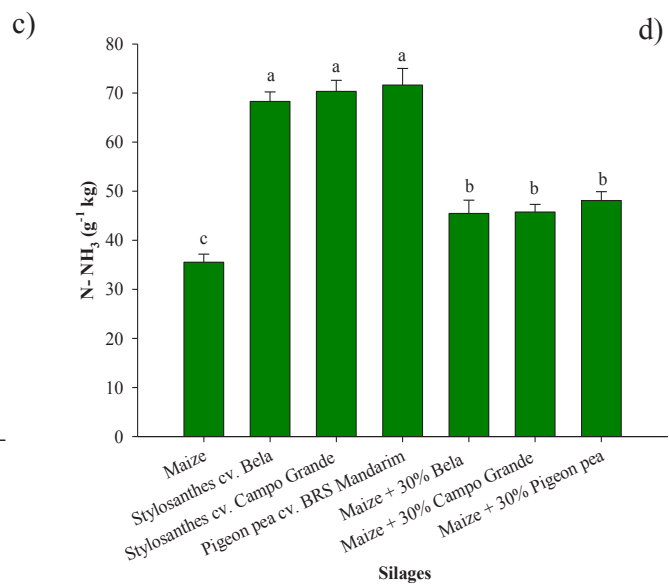
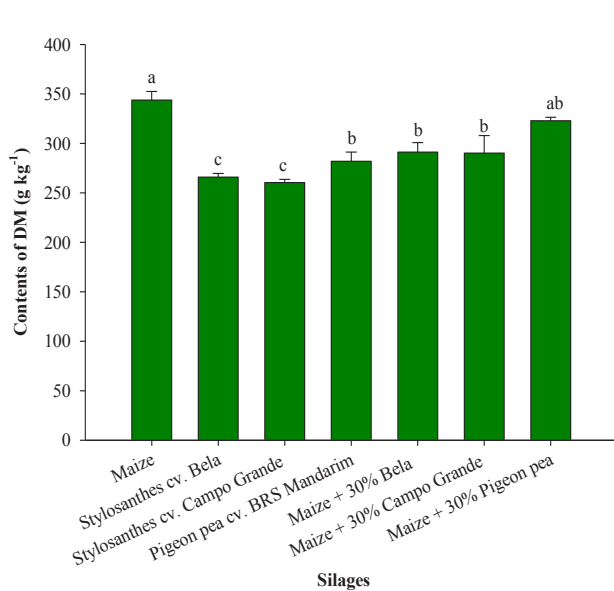
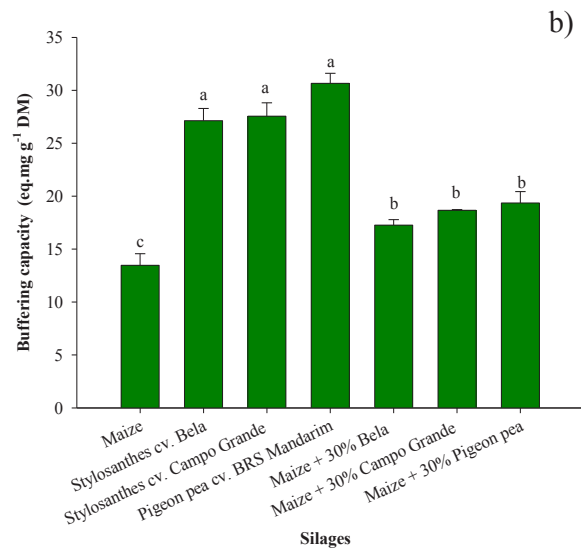
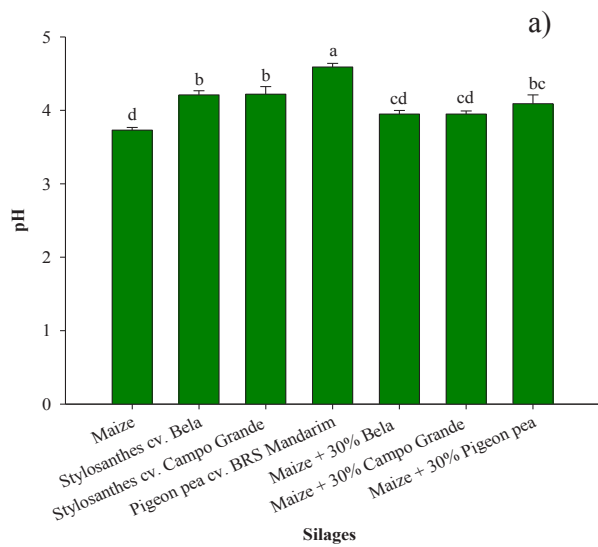
acid) were influenced ($p < 0.05$) by the use of different silages. For butyric and propionic acids, there was no significant difference ($p > 0.05$) between the silages.

Maize silage showed the lowest pH value (3.69), and Pigeon pea silage had the highest value (4.57), followed by *Stylosanthes* cv. Bela (4.23), and Campo Grande (4.25) silage (Figure 1a). The higher pH values in silage exclusive of legumes can be explained by the higher buffering capacity. Legumes, in general, present resistance to lowering of pH, because of the presence of buffering substances such as potassium (K⁺), calcium (Ca²⁺), and magnesium (Mg²⁺), which neutralize the organic acids formed by fermentation, preventing the reduction of pH (Smith, 1962), in addition to the low content of DM and soluble carbohydrates (Hawu et al., 2022). Kung et al.

(2018) reported pH values between 4.2 and 4.7 for legume silages, however, it is worth noting that because of these values, the final quality of the silage can be compromised due to the growth of undesirable microorganisms in the fermentative process.

The production of mixed maize silage with legumes lowered the pH value (Figure 1a). This reduction occurred because of the

presence of 70% maize in the composition of the silage, which ensured an adequate fermentation process owing to favorable fermentative characteristics of the crop (Bolson et al., 2022). For good-quality silage, the appropriate pH range is between 3.7 and 4.2 (Mcdonald et al., 1991), indicating that the mixed silages showed an adequate pH.



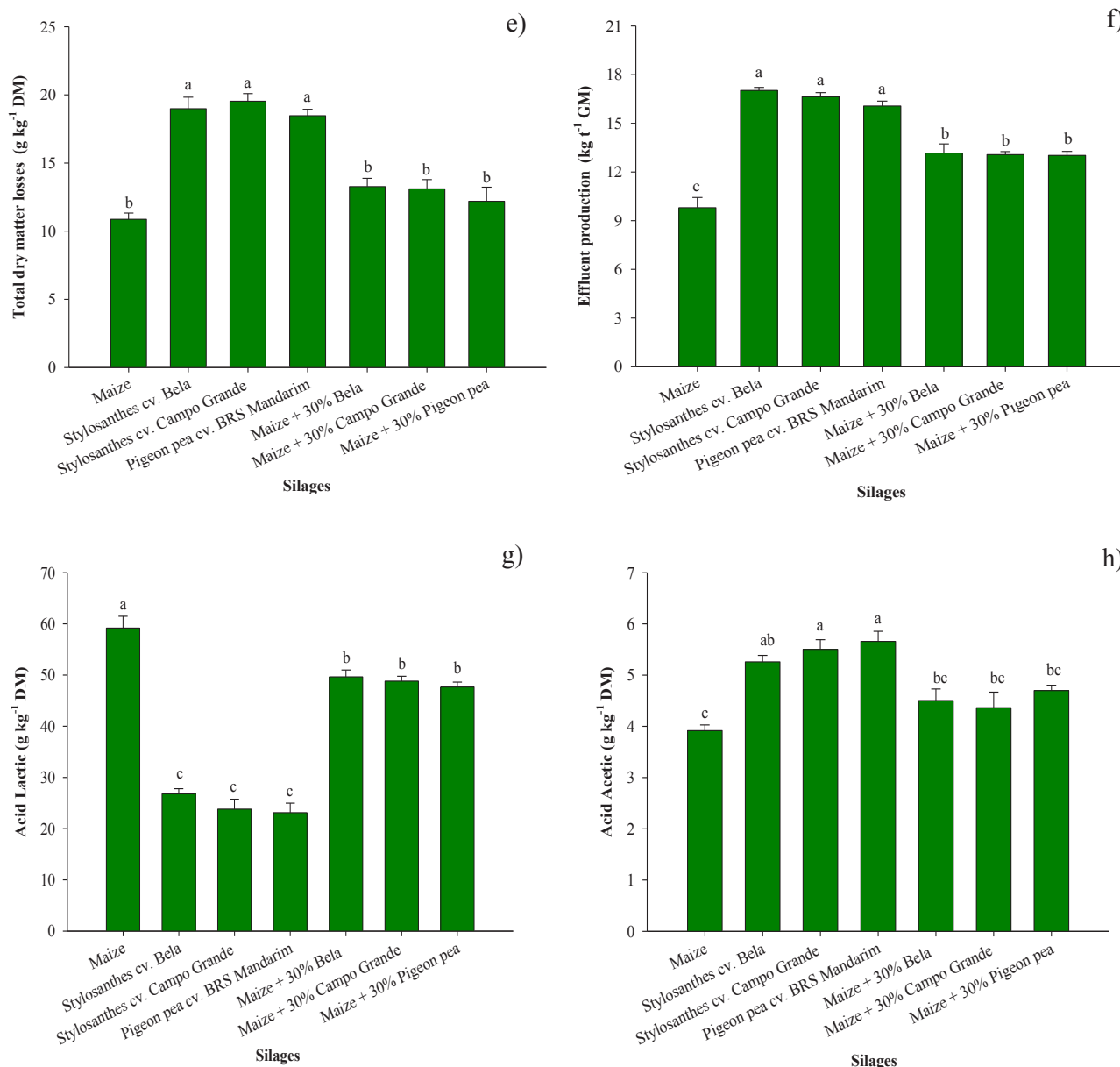


Figure 1. Values of pH (a), buffering capacity (b), dry matter (DM) (c), NH₃-NT (d), total dry matter losses (e), effluent production (f), lactic (g) and acetic (h) acid contents of maize, legume and mixed silage.

Means followed by different letters differ by Tukey test at 5% probability. Vertical bars represent standard error of mean of each point.

Exclusive legume silages have high buffering capacity, as shown in Figure (1b), making them susceptible to proteolysis

during fermentation as result of the high buffering capacity (Baghdadi et al., 2016). In contrast, mixed silages contributed to

a 35.2% reduction in buffering capacity compared to exclusive legume silages. For mixed and maize silages, the values obtained were below 20 eq.mg HCl/100 g DM (Ferrari & Lavesso, 2001), indicating that fermentation occurred adequately, ensuring high-quality silage. These results showed that fermentation occurred adequately, ensuring the production of good quality silage. Thus, it is possible to verify the importance of producing mixed silages to reduce the pH and buffering capacity of exclusive legume silages, ensuring adequate preservation of ensiled material.

The maize silage showed a higher DM content (344.4 g kg⁻¹) (Figure 1c), similar to the mixed maize silage with Pigeon pea (320.6 g kg⁻¹). These results are within the range considered ideal for proper fermentation of ensiled material, which is 270–380 g kg⁻¹ DM (McDonald et al., 1991).

In contrast, silages exclusively from *Stylosanthes* showed lower levels of DM, with an average of 260 g kg⁻¹, which is below the appropriate range. The lower DM content obtained in case of *Stylosanthes* can be explained by the large proportion of leaves that are found in legumes (Epifanio et al., 2019a). *Stylosanthes* show a high moisture content, which is undesirable in the ensiling process and can result in inadequate fermentative characteristics, thereby compromising the final quality of the feed (Hawu et al., 2022). Thus, determining the DM content of the material before ensiling is a primary factor in the fermentative process, as it directly affects the final quality of the silage produced (Borreani et al., 2018).

Analysis of N-NH₃ revealed that the silages of exclusive legumes showed higher N-NH₃ values (72.67; 69.61; 68.98, for the Pigeon pea silage, *Stylosanthes* cv. Campo Grande, and Bela (Figure 1d). The mixed silages led to a reduction in the values of N-NH₃ compared to the exclusive legume silages that presented the highest values. The higher proportion of maize (70%) in mixed silages explains this result because maize presents an adequate concentration of soluble carbohydrates, the main substrate used by lactic acid bacteria in the fermentative process which ensures a rapid fermentation and reduced loss of nutrients (Li et al., 2022).

The results observed in the present study are in agreement with Kung et al. (2018), who cited levels below 100 g kg⁻¹ of N-NH₃, for the classification of good quality silage, demonstrating that there was an action of bacteria from the genus *Lactobacillus*, allowing the efficient production of lactic acid and inhibiting the growth of undesirable microorganisms in the fermentative process, preserving the nutritional value of the ensiled material (N. C. Oliveira et al., 2021).

The silages of exclusive legumes showed the highest total DM losses (Figure 1e), which was due to lower DM content of legumes at the time of cutting for ensiling (Table 1) compared to that of maize. Legumes exhibit a higher buffering capacity, high moisture, low concentration of soluble carbohydrates, expressive water activity, and undesirable characteristics at the time of ensiling, which results in a prolonged fermentative process with high consumption of fermentable substrates, resulting in higher DM losses (Borreani et al., 2018; Castro-Montoya & Dickhoefer, 2020).

Mixed silages expressed the ability to reduce the total DM loss, exclusively in legume silages (Figure 1e). This result was possibly due to the higher proportion of maize (70%) and adequate DM content at the time of ensiling (340.1 g kg^{-1}). These results corroborate those of Parra et al. (2019), who observed lower DM losses when maize represented the largest proportion of the maize-soybean silage.

The exclusive legume silages also showed higher effluent production (Figure 1f). The lower DM content of legumes in the silage (Table 1) was positively related to this result. High effluent production entails nutrient losses by leaching, compromising the nutritive value of the silage and negatively impacting the natural soil microbiota, thereby leading to the emission of greenhouse gases, such as nitrous oxide (Araújo et al., 2020).

Mixed silage production has been efficient in reducing the effluent production of legume silage. As shown earlier, the higher proportion of maize in the composition of mixed silages and its adequate DM content during ensiling contributed to the reduction in effluent production and DM losses. Amorim et al. (2020) confirmed that the DM losses in the form of effluents is directly related to the characteristics of the ensiled material. Given these results, it is worth emphasizing the importance of producing mixed silages to improve the fermentative process of exclusive legume silages, thus allowing an adequate conservation of the ensiled material.

Analyzing the organic acids, it was possible to observe that maize silage showed the highest values of lactic acid, followed by mixed silages that showed an increase of 48,98% in lactic acid production compared

to silage of exclusive legumes (Figure 1g). Similar results were observed by Hawu et al. (2022), who evaluated the sustainable use of legumes in maize straw silage and observed an increase in lactic acid production in maize-legume silages. Under proper silage fermentation conditions, lactic acid is the main product, which contributes to the rapid stabilization of pH and preservation of the ensiled material (Carvalho et al., 2016; S. S. Oliveira et al., 2020).

The *Stylosanthes* cv. Campo Grande and Pigeon pea silages presented the highest values of acetic acid (Figure 1h), followed by *Stylosanthes* cv. Bela silage. This result may be due to the lower DM content in legumes. Mixed silages showed similar results to maize silage, showing once again the positive contribution of mixed silage in providing adequate silage fermentation. Even with these results, the acetic acid production of all silages was within the appropriate range of classification, below 20 g kg^{-1} DM, indicating that the fermentative process was efficient in preserving ensiled material (Kung et al., 2018). These results demonstrate that during fermentation, there was greater activity of bacteria of the genus *Lactobacillus*, co-producers of lactic acid, ensuring a rapid pH drop, and consequently adequate preservation of the ensiled material (Ni et al., 2018).

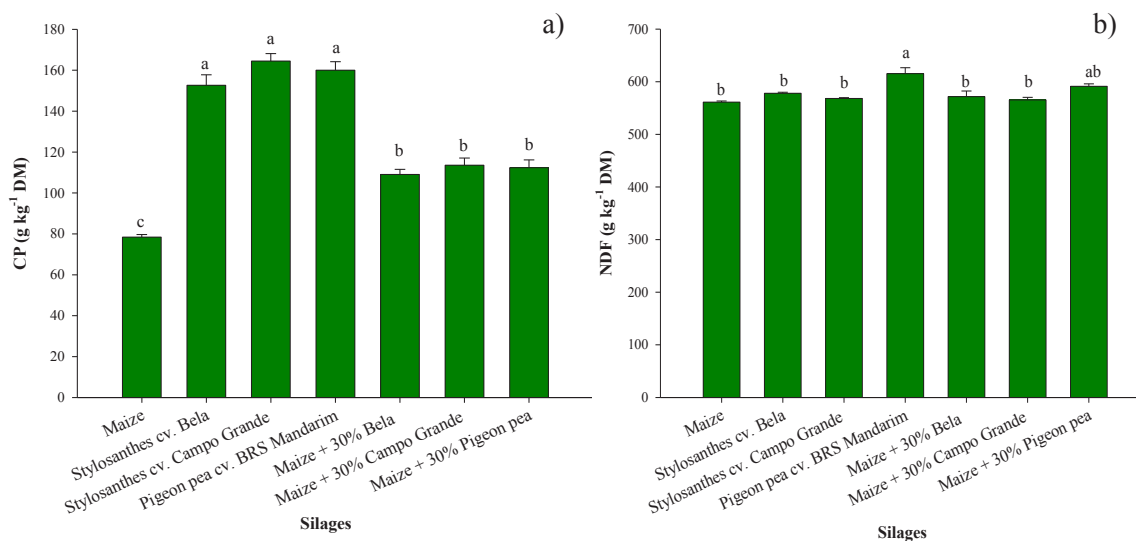
There was a significant effect ($p < 0.05$) on the nutritive values (CP, NDF, ADF, lignin, IVDMD, EE, and TDN) of the different silages. The exclusive legume silages showed higher CP values, whereas maize silage showed the lowest value (75.4 g kg^{-1} DM). The addition of 30% legumes to maize silage resulted in an increase of 38.1%, 50.12%, and 49.9% in the CP content of the mixed maize silages with *Stylosanthes* cv. Bela, Campo Grande,

and Pigeon pea, respectively (Figure 2a). As legumes present higher CP content when added to maize silage, which does not present great prominence in this value (Souza et al., 2019), it will be possible to make a forage material of better nutritional quality.

Increases in the CP content of mixed silage containing legumes were also obtained in the studies of Epifanio et al. (2016), Pereira et al. (2019), Ligoski et al. (2020) and Ludkiewickz et al. (2022), showing the benefits and relevance of mixed silage production by increasing the protein value of silages, which can contribute to the reduction in cost of purchasing protein salts and/or concentrates, aiming at protein supply. In addition, exclusive maize silage presents a high production cost (Edson et al., 2018), and mixed maize silage with legumes is a viable alternative to reduce the cost of silage production, since forage legumes (*Stylosanthes* and Pigeon pea) are of low requirement in soil fertility (Epifanio

et al., 2019a; Costa et al., 2021) and adds to the improvement of fertility through nitrogen fixation (Epifanio et al., 2019b).

The exclusive Pigeon pea silage, followed by the mixed maize with Pigeon pea silage, had a higher content of NDF (Figure 2b) and lignin (Figure 2d). This result is due to the larger diameter of the stalk in this legume, concentrating a greater amount of fiber (Pereira et al., 2019). Shrub legumes, such as Pigeon pea, have a higher lignin content than herbaceous legumes, such as *Stylosanthes*. Moreover, high lignin content of shrub legumes has a negative relationship with digestibility and thus, is considered as a limiting factor for use as an exclusive food resource for animals (Castro-Montoya & Dickhoefer, 2020). In contrast, the maize and *Stylosanthes* cv. Bela and Campo Grande silages, both exclusive and mixed, showed similar results for NDF and lignin content.



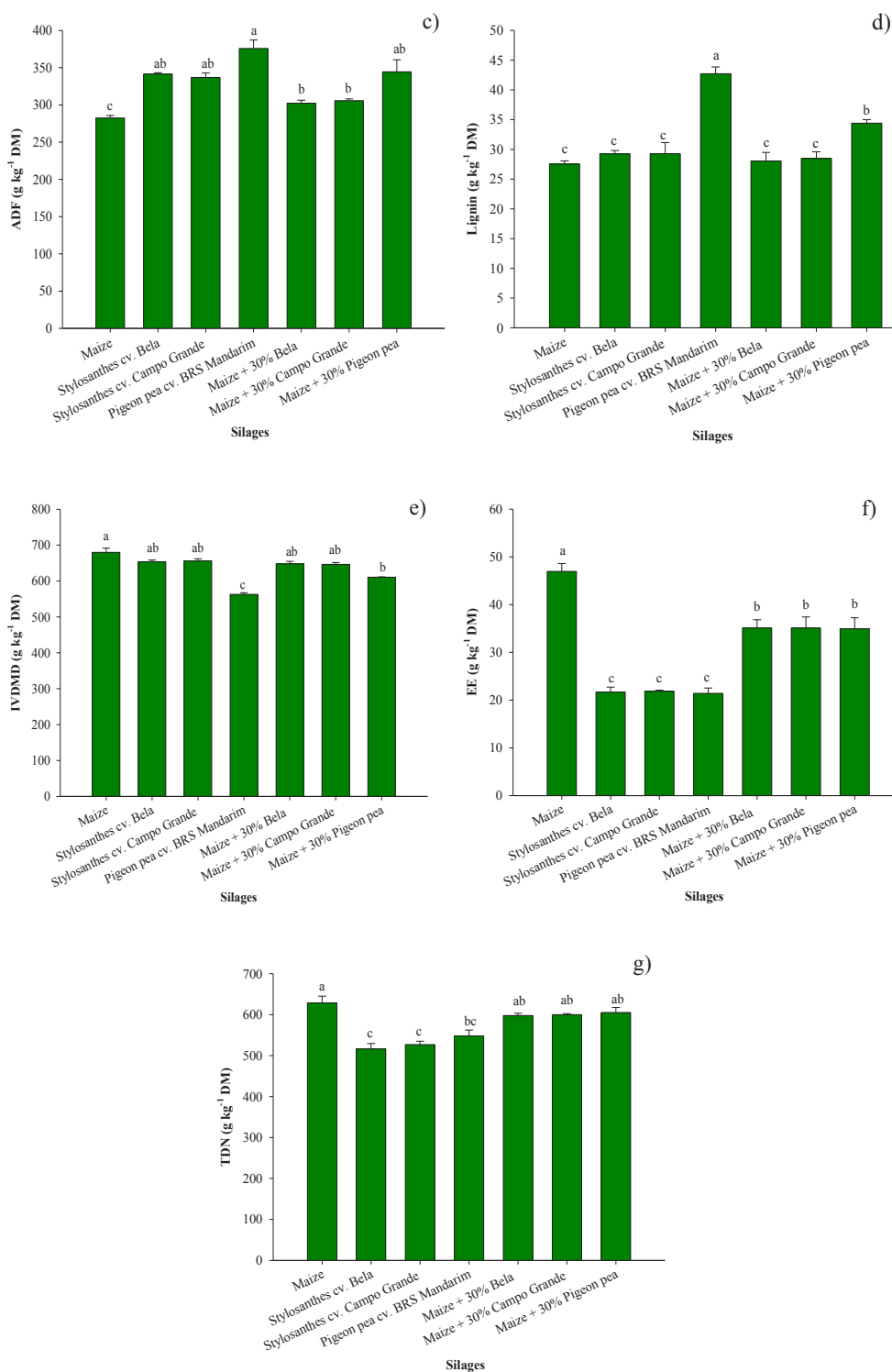


Figure 2. Crude protein (CP) (a), neutral detergent fiber (NDF) (b), acid detergent fiber (ADF) (c), lignin (d), In vitro digestibility contents of dry matter (IVDMD) (e), ether extract (EE) (f) and total digestible nutrients (TDN) (g) contents of maize, legume and mixed silage. Means followed by different letters differ by Tukey test at 5% probability. Vertical bars represent standard error of mean of each point.

Regarding the levels of ADF, it was possible to verify that the maize silage had the lowest content, followed by the mixed silages with *Stylosanthes*, once again showing the contribution of mixed silage to decrease the levels of ADF in exclusive legume silages (Figure 2c). It is worth noting that though the exclusive legume silages showed higher levels of ADF, these values were below $400 \text{ g kg}^{-1} \text{ DM}$, which, according to Van Soest (1994), results in the unavailability of degradable structural carbohydrates, due to the lignin present in the cell wall, which hinders the adherence of the rumen microbiota and consequent enzymatic hydrolysis of some components such as cellulose and hemicellulose, reducing fiber digestibility.

Maize, *Stylosanthes* cv. Bela and Campo Grande silages-exclusive and mixed provided higher levels of IVDMD, differing from the Pigeon pea exclusive silage (Figure 2e). The lower digestibility of this legume can be explained by the higher concentration of fibrous material and lignin polymers in its components, which can result in its reduced digestibility (Pereira et al., 2019). When this material is provided exclusively for the consumption of small and large ruminants, it is likely to preclude maximum performance in the production system (Rufino et al., 2022).

Maize silage showed the highest EE content, differentiating it from the other silages (Figure 2f). The mixed maize and legume silages contributed to a 12.02% increase in the EE content of the exclusive legume silages. Tropical forages have a low EE, which measures the amount of fat in food and is very important for maintaining an adequate TDN content (S. S. Oliveira et al., 2020).

The TDN represents the energy content of food (Marques et al., 2019) and results showed (Figure 2g) that maize silage showed the highest values of TDN followed by mixed silages which showed no difference in the values. Legume silages showed the lowest values, which can be explained by the lowest levels of EE. Thus, the benefits of using mixed silages are evident as they increase the energy of exclusive legume silages. In addition, it is worth noting that TDN and protein are essential variables for ruminants, and may be the most limiting (Souza et al., 2019).

The definition of the number of factors that explain the variation in the data is based on the eigenvalue. A factor contributes to explaining the data variation when it has a value greater than or equal to one. Value less than one indicates that the factor contributes little to explain the data variation. In this work, it was verified that only the first and second factors had eigenvalues greater than 1. The first factor was responsible for 79.51% and the second for 16.58%, explaining 96.09% of the total data variation (Figure 3).

Considering the value of fermentation and nutritive variables, all variables showed high correlation ($|r| > 0.70$) with the first factor, except for lignin, NDF, and IVDMD, which showed high correlation with the second factor ($|r| > 0.70$), thus demonstrating that factors I and II are relevant to explain the behavior of variables and subjected treatments. It was also observed that the communality was greater than 0.98 for all variables, indicating that the factors explain the variables.

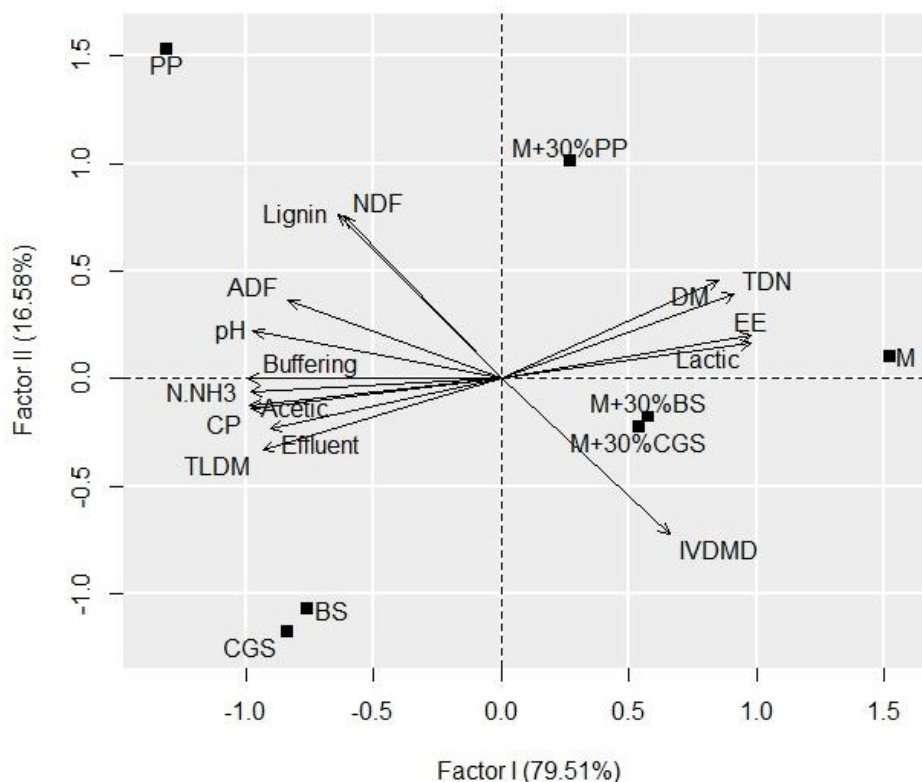


Figure 3. Two-dimensional scatter plot of the factorial loading matrix and scores of the 15 variables of fermentative characteristics and nutritive value of maize, legume and mixed silage.

M, Maize; BS, *Stylosanthes* cv. Bela, CGS, *Stylosanthes* cv. Campo Grande, PP, Pigeon pea; M+30%BS, Maize + 30% Bela; M+30%CGS, Maize + 30% Campo Grande; M+30%PP, Maize + 30% Pigeon pea; DM, dry matter; TLDM, total losses of DM; Effluent, effluent production; pH; N. NH₃, ammoniacal nitrogen; Buffering, buffering capacity; Lactic, lactic acid; Acetic, acetic acid; CP; NDF; ADF; Lignin; EE; IVDMD; and TDN.

Using multivariate factor analysis (Figure 3), it was possible to classify the variables into two groups, one including the variables that were to the left and the other to the right on the horizontal axis. From the angle between the arrows, it can be seen that, within the groups of variables, there is a high positive correlation observed between them. For the variables in different groups, there was a high negative correlation.

Factor I is represented horizontally in the scatterplot, whereas Factor II is

represented vertically (Figure 3). It can be observed that the variables were able to separate the treatments in a coherent way, thus facilitating the understanding of the results, with the discrimination of five groups of treatments forming the following groups: 1: PP; 2: CGS and BS; 3: M+30%PP; 4: M+30%BS and M+30%CGS and 5: M

The production of mixed maize silages with the inclusion of 30% legumes (Groups 3 and 4) was shown to be efficient in improving the DM, TDN, EE, lactic acid production, and

digestibility and reducing the pH, N-NH₃, lignin NDF, ADF, buffering capacity, acetic acid, DM losses, and effluent production of exclusive legume silage (Groups 1 and 2), in addition to improving the crude protein content of the exclusive maize silage.

Conclusions

Exclusive legume silage without preservatives present fermentative losses that compromise silage quality. Addition of 30% legumes in maize silage improves the nutritional value of the silage without compromising its fermentative profile.

Stylosanthes cv. Campo Grande and Bela are the most recommended locations for maize silage. Thus, mixed silages of maize and legumes can be a good alternative to improve the nutritional profile of maize exclusive silage and reduce fermentative losses of exclusive legume silage.

Acknowledgments

We thank the Federal Institute of Goiano for supporting this study and the Coordination for the Improvement of Higher Education Personnel (Capes) for granting a doctoral scholarship.

References

- Amorim, D. S., Edvan, R. L., Nascimento, R. R., Bezerra, L. R., Araújo, M. J., Silva, A. L., Mielezrski, F., & Nascimento, K. D. S. (2020). Fermentation profile and nutritional value of sesame silage in relation to usual silages. *Italian Journal of Animal Science*, 19(1), 230-239. doi: 10.1080/1828051X.2020.1724523
- Araújo, J. A. S., Almeida, J. C. C., Reis, R. A., Carvalho, C. A. B., & Barbero, R. P. (2020). Harvest period and baking industry residue inclusion on production efficiency and chemical composition of tropical grass silage. *Journal of Cleaner Production*, 266, 121953. doi: 10.1016/j.jclepro.2020.121953
- Assis, G. M., Beber, P. M., Miqueloni, D. P., & Simeão, R. M. (2018). Identification of stylo lines with potential to compose mixed pastures with higher productivity. *Grass and Forage Science*, 73(4), 897-906. doi: 10.1111/gfs.12383
- Association Official Analytical Chemists (1990). *Official methods of analysis* (15th ed.). AOAC.
- Baghdadi, A., Halim, R. A., Radziah, O., Martin, M. Y., & Ebrahimi, M. (2016). Fermentation characteristics and nutritive value of corn silage intercropped with soybean under different crop combination ratios. *Journal of Animal & Plant Sciences*, 26(6), 1710-1717.
- Bao, J., Wang, L., & Yu, Z. (2022). Effects of different moisture levels and additives on the ensiling characteristics and in vitro digestibility of *Stylosanthes* silage. *Animals*, 12(12), 1555. doi: 10.3390/ani12121555
- Barreto, R. F., Prado, R. D. M., Habermann, E., Viciado, D. O., & Martinez, C. A. (2020). Warming change nutritional status and improve *Stylosanthes capitata* Vogel growth only under well-watered conditions. *Journal of Soil Science and Plant Nutrition*, 20(4), 1838-1847. doi: 10.1007/s42729-020-00255-5

- Boddey, R. M., Casagrande, D. R., Homem, B. G., & Alves, B. J. (2020). Forage legumes in grass pastures in tropical Brazil and likely impacts on greenhouse gas emissions, a review. *Grass and Forage Science*, 75(4), 357-371. doi: 10.1111/gfs.12498
- Bolsen, K. K., Lin, C., & Brent, B. E. (1992). Effect of silage additives on the microbial succession and fermentation process of alfalfa and corn silages. *Journal of Dairy Science*, 75(11), 3066-3083. doi: 10.3168/jds.S0022-0302(92)78070-9
- Bolson, D. C., Jacovaci, F. A., Gritti, V. C., Bueno, A. V. I., Daniel, J. L. P., Nussio, L. G., & Jobim, C. C. (2022). Intercropped maize-soybean silage, Effects on forage yield, fermentation pattern and nutritional composition. *Grassland Science*, 68(1), 3-12. doi: 10.1111/grs.12323
- Borreani, G., Tabacco, E., Schmidt, R. J., Holmes, B. J., & Muck, R. E. (2018). Silage review, factors affecting dry matter and quality losses in silages. *Journal of Dairy Science*, 101(5), 3952-3979. doi: 10.3168/jds.2017-13837
- Braga, G. J., Ramos, A. K. B., Carvalho, M. A., Fonseca, C. E. L., Fernandes, F. D., & Fernandes, C. D. (2020). Liveweight gain of beef cattle in *Brachiaria brizantha* pastures and mixtures with *Stylosanthes guianensis* in the Brazilian savannah. *Grass and Forage Science*, 75(2), 206-215. doi: 10.1111/gfs.12473
- Carvalho, W. G., Costa, K. A. P., Epifanio, P. S., Perim, R. C., Teixeira, D. A. A., & Medeiros, L. T. (2016). Silage quality of corn and sorghum added with forage peanuts. *Revista Caatinga*, 29(2), 465-472. doi: 10.1590/1983-21252016v29n224rc
- Castro-Montoya, J. M., & Dickhoefer, U. (2020). The nutritional value of tropical legume forages fed to ruminants as affected by their growth habit and fed form, A systematic review. *Animal Feed Science and Technology*, 269, 114641. doi: 10.1016/j.anifeedsci.2020.114641
- Chandler, P. (1990). Energy prediction of feeds by forage testing explorer. *Feedstuffs*, 62(36), 1-12.
- Costa, N. R., Crusciol, C. A., Trivelin, P. C., Pariz, C. M., Costa, C., Castilhos, A. M., Souza, D. M., Bossolani, J. W., Andreotti, M., Meirelles, P. R. L., Moretti, L. G., & Mariano, E. (2021). Recovery of 15N fertilizer in intercropped maize, grass and legume and residual effect in black oat under tropical conditions. *Agriculture, Ecosystems & Environment*, 31, 107226. doi: 10.1016/j.agee.2020.107226
- Edson, C., Takarwirwa, N. N., Kuziwa, N. L., Stella, N., & Maasdorp, B. (2018). Effect of mixed maize-legume silages on milk quality and quantity from lactating smallholder dairy cows. *Tropical Animal Health and Production*, 50, 1255-1260. doi: 10.1007/s11250-018-1552-4
- Epifanio, P. S., Costa, K. A. P., Guarnieri, A., Teixeira, D. A. A., Oliveira, S. S., & Silva, V. R. (2016). Silage quality of *Urochloa brizantha* cultivars with levels of Campo Grande *Stylosanthes*. *Acta Scientiarum. Animal Sciences*, 38(2), 135-142. doi: 10.4025/actascianimsci.v38i2.29631
- Epifanio, P. S., Costa, K. A. P., Severiano, E. C., Simon, G. A., & Silva, V. R. (2019b). Nitrogen nutrition and changes in the chemical attributes of the soil for cultivars of *Brachiaria brizantha* intercropped with *Stylosanthes* in different forage

- systems. *Archives of Agronomy and Soil Science*, 66(8), 1154-1169. doi: 10.1080/03650340.2019.1658867
- Epifanio, P. S., Costa, K. A. P., Severiano, E. C., Souza, W. F., Teixeira, D. A. A., Silva, J. T., & Moura Aquino, M. (2019a). Productive and nutritional characteristics of *Brachiaria brizantha* cultivars intercropped with *Stylosanthes* cv. Campo Grande in different forage systems. *Crop and Pasture Science*, 70(8), 718-729. doi: 10.1071/CP18447
- Ferrari, E., Jr., & Lavezzo, W. (2001). Qualidade da silagem de capim-elefante (*Pennisetum purpureum* Schum) emurcheado ou acrescido de farelo de mandioca. *Revista Brasileira de Zootecnia*, 30(5), 1424-1431. doi: 10.1590/S1516-35982001000600006
- Guan, H., Shuai, Y., Yan, Y., Ran, Q., Wang, X., Li, D., Cai, Y., & Zhang, X. (2020). Microbial community and fermentation dynamics of corn silage prepared with heat-resistant lactic acid bacteria in a hot environment. *Microorganisms*, 8(5), 1-18. doi: 10.3390/microorganisms8050719
- Hawu, O., Ravhuhali, K. E., Mokoboki, H. K., Lebopa, C. K., & Sipango, N. (2022). Sustainable use of legume residues, effect on nutritive value and ensiling characteristics of maize straw silage. *Sustainability*, 14(11), 6743. doi: 10.3390/su14116743
- Jobim, C. C., Nussio, L. G., Reis, R. A., & Schmidt, P. (2007). Avanços metodológicos na avaliação da qualidade da forragem conservada. *Revista Brasileira de Zootecnia*, 36(Suppl), 101-119. doi: 10.1590/S1516-35982007001000013
- Kung, L., Jr., & Shaver, R. (2001). Interpretation and use of silage fermentation analyses Reports. *Focus on Forage*, 3(13), 1-5.
- Kung, L., Jr., Shaver, R. D., Grant, R. J., & Schmidt, R. J. (2018). Silage review, interpretation of chemical, microbial, and organoleptic components of silages. *Journal of Dairy Science*, 101(5), 4020-4033. doi: 10.3168/jds.2017-13909
- Li, J., Wen, X., Yang, J., Yang, W., Xin, Y., Zhang, L., Liu, H., He, Y., & Yan, Y. (2022). Effects of maize varieties on biomass yield and silage quality of maize-soybean intercropping in the Qinghai-Tibet Plateau. *Fermentation*, 8(10), 542. doi: 10.3390/fermentation8100542
- Ligoski, B., Gonçalves, L. F., Claudio, F. L., Alves, E. M., Krüger, A. N., Bizzuti, B. E., Lima, P. M. T., Abdalla, A. L., & Paim, T. P. (2020). Silage of intercropping corn, palisade grass, and pigeon pea increases protein content and reduces in vitro methane production. *Agronomy*, 10(11), 1784. doi: 10.3390/agronomy10111784
- Ludkiewickz, M. G., Andreotti, M., Modesto, V. C., Nakao, A. H., Araujo, O., Jr., & Pechoto, E. A. P. (2022). Densidade de semeadura de guandu-anão para produção de silagem de milho safrinha consorciado ou não com capim marandu em cerrado de baixa altitude. *Agrarian*, 15(55), e15281. doi: 10.30612/agrarian.v15i55.15281
- Marques, K. O., Jakelaitis, A., Guimarães, K. C., Pereira, L. S., Cardoso, I. S., & Lima, S. F. (2019). Production, fermentation profile, and nutritional quality of silage from corn and soybean intercropping *Semina: Ciências Agrárias*, 40(6), 3143-3156. doi:10.5433/1679-0359.2019v40n6Supl2p3143

- Mcdonald, P. J., Henderson, A. R., & Heron, S. J. E. (1991). *The biochemistry of silage* (2nd ed.). Mallow Chalcombe Publications.
- Mertens, D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beaker or crucibles, collaborative study. *Journal of AOAC International*, 85(6), 1217-1240. doi: 10.1093/jaoac/85.6.1217
- Ni, K., Zhao, J., Zhu, B., Su, R., Pan, Y., Ma, J., Zhong, G., Tao, Y., Liu, X., & Zhong, J. (2018). Assessing the fermentation quality and microbial community of the mixed silage of forage soybean with crop corn or sorghum. *Bioresource Technology*, 265, 563-567. doi: 10.1016/j.biortech.2018.05.097
- Oliveira, N. C., Costa, K. A. P., Rodrigues, L. G., Silva, A. C. G., Costa, J. V. C. P., Silva, S. Á. A., Assis, L. F. A., Oliveira, S. M. P., & Vieira, M. L. (2021). Fermentation characteristics and nutritive value of sweet sorghum silage with Paiaguas palisadegrass and Ipypora grass. *Semina: Ciências Agrárias*, 42(3), 1923-1940. doi: 10.5433/1679-0359.2021v42n3 Supl1p1923
- Oliveira, S. S., Costa, K. A. P., Souza, W. F., Santos, C. B., Teixeira, D. A. A., & Costa, V. (2020). Production and quality of the silage of sorghum intercropped with Paiaguas palisadegrass in different forage systems and at different maturity stages. *Animal Production Science*, 60(5), 694-704. doi: 10.1071/AN17082
- Paludo, F., Costa, K. A. P., Dias, M. B. C., Santos, F. A., Silva, A. C. G., Rodrigues, L. G., Silva, S. A. A., Souza, W. F., Bilego, U. O., & Muniz, M. P. (2020). Fermentative profile and nutritive value of corn silage with Tamani guinea grass. *Semina: Ciências Agrárias*, 41(6), 2733-2746. doi: 10.5433/1679-0359.2020v41n6p2733
- Parra, C. S., Bolson, D. C., Jacovaci, F. A., Nussio, L. G., Jobim, C. C., & Daniel, J. L. P. (2019). Influence of soybean-crop proportion on the conservation of maize-soybean bi-crop silage. *Animal Feed Science and Technology*, 257, 114295. doi: 10.1016/j.anifeedsci.2019.114295
- Pereira, D., Lana, R., Carmo, D. L. D., & Costa, Y. K. S. (2019). Chemical composition and fermentative losses of mixed sugarcane and pigeon pea silage. *Acta Scientiarum. Animal Sciences*, 41, e43709. doi: 10.4025/actascianimsci.v41i1.43709
- Prado, L. G., Costa, K. A. P., Silva, L. M., Costa, A. C., Severiano, E. C., Costa, J. V. C. P., & Habermann, E. (2023). Silages of sorghum, Tamani guinea grass, and Stylosanthes in an integrated system: production and quality. *Frontiers in Sustainable Food Systems*, 7, 1208319. doi: 10.3389/fsufs.2023.1208319
- Rufino, L. D. A., Pereira, O. G., Silva, V. P., Ribeiro, K. G., Silva, T. C., Campos Valadares, S., Fº., & Silva, F. F. (2022). Effects of mixing Stylosanthes conserved as hay or silage with corn silage in diets for feedlot beef cattle. *Animal Feed Science and Technology*, 284, 115152. doi: 10.1016/j.anifeedsci.2021.115152
- Santos, H. G., Jacomine, P. K. T., Anjos, L. H. C., Oliveira, V. A., Lumberras, J. F., Coelho, M. R., Almeida, J. A., Cunha, T. J. F., & Oliveira, J. B. (2018). *Brazilian soil classification system* (5a ed.). EMBRAPA.

- Silva, V. P., Pereira, O. G., Silva, L. D., Agarussi, M. C. N., Campos Valadares, S., Fº., & Ribeiro, K. G. (2022). Stylosanthes silage as an alternative to reduce the protein concentrate in diets for finishing beef cattle. *Livestock Science*, 258, 104873. doi: 10.1016/j.livsci.2022.104873
- Smith, L. H. (1962). Theoretical carbohydrate requirement for alfalfa silage production. *Agronomy Journal*, 54, 291-293. doi: 10.2134/agronj1962.00021962005400040003x
- Souza, W. F., Costa, K. A. P., Guarnieri, A., Severiano, E. C., Silva, J. T., Teixeira, D. A. A., Oliveira, S. S., & Dias, M. B. C. (2019). Production and quality of the silage of corn intercropped with Paiaguas palisadegrass in different forage systems and maturity stages. *Revista Brasileira de Zootecnia*, 48, e.20180222. doi: 10.1590/rbz4820180222
- Tilley, J. M. A., & Terry, R. A. (1963). A two-stage technique of the "in vitro" digestion of forage crop. *Grass and Forage Science*, 18(2), 104-111. doi: 10.1111/j.1365-2494.1963.tb00335.x
- Van Soest, P. J. (1994). *Nutritional ecology of the ruminant* (2nd ed.). Cornell University Press.