



Silage quality of *Urochloa brizantha* cultivars with levels of campo grande Stylosanthes

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ABSTRACT. The seasonality of forage production represents a limiting factor in the production of ruminants. The use of silages has been a solution for periods of low forage production, providing high quality food. This study was conducted to evaluate the fermentative characteristics and the chemical composition of silage of *Urochloa brizantha* cultivars with different levels of Campo Grande Stylosanthes. It was used a complete randomized block design with three replications in a 2 x 4 factorial design, being two cultivars of *Urochloa brizantha* (BRS Piata palisadegrass and BRS Paiaguas palisadegrass) and four levels of Campo Grande Stylosanthes (0, 10, 20 and 30%). Silages of *Urochloa brizantha* with Campo Grande Stylosanthes exhibit satisfactory quality, on the basis of the characteristics evaluated, with no significant difference between cultivars. The mixed silage of Campo Grande Stylosanthes with grasses at 30% has proven to be an interesting option, thus promoting adequate fermentation and maintaining the nutritional quality of silage.

Keywords: chemical composition, fermentation, inoculant.

Qualidade da silagem de cultivares de *Urochloa brizantha* com níveis de Estilosantes campo grande

RESUMO. A estacionalidade da produção de forragem é um dos fatores limitantes na produção de ruminantes. Sendo assim, a utilização de silagens tem sido uma solução para períodos de baixa produção de forragens, proporcionando alimento de boa qualidade. Com isso, desenvolveu-se esse estudo com o objetivo de avaliar as características fermentativas e a composição químico-bromatológica da silagem de cultivares de *Urochloa brizantha* com níveis de estilosantes Campo Grande. O delineamento experimental utilizado foi o de blocos completos ao acaso, com três repetições, em esquema fatorial 2 x 4, sendo dois cultivares de *Urochloa brizantha* (BRS Piatã e BRS Paiaguás) e quatro níveis de estilosantes Campo Grande (0, 10, 20 e 30%). Os resultados demonstraram que as silagens de *Urochloa brizantha* (BRS Piatã e BRS Paiaguás) com o estilosantes Campo Grande apresentam qualidades aceitáveis, com base nas características avaliadas, não havendo diferença entre os cultivares. A silagem mista de estilosantes Campo Grande com gramíneas na proporção de 30% demonstrou ser uma opção interessante, favorecendo o adequado processo fermentativo, bem como garantindo a manutenção da qualidade nutricional da silagem.

Palavras-chave: composição químico-bromatológica, fermentação, inoculante.

Introduction

In Brazil, the Central-West region is characterized by two marked seasons, rainy and dry, which causes the seasonality of forage production. Thus, the distribution of forage production throughout the year is uneven, due to the lack or abundance of rainfall, where production is high during the rainy season and scarce during the dry season (Santos & Zanine, 2006). Therefore, alternatives should be sought to supplement this forage deficit, since the requirements of the animals remain constant throughout the year. Thus, it is interesting the use of excess forage for ensiling for its use during periods of low forage production.

Silage of forage grasses is currently gaining ground due to the high production potential of tropical grasses. Among the grasses, stands out the genus *Urochloa*, which have attracted the interest of farmers, and has been widely used because of its appropriate nutritional characteristics for making silage (Costa et al., 2011), with positive results (Epifanio et al., 2014).

In addition, the use of legume silage has also attracted interest because of its high nutritional value. Among the tropical legumes, Campo Grande Stylosanthes has emerged as a promising species with great potential for the ensiling process, with a suitable source of bulky to ruminant nutrition (Silva

et al., 2015) due to its good characteristics of adaptation to low soil fertility, resistance to high humidity and temperature throughout the year and high nutritional value, currently being the main legume used in the Brazilian Cerrado (Barcellos, Ramos, Vilela & Martha Junior, 2008).

Considering the lack of information on silages of grass with legumes, there is need for further information, especially with regard to the legume levels added to the grass silage. In this sense, this study evaluated the chemical and fermentation characteristics of silages of *Urochloa brizantha* cultivars with different levels of Campo Grande Stylosanthes.

Material and methods

The experiment was conducted at the Federal Institute of Goiás, Rio Verde Campus, at 748 m asl., 17°48' South latitude and 50°55' West longitude. Cultivars of *Urochloa* (BRS Piata palisadegrass and BRS Paiaguas palisadegrass) and Campo Grande Stylosanthes were already planted since March 2013. The seeds of *Urochloa brizantha* BRS Paiaguas were provided by Embrapa Beef Cattle, through the signed material transfer agreement with the Federal Institute of Goiás, Rio Verde Campus (process: 20500.13 / 0013-1).

Before planting, soil samples were collected from the 0-20 cm layer to assess the physical and chemical characteristics of the experimental area. Overall, the following values were obtained: clay: 530 g kg⁻¹; silt: 90 g kg⁻¹; sand: 380 g kg⁻¹; pH in CaCl₂: 5.73; Ca: 2.78 cmolc dm⁻³; Mg: 0.82 cmolc dm⁻³; Al: 0.10 cmolc dm⁻³; Al+H: 5.2 cmolc dm⁻³; K: 0.49 cmolc dm⁻³; cation exchange capacity (CEC): 9.29 cmolc dm⁻³; P: 3.02 mg dm⁻³; Cu: 2.0 mg dm⁻³; Zn: 7.5 mg dm⁻³; Fe: 13.2 mg dm⁻³; and organic matter (OM): 23.21 g dm⁻³.

In planting of forage it was applied in all plots 80 kg ha⁻¹ P₂O₅ and 20 kg ha⁻¹ FTE BR 12, using super simple phosphate and FTE fertilizers, respectively. For single grass, it was applied 120 kg ha⁻¹ nitrogen and 60 kg ha⁻¹ of K₂O 30 days after germination, as urea and potassium chloride, respectively, divided into three applications, the first in April 2013, the second in October 2013 and third in January 2014.

The experimental design was a complete randomized block with three replications in a 2x4 factorial design, with two cultivars of *Urochloa* (BRS Piata palisadegrass and BRS Paiaguas palisadegrass) and four levels of Campo Grande Stylosanthes (0, 10, 20 and 30 %), calculated based on the natural matter.

The material used was harvested one year after the experimental implementation in the fall. For the

ensiling process, forages were harvested after 40 days of growth, using backpack mower, at 20 cm from the ground level. The material was minced in ensiling machine, model Nogueira EN-9F3B, into 10-30 mm particles.

All treatments received the commercial bacterial inoculant Katec® Bacto Silo C. Tropical. The inoculant used had the following assurance levels: *Lactobacillus plantarum* MA18/5U and *Pediococcus acidilactici* MA18/5M. We used 2g (400 g inoculant to 10 tons of material), diluted in 80 mL distilled water and homogeneously sprayed onto 3 kg forage. Then, the material was homogenized with the pre-set levels of Campo Grande Stylosanthes.

The material was stored in experimental PVC silos 10 cm diameter and 40 cm length. Subsequently, silos were compressed with iron pendulum and closed with PVC lids fitted with Bunsen valves and sealed with adhesive tape to maintain the environment anaerobic. Then, the experimental silos were kept in a roofed area, at room temperature.

After 54 days of fermentation, silos were opened and discarded the material of the top and the bottom of each. The central portion was homogenized and placed in plastic trays. Part of fresh silage after opening the silos was separated for analysis of pH, titratable acidity (TA) and buffering capacity (PT) by the method described by Silva and Queiroz (2002).

After, a silage sample was taken and divided into two parts. The first was wrapped in plastic bags and frozen. For determination of ammonia nitrogen (NH₃-N), the samples were thawed for juice extraction. Organic acids were determined by high performance liquid chromatography (HPLC), Shimadzu®, model SPD-10A VP, coupled to a ultraviolet (UV) detector, at 210 nm wavelength, according to Kung Jr. and Ranjit (2001).

The other part of the silage of about 1 kg was weighed, dried in a forced ventilation oven at 55°C, for 72 hours, and milled in a Wiley knife mill, with 1 mm sieve, and stored in plastic containers.

Chemical analyses were performed to determine dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and mineral matter (MM), using the method described by Silva and Queiroz (2002). Total digestible nutrient (TDN) was obtained by the equation (% TDN = 105.2-0.68 (% NDF)), proposed by Chandler (1990).

The *in vitro* dry matter digestibility (IVDMD) was assessed using the method described by Tilley and Terry (1963) and was adapted to the artificial rumen developed by ANKON® using a 'Daisy incubator' device from Ankom Technology (*in vitro*

true digestibility- IVTD). Rumen fluid was collected from two male bovines cannulated with an average weight of 500 kg, fitted with rumen fistula, where the animals were kept on pasture of *Urochloa brizantha* cv. Piata for 10 days. For the analyses, it was weighed 0.25 g of each material, placed in nylon bags (F57 model) for use in Ankon, and packaged in jars, containing ruminal fluid and buffer solution. The specimens were incubated for 48 hours when it was added a pepsin and HCl solution and allowed to ferment for 24 hours. Bags were removed from the rumen fermenter, washed with distilled water to remove the adherent materials and after the filter were dried in a forced circulation oven for 8 h 105°.

Before the ensiling process, Piata palisadegrass, Paiaguas palisadegrass and Campo Grande Stylosanthes were analyzed for chemical analysis (Table 1), according to methods described above.

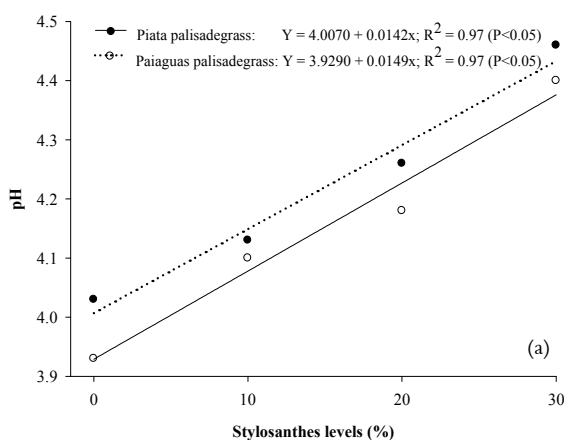
Table 1. Chemical composition of *Urochloa brizantha* cultivars and Campo Grande Stylosanthes used for silage production.

Chemical composition	Piata palisadegrass	Paiaguas palisadegrass	Stylosanthes
DM (%)	21.87	20.52	25.66
CP (%)	12.60	12.64	17.75
NDF (%)	64.51	64.80	58.32
ADF (%)	39.16	40.73	37.20
Hemicellulose	25.35	24.08	21.12
TDN (%)	57.42	58.07	55.31
Ash (%)	3.18	3.61	2.87
IVDMD (%)	55.64	56.52	59.05

DM: Dry matter; CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; TDN: Total digestible nutrients; IVDMD: *in vitro* dry matter digestibility.

Source: Elaboration of the authors

For comparison of forages, data were subjected to analysis of variance and mean values were compared by Tukey's test at 5% probability using the software SISVAR (Ferreira, 2011) and for Stylosanthes levels, regression analysis was run, and graphics were made in Sigma Plot.



Results and discussion

The results of analysis of variance indicated no significance ($p > 0.05$) for the interaction between *Urochloa brizantha* cultivars (BRS Piata palisadegrass and BRS Paiaguas palisadegrass) and Stylosanthes levels, for the variables studied, except for the pH values. The same was observed for the isolated effect of cultivars, which showed similar results ($p > 0.05$).

In turn, when analyzed the Stylosanthes levels, there was a significant effect ($p < 0.05$) for titratable acidity, buffering capacity, NH_3/TN , lactic acid, acetic acid, CP, NDF, ADF and IVDMD. And for DM content, butyric acid, propionic acid, hemicellulose, NDT and MM, there was no significant effect ($p > 0.05$) of the interaction and of isolated factors, with mean values of 23.9; 0.03; 0.22; 26.82; 55.16 and 2.25%, respectively.

In relation to pH, there was a linear increase in the values of silages of *Urochloa brizantha* cultivars with the inclusion of levels of Campo Grande Stylosanthes in the ensiled mass (Figure 1a), demonstrating that the legume was less susceptible to pH reduction when combined with grasses. It is recognized that legume silages stabilize at higher pH, as reported by Lima Orozco, Castro-Alegría and Fievez (2013). This is because legumes have higher buffering capacity over the grasses, tending to stabilize at higher pH with values greater than 4.5 for legume silages (Dewhurst, Fisher, Tweed, & Wilkins, 2003).

With regard to *Urochloa brizantha* cultivars, although belonging to the same genus and species, the Piata palisadegrass presented greater restrictions to reduce the silage pH compared to Paiaguas palisadegrass (Figure 1a).

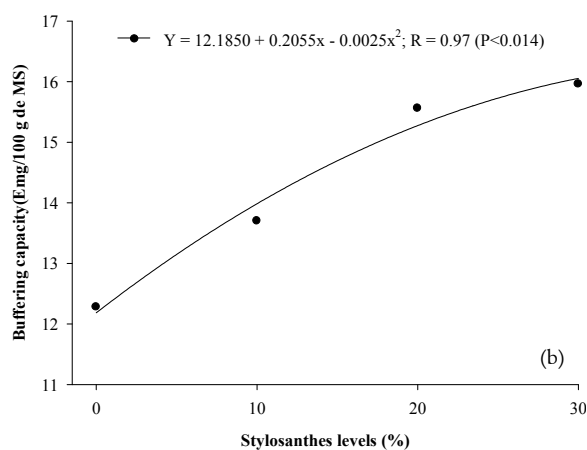


Figure 1. Values of pH of silages of Piata palisadegrass and Paiaguas palisadegrass (a) and silage buffering capacity (b) according to different levels of Campo Grande Stylosanthes.

Source: Elaboration of the authors.

The addition of *Stylosanthes* to the ensiling process also provided a greater buffering capacity in the silages of *Urochloa brizantha* cultivars (Figure 1b). This is due to the great presence of buffering substances, such as potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}), which neutralizes the organic acids formed by fermentation, preventing the reduction in pH (Smith, 1962). Legumes have high buffering capacity and low amounts of soluble carbohydrates, which require the addition of sugars or the removal of part of its moisture by wilting to improve their fermentation process (Liu, Zhang, Shi & Sun, 2011).

In general, values of buffering capacity found herein are within the ideal range recommended by the literature, which did not constitute a barrier to the rapid reduction in pH, the ideal values are below 20 eq.mg HCl/100 g DM (Ferrari Junior and Lavezzo, 2001).

Considering the titratable acidity, Figure 2a illustrates the influence ($p < 0.05$) of the addition of Campo Grande *Stylosanthes*, with a linear reduction in acidity as increased *Stylosanthes* levels in silage of *Urochloa brizantha* cultivars. In accordance with Silva and Queiroz (2002), titratable acidity analysis indicates the overall aspect of fermentation quality of silages, which influence taste, odor, color and stability, because it is directly related to the acids that determine the pH, especially acid lactic.

Silage without *Stylosanthes* showed the highest titratable acidity when compared the inclusion levels in the ensiling process. The titratable acidity is inversely proportional to pH values, which was also observed in the present study (Figure 1a), because the silage without the inclusion of legume reached, on average, a lower pH (3.93). Therefore, there was an inverse correlation between pH and titratable acidity with the addition of the legume, since the higher the pH the lower the acidity.

According to Andrade et al. (2012), titratable acidity is a sensitive indicator that, along with pH, should be used in the evaluation of silage fermentation. These results corroborate the recommendations in the literature, as works carried out by Figueredo et al. (2014) showed this relationship.

A similar trend in titratable acidity was observed for NH_3 -N (Figure 2b). There was a linear decrease of 1.93 percentage units with increasing levels of *Stylosanthes* in the silage. These results highlight the importance of the legume in the ensiling of tropical grasses, because the higher the NH_3 -N, the lower the quality of silage, in other words, there is degradation of protein compounds (true protein, peptides, amino acids, amines and amides), by *Clostridium* bacteria, to ammonia, known as promoters of proteolysis, which is lost by evaporation at the opening of the silo (Teixeira, Veloso, Pires, Silva & Nascimento, 2008). Therefore, the quantification of NH_3 -N values of the silage can be used to determine the efficiency of the fermentation process (McDonald, Henderson & Heron, 1991).

Even in treatments without addition of Campo Grande *Stylosanthes*, values of NH_3 -N were within the ideal range, because, according to Woolford (1984), the values are considered adequate when less than 10%, acceptable between 10 and 15% and unsatisfactory when above 20%. Based on these data and the above criteria, all silages can be classified as having good quality. In this case, it is evident that the silages of grasses even without addition of *Stylosanthes* did not result in excessive breakdown of protein into ammonia, causing a lower activity of *Clostridium* bacteria and hence lower production of butyric acid.

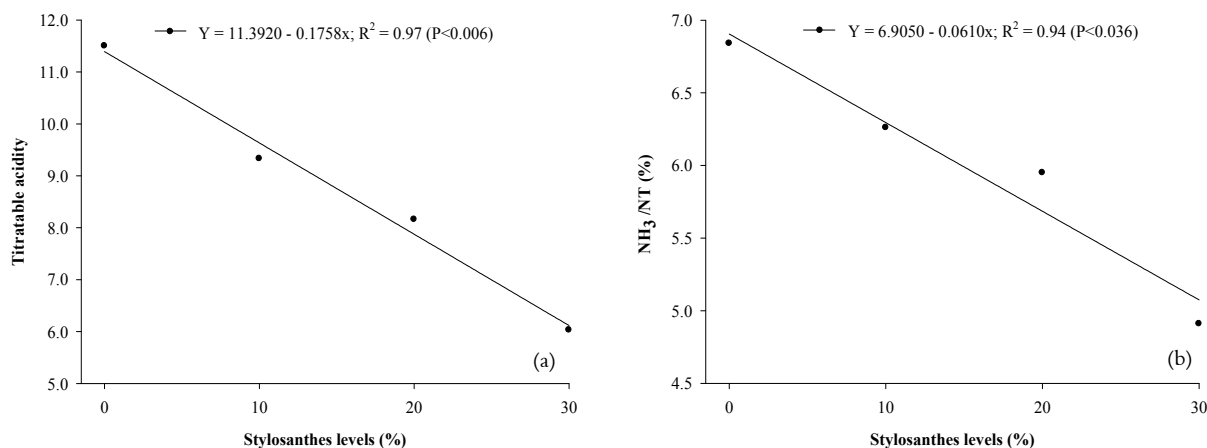


Figure 2. Titratable acidity (a) and NH_3 -N (b) of silage according to different levels of Campo Grande *Stylosanthes*.

Source: Elaboration of the authors.

Analyzing the concentration of organic acids (Figure 3), only lactic acid (a) and acetic acid (b) were influenced by the levels of Campo Grande Stylosanthes. The average concentration of propionic acid of silages was 0.04%, remaining within the proper range (less than 0.10%) for a good conservation of silage (Kung Jr. & Shaver, 2001). For butyric acid, the average concentration was 0.22% and below 0.5%, as recommended by Kung Jr. and Shaver (2001).

There was a linear increase in lactic acid with increasing levels of Stylosanthes in the grass silage (Figure 3a), showing an increase of 2.0 percentage units to the level of 30% compared to the silage without addition of Stylosanthes.

Values of lactic acid determined herein were higher when compared to acetic acid, corroborating Kung Jr. and Ranjit (2001), which reported that lactic acid should be the main acid in good silage and its content should remain at higher concentration than the other acids (acetic, propionic, butyric), once all the acids produced during the fermentation process contribute to reducing silage pH and lactic acid plays a key role in this process, as it shows a greater dissociation constancy than the others.

For grass silage, regarding the criteria set by Kung Jr. and Shaver (2001), silages with lactic acid values in the range 4-6% have good quality. In this study, the measured values ranged from 2.06 to 3.66%, which classify them as satisfactory silage, not only because of the lactic acid values, but mainly due to other parameters.

For the concentration of acetic acid (Figure 3b), there was a quadratic increase with the addition of Stylosanthes levels in silages of *Urochloa* cultivars. Acetic acid values are within the recommended by the literature, i.e. below the

2.0% limit, which, according to Kung Jr. and Shaver (2001), is the reference to classify the silages as having good quality. Still according to these authors, high acetic acid values can interfere with lactic fermentation. The concentrations of acetic and butyric acids are related with lower rates of decrease of pH values. This corresponds mainly to the more prolonged action of enterobacteria and heterofermentative lactic bacteria, although also produced by clostridia, on a smaller scale.

McDonald et al. (1991) claimed that fermentation mechanism of enterobacteria is similar to that of heterofermentative bacteria, causing losses of dry matter and small losses of energy. Therefore, well-preserved silages should have low levels of acetic acid, confirming the results obtained in this study, with values below 2.0%.

The inclusion of Stylosanthes in silages of *Urochloa brizantha* cultivars promoted a quadratic reduction in NDF, when increasing the levels in silage, where the minimum point was estimated at the level of 24 with 62.6% content. This decrease can be explained by the lower content of NDF of Stylosanthes compared to grasses (Table 1), occurring the dilution of the fiber, with the Stylosanthes levels added to the silage.

It is known that the NDF content in forages is inversely related to the dry matter intake by the animal, that is, the higher the NDF content the lower the total intake. In this way, the progressive increase in NDF content can reduce the intake of dry matter, due to the physical effect of rumen filling by excessively fibrous material, reducing the rate of passage of food through the digestive tract (Bosa et al., 2012).

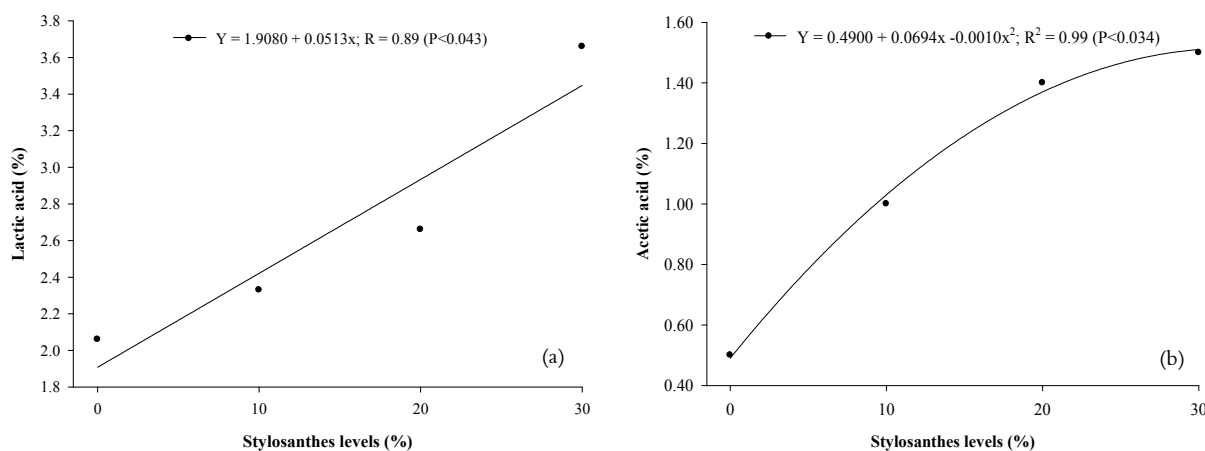


Figure 3. Concentration of lactic acid (a) and acetic acid (b) of silage according to different levels of Campo Grande Stylosanthes.

Source: Elaboration of the authors.

Figure 4b shows that the addition of Campo Grande Stylosanthes also contributed to linearly reduce ADF content in silage of *Urochloa brizantha* cultivars. With addition of levels 10; 20 and 30% of Stylosanthes, the contents of ADF were 40.73; 39.16 and 37.20% respectively, according to the recommendations by Van Soest (1994), which is below 40%. High ADF contents lead to the unavailability of potentially degradable structural carbohydrates, since a component of the cell wall, lignin, forms a barrier that prevents microbial adherence and enzymatic hydrolysis of cellulose and hemicellulose, and consequently decreases fiber digestibility and quality and the use of forage.

This indicates that the production of silage of *Urochloa brizantha* with Stylosanthes can be a good indicator, because, in agreement with Van Soest (1994), ADF content is negatively correlated with digestibility, and consequently, better nutritional value, a fact confirmed in this study.

Differences in fiber content between silage of grasses and legumes were also reported by Silva, Jobim, Nascimento, Ferreira and Oliveira (2014), which is indicative of higher digestibility and hence better nutritional value of the silage. Studies performed by Evangelista et al. (2005) evidenced decreases in ADF content of sorghum silages when added 10, 20, 30 and 40% of Leucena.

There was a linear increase in CP content with the increase in Stylosanthes levels in silage grass, with an increase of 43.3% when comparing the silage without Stylosanthes with that containing 30% Stylosanthes. This increase is due to higher crude protein content in Campo Grande Stylosanthes (17.75%), compared to Piata palisadegrass and Paiaguas palisadegrass (Table 1). In this sense, when added to the silage, it improves the quality of the

grass silage, and restricts *Clostridium* activity, preserving the protein fraction of the forage. Thus, the association of silage of grasses with legumes becomes advantageous to improve the CP content of grass silages.

Souza, Pereira, Ribeiro Santos and Valadares Filho (2014) stated that Campo Grande Stylosanthes can be used in the form of silage, once it represents a good alternative to increase the protein content, being a promising dietary option to be used as a forage source in beef cattle diets during the growth and finishing phases, and its use depends on the availability and economic factors.

Values of CP content, even without the addition of Stylosanthes, were higher than 7%, which, according to Van Soest (1994), are sufficient to provide nitrogen for a proper microbial fermentation in the rumen. This observation is explained by the high leaf: stem ratio of Piata palisadegrass and Paiaguas palisadegrass, considered forages with high productive and nutritional potential for the ensiling process (Perim et al., 2014).

Considering the IVDMD, Figure 5b shows that there was a quadratic increase in IVDMD with the increase in Stylosanthes levels. Van Soest (1994) explains that there is a rise in IVDMD with the addition of a material containing a higher content of non-structural carbohydrates (starch, pectin and sugars), which are more digestible than structural ones (cellulose and hemicellulose) and may be related with that found in this study, with inclusion of the Campo Grande Stylosanthes. In this case, this increase is justified by lower fiber content of the legume, contributing to higher digestibility of silage produced.

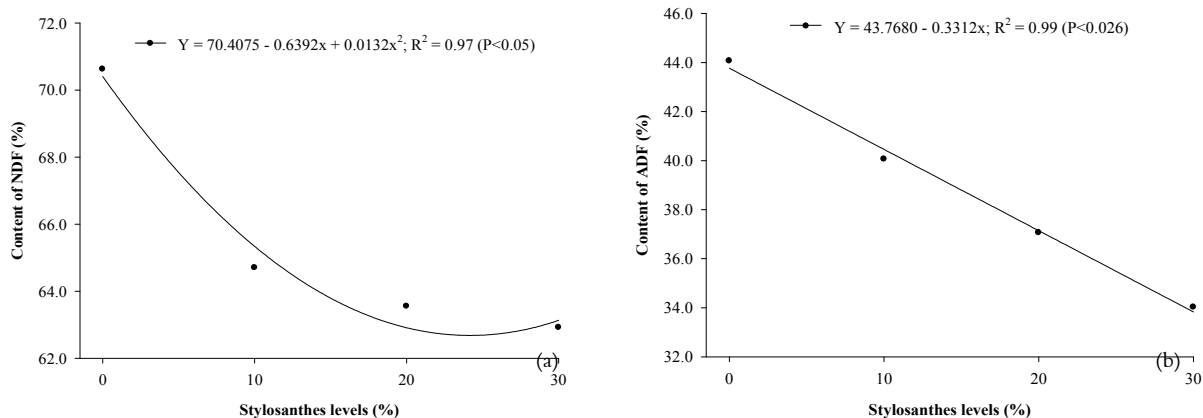


Figure 4. Content of NDF (a) and ADF (b) of silage according to different levels of Campo Grande Stylosanthes.

Source: Elaboration of the authors.

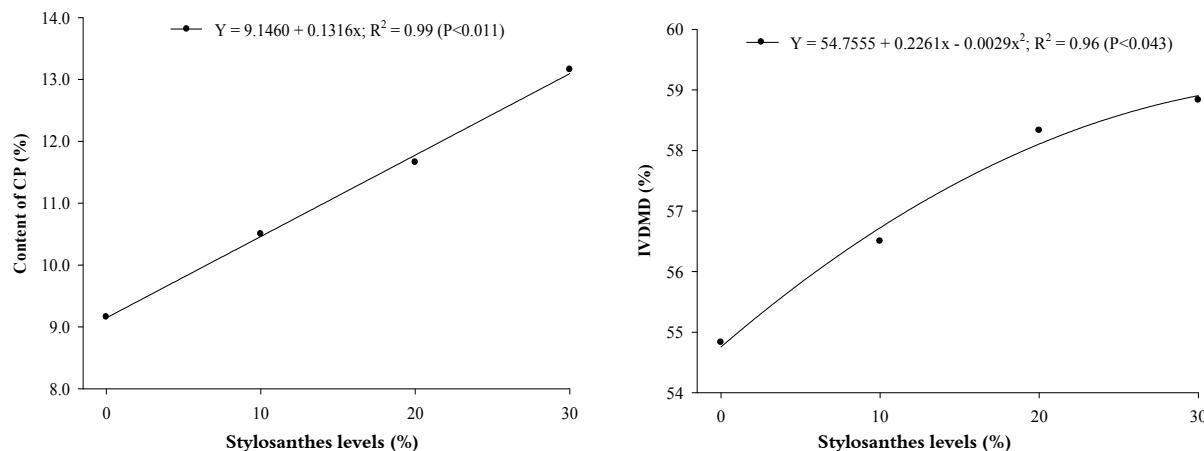


Figure 5. Content of CP (a) and IVDMD (b) of silage according to different levels of Campo Grande Stylosanthes.

Source: Elaboration of the authors.

Lower values of IVDMD (54.83%) were obtained in the silage without the addition of Stylosanthes. Meanwhile, it was registered that the largest increases were provided from the level 10 to 20% with 1.83 percentage units, and from the level of 20 to 30%, this increase was smaller: 0.5 percentage units.

Additionally, Silva et al. (2014) examined the use of additives in silages of Campo Grande Stylosanthes and verified IVDMD of 66.57% in the silage of Stylosanthes alone. This value was higher than that found in this study, which was 58.83%, when added 30% of Stylosanthes in the ensiling process.

Conclusion

Silages of *Urochloa brizantha* (BRS Piata palisadegrass and BRS Paiaguas palisadegrass) with Campo Grande Stylosanthes exhibit satisfactory quality, on the basis of the characteristics evaluated, with no significant difference between cultivars.

The mixed silage of Campo Grande Stylosanthes with grasses at 30% has proven to be an interesting option, thus promoting adequate fermentation and maintaining the nutritional quality of silage.

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