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ORIGINAL ARTICLE

Nutritive value and fermentation quality of palisadegrass and stylo mixed silages

Juliana S. da SILVA,[1] Karina G. RIBEIRO,[1] Odilon G. PEREIRA,[1] Hilário C. MANTOVANI,[2] Paulo R. CECON,[3] Rosana C. PEREIRA[4] and Janaina de L. SILVA[5]

[1]*Departments of Animal Science,* [2]*Microbiology,* [3]*Statistics, Universidade Federal de Viçosa, Viçosa,* [4]*Fluminense Federal Institute of Education, Science and Technology, Bom Jesus do Itabapoana Campus, and* [5]*Multidisciplinar Center of Barra, Universidade Federal do Oeste da Bahia, Barra, Brazil*

ABSTRACT

The nutritive value and fermentation quality of palisadegrass (*Brachiaria brizantha* cv. Xaraes) and stylo (*Stylosanthes capitata* × *S. macrocephala* cv. Campo Grande) mixed silages were evaluated. The experiment was analyzed in a factorial scheme (5 × 2) in a completely randomized design using increasing levels of stylo (0, 25, 50, 75 and 100% on a fresh matter basis) on palisadegrass silages, with and without microbial inoculants (MI). With the increased ratio of stylo in mixed silages, dry matter (DM), crude protein (CP), acid detergent fiber (ADF), and lignin content increased in silages. The presence of MI promoted lower DM content, and higher neutral detergent fiber corrected for ash and protein, ADF and lignin content. The acid detergent insoluble nitrogen content and the lactic acid bacteria populations were not affected by treatments. The *in vitro* DM digestibility was affected by the interaction of levels of the stylo and MI. The pH, NH_3-N/total nitrogen and butyric acid concentrations decreased with increasing levels of stylo. Better nutritive value and quality of fermentation was found in the silage containing higher proportions of this stylo mixed with palisadegrass. The microbial inoculant evaluated did not alter the nutritive value or quality of the fermentation of the silages in this experiment.

Key words: *fermentative capacity,* in vitro *dry matter digestibility, lactic acid bacteria, microbial inoculant, organic acids.*

INTRODUCTION

Brazil has wide areas of pasture with tropical climate grasses, and the silage of these grasses has been used in the animal production system. Ensiling of grasses mixed with legumes has become a promising method of increasing the concentration of crude protein (CP) and digestibility of silages (Ajayi 2011; Contreras-Govea *et al.* 2011). However, the literature is still lacking on mixed silages of tropical grasses and legumes.

Brachiaria brizantha cv. Xaraes has a good nutritive value and produces more forage, exhibits more rapid regrowth after grazing, and has a greater carrying capacity during the rainy season and greater annual productivity than *B. brizantha* cv. Marandu. The former variety is also more adapted to Cerrado soils with medium fertility (Valle *et al.* 2004).

Stylo cv. Campo Grande is derived from a physical mixture of the seeds of *Stylosanthes capitata* and *S. macrocephala* (80:20 by weight) through the genetic combination of several accessions, selected by outcrossing over more than six generations (Grof *et al.* 2001). This legume adapts well to conditions of low fertility, particularly in sandy soil; is tolerant of anthracnose; has good persistence in pasture systems; and is more competitive than other crops in pastures intercropped with grasses (Fernandes *et al.* 2005). Magalhães and Corrêa (2012) reported a high nutritive value for stylo Campo Grande, which suggests a high intake by cattle.

Microbial inoculants (MI) have been used to control and improve fermentation during the ensilage process. These inoculants are mostly lactic acid bacteria (LAB) that promote rapid and efficient fermentation by producing high concentrations of lactic acid. Thus, CP losses could be reduced because the

Correspondence: Karina Guimarães Ribeiro, Departamento de Zootecnia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, 36570-900, Brazil.
(Email: karinaribeiro@ufv.br)
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formation of ammonia is inhibited when increased lactic acid concentration reduces pH. Further studies are still necessary to elucidate the appropriate dosage for each crop and the proper production conditions (Heinritz *et al.* 2012).

The objective of this study was to investigate the effects of the different mixture ratios of palisadegrass and stylo with and without lactic acid bacteria inoculation on the nutritive value and fermentation quality of silages.

MATERIALS AND METHODS

Silage material and treatments

The trial was performed at the Experimentation, Research and Extension Center of Triângulo Mineiro (Central de Experimentacão, Pesquisa e Extensão do Triângulo Mineiro, CEPET) of the Federal University of Vicosa (Universidade Federal de Viçosa, UFV), Minas Gerais, Brazil (18.41°S, 39.34°W, 520 m above sea level, annual mean temperature 23°C, and annual average rainfall 1530.2 mm).

Palisadegrass (*Brachiaria brizantha* cv. Xaraes) was collected after 60 days of regrowth, and stylo (*Stylosanthes capitata* × *Stylosanthes macrocephala* cv. Campo Grande) was collected at the beginning of flowering with a JF Z10 forage harvester (JF Agricultural Machinery, SP, Brazil) fitted with a model FAHARA-100 harvesting platform (Harama Q, Hinterland, RS, Brazil), with particle size of approximately 2 cm. The fresh forage was then weighed, and palisadegrass and stylo were mixed in the following ratios: 100:0, 75:25, 50:50, 25:75 and 0:100 with and without MI. Thirty buckets were used, three buckets of each combination to provide the replications.

The chemical composition, buffering capacity (BC), fermentative capacity (FC), and microbial populations of palisadegrass and stylo, determined before ensilage and without MI, are shown in Table 1.

Table 1 Chemical and microbial compositions of palisadegrass and stylo as fresh material (FM)

	Palisadegrass	Stylo
DM (g/kg)	208.5	264.9
CP (g/kg DM)	95.5	122.4
NDFap (g/kg DM)	674.9	601.7
ADF (g/kg DM)	369.3	418.2
ADIN (g/kg DM)	53.9	68.6
Lignin (g/kg DM)	70.9	125.4
WSC (g/kg DM)	41.9	22.5
BC (e.mg HCl/100 g DM)	44.4	43.7
WSC:BC ratio	0.94	0.51
FC	28.4	30.6
LAB (log cfu/g FM)	5.03	5.08
Enterobacteria (log cfu/g FM)	6.02	6.34
Molds plus yeasts (log cfu/g FM)	5.52	5.49

ADF, acid detergent fiber; ADIN, acid detergent insoluble nitrogen; BC, buffering capacity; cfu, colony-forming units; CP, crude protein; DM, dry matter; FC, fermentative capacity; FM, fresh matter; LAB, lactic acid bacteria; log, denary logarithm of the numbers; NA, unvalued; NDFap, neutral detergent fiber corrected for ash and protein; WSC, water soluble carbohydrates.

Silage making

The microbial inoculant Sil-All4x4 Water Soluble (Alltech, Paraná, Brazil) contained *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Lactobacillus salivarius* subsp. *salivarius* (min. 1.89×10^{10} colony-forming units (cfu)/g), and *Enterococcus faecium* (min. 2.10×10^{9} cfu/g); enzymes (xylanase (min. 16 U/g), amylase (min. 200 UI/g), cellulase (min. 10 UI/g) and hemicellulotytic enzyme (min. 16 U/g)); silicon dioxide, and saccharose. The MI was used at the recommended rate of 5 g/t of fresh forage. It was diluted in deionized water and applied using a 2 L hand sprayer by spraying uniformly onto the forage that was constantly hand-mixed. The untreated material received a volume of water equal to the amount of inoculant.

After the treatment application, the untreated and treated forages were ensiled in plastic silos with 20 L capacities and equipped with snap-on lids fitted with a Bunsen valve that only enabled gas release by the fermentation. At the bottom of the silos, 4 kg of sand were placed inside a bag with cotton tissue to capture the effluent, and the forage mass ensiled was 16.2 kg on average in each silo. The 30 silos were kept in a covered area at room temperature. The silos were weighed, conserved at 25 ± 1°C, and opened after 150 days. At the opening, each silo was weighed, and the contents were mixed thoroughly and sub-sampled (300 g) to determine dry matter (DM) content, chemical composition, *in vitro* dry matter digestibility (IVDMD), fermentation profile and microbiological counts.

Chemical and microbial analyses

To determine the chemical composition, the pre-ensilage forage and silage samples were dried in an oven at 55°C and ground in a Wiley mill with a 1 mm sieve. These samples were used to determine the concentrations of DM (Association of Official Analytical Chemists – AOAC (2005); method number 930.15); CP, obtained by total nitrogen determination, according to the Kjeldahl method (AOAC 2005; method number 976.05); acid detergent insoluble nitrogen (ADIN), according to Licitra *et al.* (1996); NH_3/TN (ammonia nitrogen as part of the total nitrogen), according to Bolsen *et al.* (1992); neutral detergent fiber corrected to ash and protein (NDFap) according to the methods of Van Soest *et al.* (1991); and acid detergent fiber (ADF) and lignin (AOAC 2005; method number 973.18).

In vitro DM digestibility was determined by the technique proposed by Tilley and Terry (1963), including second stage using NDF solution according to Silva and Queiróz (2004). The samples were placed in contact with the rumen fluid in test tubes and incubated for 48 h, simulating the pH conditions, microorganisms, buffering capacity, anaerobiose and temperature of the rumen. After that period of incubation, NDF analysis was carried out to obtain the indigestible residue.

To microbial counts, 25 g of wet sample were transferred into sterile homogenization bags containing 225 mL of sterile solution (Ringers Solution®, Oxoid, Basingstoke, UK) to obtain a dilution of 10^{-1} and homogenized for 4 min in an industrial blender. Serial dilutions were prepared with de Man, Rogosa and Sharpe (MRS) agar-*Lactobacillus* MRS Broth® (Difco Laboratories, Detroit, MI, USA) to determine LAB numbers at 37°C for 48 h (Difco Laboratories) and to determine enterobacteria numbers after incubation at 37°C for 24 h in Violet Red Bile agar medium using a pour plate technique. Mold and yeast numbers were determined using the technique (Petrifilm™, 3M Microbiology Products, St. Paul, MN, USA) after incubation at 25°C for 3 and 5 days for yeast and mold, respectively. The mold and yeast cfu were enumerated separately, according to their macromorphological features, using values between 30 and 300 cfu for counting. The results were transformed into log X to achieve normal distribution.

The FC of the pre-ensilage forage was calculated according to the following equation proposed by Weissback and Honig (1996) and cited by Oude-Elferink *et al.* (2000): $FC = DM + 8 \times (WSC/BC)$, where DM is the dry matter (g/kg), WSC is the water soluble carbohydrates (g/kg), and BC is the buffering capacity (eq.mg of HCl/100 g DM). The WSC were determined using the anthrone method, and the samples were run in a colorimetric spectrometer, according to the technique described by Silva and Queiróz (2004). The BC of silages was determined as described by Playne and McDonald (1966).

The pH was measured with a glass electrode pH meter. To determine the fermentation profile of the silages, an extract was prepared using 20 g of fresh sample that was diluted in deionized water (1:10) and homogenized for 30 s in an industrial blender. After homogenization, the mixture was filtered through four layers of gauze. An aliquot of 20 mL of this filtered material was centrifuged at 48 400 g for 20 min at −20°C (Filya *et al.* 2007) to quantify the organic acids according to Siegfried *et al.* (1984). The organic acids used for standard calibration curve were: acetic acid, propionic acid, butyric acid and lactic acid, all in an initial concentration of 10 mmol/L, except acetic acid, which had an initial concentration of 20 mmol/L. The samples were analyzed in an Ultimate 3000 dual chromatograph (Dionex, Sunnyvale, CA, USA) coupled to a Shodex RI-101 refractive index (RI) detector (Showa Denko, Kawasaki, Kanagawa, Japan) at 45°C and equipped with a 300 × 7.8 mm Rezex ROA ion-exchange column (Phenomenex, Torrance, CA, USA) maintained at 45°C. The mobile phase used was 4.2 mmol/L sulfuric acid (H_2SO_4) and sodium-free 0.35 mmol/L ethylenediaminetetraacetic acid with a flow rate of 0.7 mL/min.

Statistical analysis

The experiment was analyzed in a factorial scheme (5 × 2) in a completely randomized design using increasing fresh matter levels of stylo (0, 25, 50, 75 and 100%), with or without MI, with three repetitions.

The results were subjected to analysis of variance, with the means of the quantitative factors subjected to regression analysis and the means of the qualitative factors compared by F-test at 5% probability of a type I error, using the statistical program SAEG 9.1 (SAEG 2007). For the quantitative factor, the models were chosen based on the significance of the regression coefficients using the *t*-test adopting the 10% level of probability, the coefficient of determination (R^2), and the biological behavior of the phenomenon.

RESULTS

Chemical composition and *in vitro* DM digestibility of silages

There were no significant effects ($P > 0.05$) of the interaction between mixture ratio and MI for any of the variables evaluated (Table 2) on silages, except for IVDMD ($P < 0.05$). There was no significant effect ($P > 0.05$) of MI on the CP, ADIN or WSC content of silages, with average means of 108.5; 80.5 and 7.3 g/kg DM, respectively (Table 2). The MI significantly affected the contents of DM, NDFap, ADF and lignin ($P < 0.01$). There was a significant effect of mixture ratio for DM ($P < 0.01$), CP ($P < 0.01$), NDFap ($P < 0.01$), ADF ($P < 0.05$), lignin ($P < 0.01$), and WSC ($P < 0.01$) content (Table 2).

The chemical composition of silages was changed by increasing levels of stylo (Table 4); the DM, CP and lignin content increased linearly ($P < 0.01$), and NDFap content decreased linearly ($P < 0.01$). No statistical model was adjusted to the ADF content the average of which was 424.3 g/kg. IVDMD for non-inoculated silage showed a quadratic effect, with a maximum value of 558.2 g/kg for silage containing 49.3% of stylo. In contrast, no statistical model was adjusted to the IVDMD coefficient for inoculated silage which averaged 424.3 g/kg (Table 4).

Table 2 Chemical composition of palisadegrass silage with increasing levels of stylo ensiled with (I) or without (NI) microbial inoculant

Item	Level of stylo (%)					SEM	Significance		
	0	25	50	75	100		L	I	L × I
DM (g/kg)									
NI	210.1	223.4	234.0	242.0	248.0	0.29	**	**	NS
I	201.6	215.2	223.2	234.8	248.7				
CP (g/kg DM)									
NI	74.9	95.6	105.7	124.5	142.1	0.41	**	NS	NS
I	76.5	104.9	102.5	119.2	136.7				
NDFap (g/kg DM)									
NI	699.9	654.1	636.8	609.9	598.2	0.72	**	**	NS
I	717.3	674.0	676.3	638.3	615.4				
ADF (g/kg DM)									
NI	410.3	417.6	417.8	424.9	419.8	0.21	*	**	NS
I	418.9	429.2	430.1	442.8	432.0				
ADIN (g/kg DM)									
NI	82.3	77.8	82.9	86.1	81.5	0.14	NS	NS	NS
I	66.5	79.3	78.1	81.6	89.9				
Lignin (g/kg DM)									
NI	67.6	70.8	94.1	101.1	122.7	0.39	**	**	NS
I	76.2	94.0	107.2	114.3	130.7				
WSC (g/kg DM)									
NI	9.0	8.0	6.7	6.2	7.2	0.01	**	NS	NS
I	8.1	7.3	6.8	6.6	6.8				
IVDMD (g/kg DM)									
NI	523.7[a]	534.5[a]	554.0[a]	566.0[a]	505.2[b]	0.72	NS	NS	*
I	495.6[a]	566.5[a]	493.3[b]	541.0[a]	570.7[a]				

Values within the same row or column with different superscripts in lowercase letters differ significantly from each other at $P < 0.05$; * and **: significant at $P < 0.05$ and 0.01, respectively; NS: not significant. DM, dry matter; CP, crude protein; NDFap, neutral detergent fiber corrected for ash and protein; ADF, acid detergent fiber; ADIN, acid detergent insoluble nitrogen; WSC, water-soluble carbohydrate; IVDMD, *In vitro* dry matter digestibility; L, level of stylo; I, inoculated; L × I, interaction of stylo levels and microbial inoculant; SEM: standard error of the mean.

Fermentation quality of silages

There were no significant effects ($P > 0.05$) of the proportional ratio of stylo, MI and the interaction of both treatments on the LAB population (Table 3) which presented a general average of 6.8 log cfu/g. Enterobacteria and fungal (molds and yeasts) populations were not detected in the silages. However, there was a linear decrease ($P < 0.01$) of pH with increasing ratios of stylo, ranging from 4.4 to 3.8 (Table 4). The NH_3/TN ratio decreased linearly ($P < 0.05$) with increasing ratios of stylo, ranging from 152.7 to 85.5 g/kg in silages (Table 4).

As shown in Table 3, there were no significant effects ($P > 0.05$) of the interaction between stylo level and MI on the organic acids. Except for butyric acid ($P < 0.05$), the stylo level and MI did not affect ($P > 0.05$) the concentrations of organic acids. The butyric acid concentrations decreased linearly by increasing the stylo level in silage (Table 4).

DISCUSSION

The DM and WSC are critical for the production of good quality silages since WSC is the main source of nutrients for microorganism growth and lactic acid production. The DM content obtained for fresh forages in our study (Table 1) was suitable to produce lactic acid content near to 60 g/kg (Table 3). According to Mahanna (1994), silages with DM content below 350 g/kg present lactic acid content of 60–80 g/kg, and the WSC content of 40–60 g/kg in grass and legumes is desirable for supporting a good fermentation of silage. In our study, stylo had lower WSC concentration before ensiling than the minimum value reported by Mahanna (1994), while palisadegrass presented 41.9 g/kg DM of WCS. In general, legumes have a lower WSC content (McDonald *et al.* 1991), which can explain these results.

The BC of the forages was similar before ensiling, whereas the WSC:BC ratio was lower for stylo in the forage material, which could be attributed to the lower concentration of WSC in stylo (Table 1). Woolford (1984) recommended that the DM content of ensiled forage exceed 350 g/kg, when the WSC:BC ratio is less than 1.0, to provide adequate fermentation and good quality silage. However, in the present study, that ratio was less than 1.0 for the forages, and the DM contents were 209 and 265 g/kg

Table 3 Lactic acid bacteria (LAB), pH, ammonia nitrogen as part of the total nitrogen (NH_3/TN), and organic acids content of palisadegrass silage with increasing levels of stylo insiled with (I) or without (NI) microbial inoculant

Item	Level of stylo (%)					SEM	Significance		
	0	25	50	75	100		L	I	L × I
LAB (log cfu/g)									
NI	7.1	7.4	7.2	6.9	6.9	0.24	NS	NS	NS
I	6.8	4.7	6.9	6.7	7.2				
pH									
NI	4.6	4.2	4.1	4.0	3.8	0.05	**	NS	NS
I	4.3	4.1	4.0	4.0	3.9				
NH_3/TN (g/kg)									
NI	155.6	121.6	103.3	96.2	92.3	0.75	*	NS	NS
I	168.4	131.1	128.4	105.3	89.1				
Lactic acid (g/kg dry matter)									
NI	67.3	57.2	62.0	65.9	62.1	0.31	NS	NS	NS
I	33.9	57.2	53.0	59.3	52.9				
Acetic acid (g/kg dry matter)									
NI	16.7	16.2	17.5	19.3	19.5	0.05	NS	NS	NS
I	15.5	20.8	19.8	20.3	16.8				
Propionic acid (g/kg dry matter)									
NI	1.1	0.7	1.1	0.8	0.9	0.88×10^{-2}	NS	NS	NS
I	0.5	0.5	0.9	1.1	1.1				
Butyric acid (g/kg dry matter)									
NI	13.1	6.2	3.7	0.8	0.7	0.18	*	NS	NS
I	22.4	9.5	10.7	5.8	0.0				

Values within the same row or column with different superscripts in lowercase letters differ significantly from each other at $P < 0.05$; * and **: significant at $P < 0.05$ and 0.01, respectively; NS: not significant; log, denary logarithm of the numbers; cfu, colony-forming units; L, level of stylo; I, inoculated; L × I, interaction stylo levels and microbial inoculant; SEM, standard error of the mean.

Table 4 Regression equations for variables on palisadegrass silages with increasing levels of stylo

Variables	Regression equation	r^2
DM (g/kg)	$\hat{Y} = 207 + 0.412^{**}X$	0.93
Crude protein (g/kg DM)	$\hat{Y} = 78.5 + 0.596^{**}X$	0.93
NDFap (g/kg DM)	$\hat{Y} = 701 - 0.975^{**}X$	0.90
ADF (g/kg DM)	$\hat{Y} = 424$	–
Lignin (g/kg DM)	$\hat{Y} = 68.9 + 0.312^{**}X$	0.89
pH	$\hat{Y} = 4.40 - 0.00572^{**}X$	0.54
NH_3/TN (g/kg)	$\hat{Y} = 153 - 0.672^{*}X$	0.46
Butyric acid (g/kg DM)	$\hat{Y} = 15.1 - 0.157^{**}X$	0.89
IVDMD (NI) (g/kg DM)	$\hat{Y} = 516 + 1.69^{*}X - 0.0172^{*}X^2$	0.70
IVDMD (I) (g/kg DM)	$\hat{Y} = 533$	–

DM, dry matter; NDFap, neutral detergent fiber corrected for ash and protein; ADF, acid detergent fiber; NH_3/TN, ammonia nitrogen as part of the total nitrogen; IVDMD, *in vitro* dry matter digestibility; X, level of stylo (0; 25; 50; 75 and 100%). * and **: significant at $P < 0.05$ and 0.01, respectively.

for palisadegrass and stylo, respectively. In addition, the ensiled forages in the present study also had similar FC that was slightly lower than 35, a value that is considered to be satisfactory for adequate fermentation (Oude-Elferink *et al.* 2000). According to these authors, limited fermentable sugar concentrations and microorganism populations, along with low DM concentrations, could interfere with the fermentation process.

The inclusion of stylo increased DM and CP content of silage (Table 4). Mixed ensilage of palisadegrass and stylo showed the possibility of increments between 20% and 60% in the CP content, with the addition of 25–75% of stylo in the silage mass in relationship to the exclusive grass silage. Also, Contreras-Govea *et al.* (2011) observed the increase in CP concentration in maize silage and sorghum silage mixed with legumes.

The decrease in NDFap content with increasing ratios of stylo in silages is explained by the lower concentration of structural carbohydrates in the cell wall of legumes compared to the grass (Van Soest 1994), contributing to improving the digestibility of mixed silage. However, there was also an increase in lignin content, which was expected since the cell wall of legumes has a higher lignin concentration compared to grasses (Van Soest 1994), and it resulted in lower digestibility in silages containing a higher ratio of stylo. Moreover, the higher ADF content in silages compared with material before ensilage may be associated to the heating during the respiration process (Muck *et al.* 2003) resulting in reducing sugars bound to amino acids, a process known as the 'Maillard raction', thereby affecting the analyzed content of ADF because glycated amino acids are detected as ADF (Bolsen 1995; Bureenok *et al.* 2016). These results in

part could explain the IVDMD coefficient behavior. The maximum value of IVDMD (558.2 g/kg) estimated with 49.3% of stylo silage obtained without MI (Table 4) was a little higher than observed by Silva *et al.* (2013) for the whole stylo plant (526.1 g/kg).

Muck (1988) stated that approximately 10^8 LAB per gram of crop are required before a noticeable drop in pH occurs, and our findings with commercial inoculant (10^5 to 10^6 cfu/g) demonstrated that this cell density was not sufficient to result in rapid pH decrease and overcome the epiphytic population in palisadegrass and stylo mixed silages. Additionally, no benefit on LAB population was obtained when the commercial inoculant was used.

In general, legume silages have a higher pH than grass silages and take longer to ensile because of the higher buffering capacity (McDonald *et al.* 1991), including silage of stylo (Liu *et al.* 2012). Therefore, the decrease in pH from 4.4 to 3.8 with increasing the proportion of stylo in the silages was a surprising result, mainly because the lactic acid content was not affected by treatments. However, we registered higher DM, lower butyric acid, and lower ammonia nitrogen contents with increasing of stylo in the silages, and this could explain the decreasing silage pH.

According to Mahanna (1994), high-quality grass and legume silages are represented by lactic acid concentration between 60 and 80 g/kg DM, and maximum acetic acid concentration of 20 g/kg DM. In our study, the silages presented almost 60 g/kg of lactic acid and below 20 g/kg of acetic acid.

Regarding propionic acid concentration in the mixed silages, all values obtained are within or close to the optimal range for propionic acid production, which should be between 1.0 and 10 g/kg DM for good quality silage, according to Mahanna (1994).

The butyric acid concentrations were higher in the silage with higher proportions of palisadegrass and closer to zero in the silage with stylo, which agrees with Liu *et al.* (2012). These authors did not detect the presence of butyric acid in most of the silages studied. The butyric acid concentration established for high-quality silage is below than 1.0 g/kg (Mahanna 1994).

A high concentration of butyric acid (>5.0 g/kg of DM) indicates that the silage has undergone clostridial fermentation, which is one of the poorest fermentations (Kung & Shaver 2001). Thus, the lower concentration of butyric acid found in mixed silage with stylo indicates a good fermentation and quality of silages, probably due to the increase of DM content and the reduction of pH with increasing ratios of stylo. The greater presence of NH_3/TN in palisadegrass silage can also be indicative of the clostridial presence in the silage and the use of such intermediary metabolic pathways. In contrast, Uchida and Kitamura (1987) found that stylo (*Stylosanthes guianensis* cv. Schofield) and siratro (*Macroptilium atropurpureum* cv. Siratro) included with rhodes grass (*Chloris gayana*) increased n-butyric acid content from 1.5 to 10.5 g/kg in silage. Also, these authors observed lower IVDMD with increasing legume levels in the silage.

The MI did not alter positively the nutritive value or fermentation characteristics of the silages in this experiment. Most enzymes used in silage additive are microbial by-products having enzymatic activity. All of these enzymes, except proteases, are fibrolytic enzymes that are components of many silage additives. These products have a dual purpose: (i) to provide extra sugars for fermentation; and (ii) to reduce fiber content so that the silage is digested more like a higher quality, less mature silage (Muck & Kung 1997). However, unfortunately, these products have not been as effective in silage as one would expect. A reduction of forage cell-wall fiber by an enzyme silage additive requires the presence of the synergistic activities of multiple enzymes in the cellulase and hemicellulase enzyme complexes. The poor effectiveness of some enzyme combinations in modifying silage composition or fermentation may be attributed to lack of, or lower levels of, one or more critical enzymes in the complexes (Kung 2009).

We recommend at least 50% of stylo mixed to *Brachiaria* to provide silages with better nutritive value and quality of fermentation.

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